A Comparison of Life-history Characters of Arctic and Alpine Populations of the Annual *Koenigia islandica*

by

Ioan Wagner

Thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of Doctor in Philosophy

Department of Biology

Carleton University
Ottawa, Ontario

November 2007

Your file Votre référence
ISBN: 978-0-494-36797-1
Our file Notre référence
ISBN: 978-0-494-36797-1

ABSTRACT

Temperature limits biological activity in tundra environments. Because of the gradients of latitude and elevation along their extensive range, however, many differences in environmental characteristics exist among arctic and alpine tundra ecosystems. Species inhabiting both environments are expected to adapt to this range of environmental conditions by evolving either ecotypes or phenotypically plastic populations.

Annual plants are rare in tundra environments because of having to complete their entire life cycle in a short, cold summer. The few annual species that grow in these harsh habitats reveal a number of prominent characteristics, such as very small habit, rapid development and low temperature optima for growth and reproduction. Yet, despite the theoretical importance of their biology and ecology, arctic and alpine annuals have been little studied.

This study focuses on dissimilarities between arctic and alpine environments, and corresponding evolved differences in germination characters, morphology, phenology and life histories of six arctic and alpine populations of the widely distributed arctic-alpine annual *Koenigia islandica* L.

Striking dissimilarities, suggesting ecotypic differentiation among the six populations, were found in all studied traits, including temperature and light requirements for seed germination, as well as the effect of winter-like cold treatment (stratification) on germination. Morphology, phenology and life-history characters were also found to be strikingly dissimilar among the six populations of *K. islandica* grown in simulated arctic and alpine conditions. On the basis of phenology, the six populations can be clearly grouped into arctic, high latitude alpine and alpine populations: arctic plants develop and

flower earliest, and alpine plants latest. Populations from high latitude sites and one alpine population performed better in arctic conditions, whereas the lowest latitude alpine population and, surprisingly, a high arctic population showed no enhanced performance under simulated arctic conditions.

Arctic and alpine populations of *K. islandica* offers a unique opportunity to gain insight into divergent life-history strategies found within species, and provides evidence for strong ecotypic differentiation. Furthermore, the dynamics of this extreme heat-intolerant species makes it ideal for use as a tool in the study of global climate change.

ACKNOWLEDGEMENTS

I would first like to thank my supervisor, Dr. Andrew Simons for the opportunity of working in his laboratory. Thank you, Andrew, for your guidance, support and great patience. You are a great supervisor and friend. I am also grateful to the members of my advisory committee: Dr. Naomi Cappuccino and Dr. Lynn Gillespie. Thank you for your support and advice.

I would like to express my special gratitude to Dr. Ola M. Heide from the Norwegian University of Life Sciences, who, by providing the first seeds from southern Norway, "jump-started" this study. His advice was invaluable also in constructing the thermogradient incubator. Many thanks to Dr. Mary Vetter from Luther College, University of Regina for collecting the seeds of the Yukon population and for advice with the iButton experiment. I would also like to thank Dr. Susan G. Aiken and Moshi Kotierk for collecting plants and seeds of the Iqaluit population, and Dr. Ingibjörg S. Jónsdóttir for collecting the seeds from Svalbard, as well as for help with the iButton experiment.

Collecting plant material of the southernmost population from Colorado was a critical step of this research; an endeavour associated with much incertitude and anguish. I am therefore much indebted to all the great people and friends that helped meeting my goals there. Thanks to Steve Popovich for permission and help to collect seeds in the Mount Evans Wilderness, and assistance with the iButton experiment, Dr. Terry Terrell for permission to work in the Rocky Mountain National Park, as well as Richard Bray and Stephanie Mason for assistance with collecting seeds and help with the iButton experiment.

I would also like to thank Brenda Dobson for permission to work in Jasper National Park and assistance with seed collection, Jennie McLaren for help with the iButton experiment, and Dr. Lennart Nilsen for help with the iButton experiment and climate data. I am grateful to Dr. Mark Forbes for lending the iButtons and to Yemisi Dare for explaining how to work them. I am also grateful to Dr. Myron Smith for permission to use his lab's thermogradient incubator for the preliminary thermogradient experiments. Many thanks to Claudia Buttera for being such a nice lab coordinator during my teaching assistantship, and for lending me lab equipment for the light requirement experiment. Thanks also to Jason Moffatt, our former lab technician.

This work was supported by a Domestic Scholarship, a Faculty of Graduate Studies Scholarship, as well as Andrew's NSERC Discovery Grant. Many thanks to Chica and Robert Bonneville; they provided me for several years with the Bonneville Family Scholarship in Botany.

Finally, I would like to thank my wife, Simona Wagner, for her support during all these years (and of course, for helping me collect the seeds from Jasper). Her kind heart and bright nature has kept me going. Thank you, Simona, for being in my life.

PREFACE

Figure 1.1 has been redrawn with kind permission of Springer Science and Business Media, as well as Dr. Christian Körner, after Fig. 1 on page 47 of Körner, C., 1995: Alpine plant diversity: a global survey and functional interpretations. *In* Chapin, F. S. and Körner, C. (eds.), *Arctic and alpine biodiversity: patterns, causes and ecosystem consequences*. Berlin Heidelberg: Springer-Verlag, 45-62. © Springer-Verlag Berlin Heidelberg 1995.

Figure 1.4 has been redrawn with permission of the Royal Swedish Academy of Science after Map 55 on page 65: Distribution of *Koenigia islandica* of Hultén, E., 1971: *The circumpolar plants. II.* Dicotyledons. Kungl. Svenska Vetenskapsakademiens handlingar. Fjärde Serien. Band 13. Nr 1. Stockholm: Almqvist & Wiksell, 463 pp. © The Royal Swedish Academy of Science 1971.

At the time of printing this thesis, a manuscript based on the third chapter is in press at *Arctic, Antarctic, and Alpine Research* as:

Wagner, I. and Simons, A. M., 2008: Intraspecific divergence in seed germination traits between high and low latitude populations of the arctic-alpine annual *Koenigia islandica*. *Arctic, Antarctic, and Alpine Research*, **40**: XXX-XXX.

Permission to include this publication in the thesis has been obtained from the journal office.

Manuscripts based on Chapters 4 and 6 have been submitted to the journals *Oecologia* and *Ecography*:

Wagner, I. and Simons, A. M.: Divergent norms of reaction for germination in response to temperature among arctic and alpine populations of *Koenigia islandica*. *Oecologia*.

and

Wagner, I. and Simons, A. M.: Divergence in life-history, morphology and phenology among arctic and alpine populations of the annual, *Koenigia islandica*. *Ecography*.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Arctic and alpine environments

Arctic and alpine ecosystems, usually synonymized with tundra, can be defined as treeless areas in high latitude and high altitude regions, experiencing short and cool growing seasons, and having a plant cover consisting of low, herbaceous or shrubby vegetation, in addition to extensive mats of lichens and mosses (Billings and Mooney, 1968; Löve and Löve, 1974). The figures given on the approximate area of land covered by tundra vary widely: from 8 million km^2 (5% of the terrestrial surface of the globe; Webber, 1974), through 11 million km^2 (Chapin and Körner, 1995), to as much as 24.8 million km^2 (16.7% of the total land surface; Billings and Mooney, 1968). These discrepancies reflect the ongoing debate on exactly which habitats can be included in the tundra biome (to which subarctic habitats are sometimes added), as well as the difficulties encountered in some situations in delimiting the boundaries of arctic and alpine ecosystems (Billings, 1974).

The word *tundra* (from Russian, but originally probably from Finno-Ugric languages), referred initially to a marshy, treeless, Siberian or Laplander plain (Billings, 1974), and for some authors has therefore a northern hemisphere, arctic connotation (Troll, 1960). As well, the high arctic, arid polar deserts are sometimes excluded from the definition of tundra, and hence also from the its total landmass (Billings, 1974). Lastly, while timberline is a good guide for delimiting the southern or the lower limits of tundra in the northern hemisphere, it is much less reliable for this purpose in the southern hemisphere. Here alpine timberline might occur at elevations lower than expected, such as in alpine regions of New Zealand, or it might be lacking altogether, like on arid, western slopes of the Andes (Billings, 1974).

The vast majority (95%) of the tundra environments occur in the

northern hemisphere (Billings and Mooney, 1968). Because of the moderating influence of the Arctic Ocean on the climate, tundra does not seem to have a northern latitudinal limit; Peary Land, at the northern tip of Greenland, at 83°N latitude has a comparatively rich vascular flora, including the species which is the subject of this thesis, *Koenigia islandica* (Hultén, 1964, 1971). Tundra environments presently occupy extensive areas at the northern fringe of North America and Eurasia—arctic[1] tundra (Figure 1.1)—as well as in the temperate and subtropical mountain ranges of the northern hemisphere—alpine[2] tundra (Figure 1.1). Tundra environments occur in areas with climates in which the average temperature of the warmest month is less than 10°C (Barry et al., 1981; Callaghan et al., 2005). The southern limits of arctic tundras can be defined climatically also based on air masses; in both North America and Eurasia the boundary between tundra and forest corresponds with the position in July of the arctic front. This approach, however, cannot be applied to alpine tundra (Barry et al., 1981). Wherever mountain ranges parallel to the meridians reach north into the Arctic—as in the case of the Rocky, Scandinavian or Ural Mountains—alpine tundra gradually gives way to arctic habitats (Billings, 1974), which adds considerable difficulty and incertitude in delimiting arctic from alpine environments in these situations. The upper altitudinal limit of tundra seems to be reached in the Himalayas in which vascular plants were recorded growing at altitudes as high as 6400 m, although closed tundra vegetation occurs at somewhat lower elevations

1 The term "*arctic*" derives from the Greek *αρκτως* (bear) and refers to the constellations *Ursa Major* and *Ursa Minor*, of which the North Star (*Polaris*) is part. Later, *arctic* came to denote high northern latitudes (Barry and Ives, 1974).

2 The origin of "*alpine*" might be from Latin ("*albus*" = white), referring to the snow-covered peaks of the North Italian Alps, as seen by the Romans. Another etymology might derive from pre-Roman times. Regardless, the term is applied today to entire mountain regions, including inhabitants, valleys, townships (Körner, 1999). In the context of phytogeography and of this thesis however, the meaning of *alpine*, as presented in text above, is much narrower, referring to vegetation above the natural high altitude treeline.

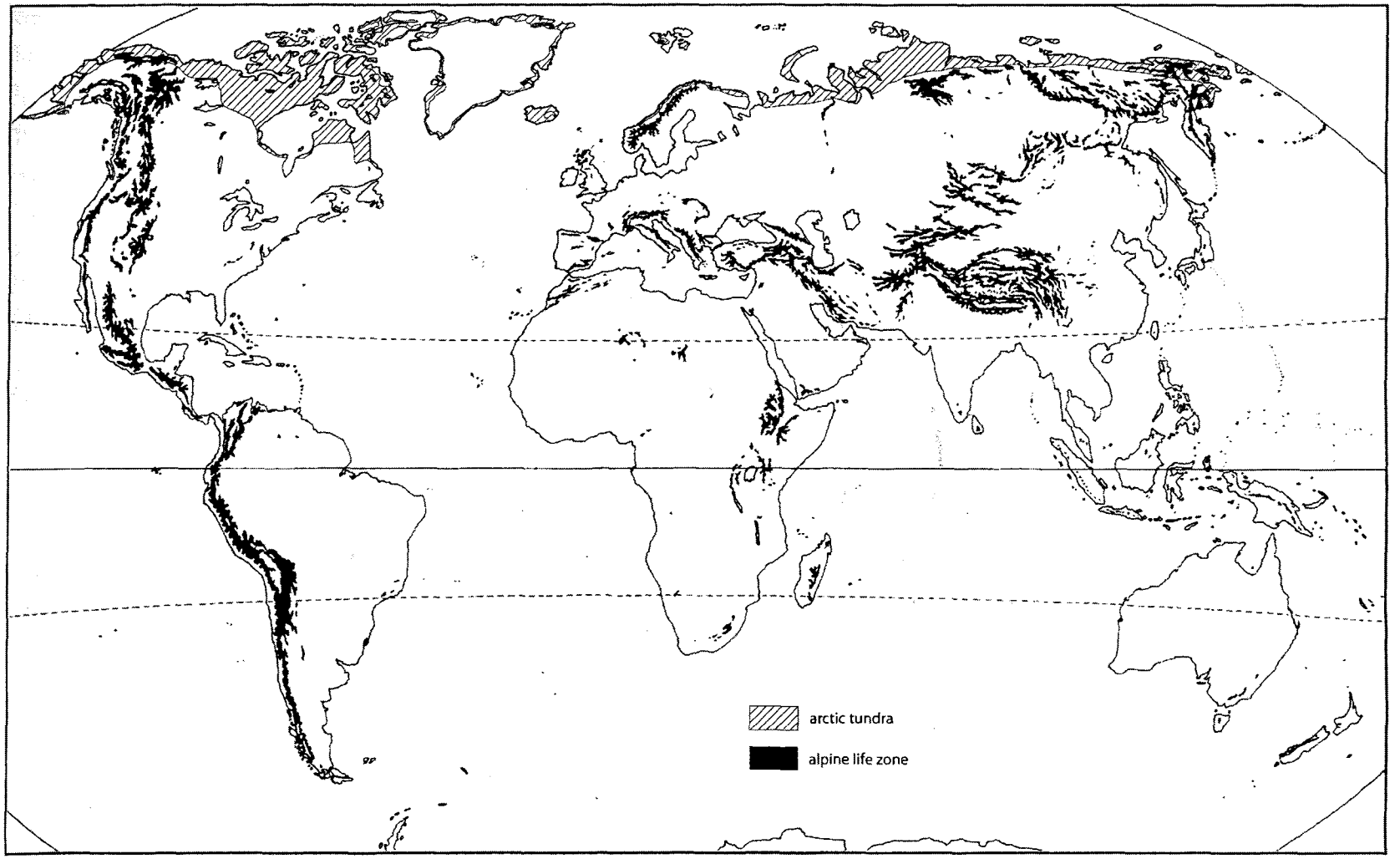

Figure 1.1. The global distribution of arctic and alpine life zones (after Webber, 1974 and Körner, 1995).

(Körner, 1999).

In spite of the extensive latitudinal and altitudinal range of tundra environments in the northern hemisphere, due to which a great many of the environmental conditions are dissimilar (Bliss, 1956), there are obvious connections between the flora and vegetation of arctic environments and those of temperate mountain ranges of North America and Eurasia (Löve and Löve, 1974). A considerable number of plant species occur in both arctic and mid-latitude, alpine regions of the northern hemisphere; for instance, 45–75% of the vascular alpine flora of some North-American mountain ranges also occur in the Arctic (Billings, 1973). Further south however, in the tropical and equatorial mountains of Africa, South America and New Guinea, the alpine regions bear little resemblance in flora and vegetation to the above-mentioned, more northern tundra environments (Billings and Mooney, 1968). As well, the southern hemisphere alpine regions in Chile, Argentina, New Zealand and Australia are superficially similar to northern hemisphere tundra, but besides a few species with bipolar distribution (including again *K. islandica*), have very different floras (Billings, 1974). This applies also to the tundra vegetation of subantarctic islands, as well as to the very depauperate, antarctic tundra (with a vascular flora consisting of only two species; Billings, 1974). For this reason, equatorial and southern hemisphere tundra environments will not be considered further; when referring to arctic and alpine tundra environments—unless otherwise stated—only arctic, and northern hemisphere, temperate alpine habitats will be implied.

At the end of the Tertiary, arctic tundra with a circumpolar flora similar to the present one must have already been established in the northernmost parts of North America and Eurasia (Löve and Löve, 1974). During the subsequent cycles of glacial maxima and warm interglacial periods however, the tundra

biota in the northern hemisphere went through extensive reorganization and migration, which led to the present distribution of arctic and alpine plant species and communities (Billings and Mooney, 1968). During the glacial maxima, much of the tundra's present range was covered by continental ice-sheets and alpine glaciers (McBean et al., 2005), with tundra habitats existing only on nunataks[3] and south of the ice caps, in present day plains and low mountains (Brubaker et al., 1995). In these conditions there must have been a substantial blending of the arctic and alpine floras, which explains the strong relationships today between these, and the disjunct occurrences of arctic-alpine plant species (Billings and Mooney, 1968). This must have been particularly true for the vast expanses of unglaciated areas of Eurasia, where tundra could have covered very large areas (Billings and Mooney, 1968). A particular situation existed in parts of Alaska and the Yukon, which were unglaciated and separated by extensive ice caps by the rest of North America (Andrews, 1974; McBean et al., 2005). For much of the Pleistocene, these regions had better connections with the Eurasian tundra, through Beringia, than with the tundra from the rest of North America. This is still reflected by the species composition of the Alaskan tundra (Billings and Mooney, 1968).

Polar and alpine environments are sometimes perceived by members of modern, industrial societies as harsh and inhospitable; exactly for this reason often they represent some of the only remaining unspoiled wildernesses, with some of the last traditional human societies, and are of special importance for this. Arctic and alpine life zones harbour a considerable share of the global biodiversity, and some animals and plants of these regions are some of the most impressive examples of adaptation to extreme environments. While some

3 The term "*nunatak*" (from the Inuit *nunataq*) refers to an exposed, unglaciated mountain peak or ridge, completely surrounded by glacial ice.

alpine vegetation coevolved with human societies and have been considerably shaped by human action, most arctic and antarctic seas are relatively unpolluted and most arctic land areas have suffered little anthropogenic disturbance. With the globalisation not only of economies, but also of pollution and environmental degradation, and the intensification of economic activity, this might be changing, however; arctic and alpine environments are especially vulnerable to the effects of industrial activities and global climate change, and would require therefore a special protection. Because of this increased vulnerability, arctic and alpine environments can also serve as models for the impact of global environmental change on other ecosystems.

1.2. Plant growth in arctic and alpine environments: limitations, characteristics and general adaptations

The most important limiting factors for plant growth in arctic and alpine environments are low summer temperatures and duration of snow cover (Billings, 1974; Wielgolaski and Karlsen, 2007). Indeed, in no other biomes is less energy available than in tundras (Bliss, 1971), and low growth season temperature is the main stressor for plant life, and probably the only common environmental factor to all arctic and alpine habitats (Billings, 1987). Even though plants from temperate seasonal environments are also able to tolerate very low winter temperatures, it is the polar and alpine plants' unique ability not only to metabolize, grow and reproduce, but also to produce relatively large amounts of photosynthates at these low temperatures in short periods of time (Billings and Mooney, 1968).

Besides temperature, nitrogen availability can be an important limiting factor for plant growth in arctic and alpine environments (Bliss, 1971). Nitrogen inputs to the soil usually have an immediate and visible effect on tundra plants;

the lush vegetation near human settlements, bird colonies and around animal carcasses is an example of this (Derry et al., 1999). Low available nitrogen levels in tundra communities are, however, largely a consequence of the low temperatures, which restrict bacterial activity, organic decomposition and microbial transformations of nitrogen in arctic and alpine soils (Wojciechowski and Heimbrook, 1984; Lennihan et al., 1994).

Water availability is not a common limiting factor in arctic and alpine environments (Billings and Mooney, 1968). High mountains in temperate regions usually receive large amounts of precipitation (Körner, 1999). Although most of the Arctic receives an order of magnitude less precipitation than temperate alpine regions, because of limited evapotranspiration arctic soils are usually moist (Billings and Mooney, 1968). Water stress can, however, occur in certain arctic and alpine locations, caused by various climatic, physical and chemical factors in the environment. Some alpine locations are subject to summer droughts of varying degrees of intensity (Neuner et al., 1999b). Also, the polar deserts of the High Arctic are characterized by low soil moisture contents, droughts that are unpredictable in both occurrence and duration, and low precipitation (Teeri, 1973). Low soil temperatures and poorly aerated, waterlogged soils in the Arctic may induce water stress in some species even when soil water is abundant, limiting photosynthesis and growth even in relatively warm summers (Stoner and Miller, 1975; Nosko and Courtin, 1995). Water limitations also can have synergistic interactions with other stressors including excessive light, low temperatures and low nutrients; having greater impact on the plant than either stressor alone (Nilsen and Orcutt, 1996). During the winter, in both alpine and arctic habitats in exposed snowless sites, evergreen shrubs and small trees are subjected to severe drought stress. In alpine environments, the effect of very low temperatures and desiccating

winds are worsened by strong visible and UV light; the combined factors of chilling, drought and high irradiation induces severe photoinhibition and embolism (Neuner et al., 1999a; Mayr et al., 2002).

Most arctic and alpine plants have low statures (Billings, 1974). The most common growth forms are chamaephytes—low shrubs and cushion plants, and hemicryptophytes—graminoid tussocks and herbaceous dicotyledons (Bliss, 1971; Billings, 1974, 1987; Körner, 1995). Trees are absent from tundra environments not only because of the severe winter conditions that kill any growth above the snow cover, but also because the short and cold growing seasons permit the production of very little spare photosynthates that could be permanently invested in aerial wood mass (Billings and Mooney, 1968). Conversely, the timberline can be defined as the location beyond or above which no more photosynthates can be spared for tree growth. The cushions of arctic and alpine plants, growing densely and closely to the soil—a common sight in tundra environments—are evolved growth forms (Körner, 1999), and probably adaptations for maximising the heat absorption and creating a considerably warmer, more favourable microenvironment for photosynthesis (Wielgolaski and Karlsen, 2007). Because of the advantages of having a set of leaves ready to photosynthesize shortly after snowmelt, many arctic and alpine shrubs are evergreen (Billings and Mooney, 1968). Due to the dangers of winter desiccation presented above, however, most shrubs have an absolute need of the thermal protection of the snow blanket; branches growing beyond the average snow cover are promptly killed the next winter (Neuner et al., 1999a). The very few dwarf shrubs that can survive in sites with little snow cover, such as the circumpolar *Loiseleuria procumbens*, go into deep physiological dormancy during the winter (Billings and Mooney, 1968).

The vast majority of the plants growing in arctic and alpine environments

are perennial, with large, underground storage systems in roots and rhizomes (Bliss, 1971; Billings, 1987). As much as 98% of the living plant material is beneath the soil in arctic regions, and 47–79% of plant biomass is underground in alpine environments (Billings, 1973); thus explaining the very low shoot:root ratios, of 0.2–0.03, characteristic for these habitats (Webber, 1974). There are few biennials in tundra environments, and even fewer annuals (only 1–2% of the arctic and alpine floras; Billings and Mooney, 1968), because of their disadvantage of having to complete their entire life-cycle in one cold, short growing season (Billings, 1974).

Because of the short growing seasons, which become increasingly shorter towards the latitudinal and altitudinal limits of arctic and alpine environments, the overall growth of the plants is slow; fairly small shrubs and cushions have ages of decades, and sometimes centuries (Billings and Mooney, 1968). In particularly unfavourable summers there might be no growth at all (Billings, 1987), and in extreme situations plants are capable of surviving several years under ice and snow, before resuming growth and flowering, as in the case of *Ranunculus glacialis* of the Alps (Körner, 1999). In the short growing season, however, growth occurs in an early, rapid burst soon after snow melt. This is accomplished by rapid translocation of energy and nutrients from the storage locations in roots, rhizomes and, in the case of evergreen shrubs, also leaves (Billings, 1987). The rapid translocation is permitted by the short translocation distances from underground storage systems to the active buds, which is again facilitated by the low growth forms (Wielgolaski and Karlsen, 2007).

A general feature of arctic and alpine plants is the presence of preformed perennating shoot and floral buds which are initiated early in the growing season of the year prior to flowering (Billings and Mooney, 1968; Bliss, 1971; Billings, 1987). In extreme cases it can take up to four years of bud

growth and formation before flowering (Wielgolaski and Karlsen, 2007). The obvious advantage of bud preformation is that it allows early, rapid growth and flowering shortly after snowmelt (Bliss, 1971). Despite this, seed production cannot be accomplished on a yearly basis (Billings and Mooney, 1968; Bliss, 1971). Some species, such as *Braya humilis*, can overwinter their immature fruits and complete their development the following growing season (Billings and Mooney, 1968).

Because of these difficulties, however, reproduction by seed is not common in arctic and alpine environments; most arctic and alpine plants (up to 90%; Körner, 1995) have some sort of vegetative, clonal propagation, usually by rhizomes. Reproduction by clonal propagation is more important in arctic than in alpine areas (Billings and Mooney, 1968; Billings, 1974), and some of the resulted clonal colonies can be very long-lived; e.g. genets of a Siberian *Carex* species were found to be well over 3000 years in age (Jónsdóttir et al., 2000). Some species are viviparous, reproducing by bulbils which have the advantage of germinating at very low temperatures (Dormann et al., 2002), while others show apomixis (Murray, 1995). From the sexually reproducing species, some are inbreeding—species with "seed-risking" strategies, as in the classification of Molau (1993), but despite the scarcity of pollinators, many plants are outcrossing and insect pollinated—"pollen-risking species" (Molau, 1993). They usually have early blooming, large and bright coloured flowers (Billings and Mooney, 1968) with a parabolic shape that concentrates sunlight, and thus temperature, to the centre of the flower. These adaptations facilitate ovary development and pollination, and are commonly seen in species of *Dryas*, *Papaver* and *Ranunculus* (Wielgolaski and Karlsen, 2007).

Arctic and alpine plant species do not seem to have specific adaptations in seed characteristics and germination. Contrary to expectations for environments with such severe climates, the seeds of arctic and alpine plants do not generally have deep dormancies (Amen, 1966; Billings and Mooney, 1968), and require surprisingly high temperatures for germination (Mooney and Billings, 1961; Billings and Mooney, 1968; Wagner and Simons, 2008b). Because of the short, cold summers however, seedlings grow slowly, and seedling establishment is not successful every year due to high seedling mortality (Billings and Mooney, 1968). Even though some arctic and alpine plants germinate readily in favourable conditions (Sørensen, 1941), the unsuitability of tundra conditions for germination on a yearly basis, and the conservative germination strategies of some species lead to large seed banks in arctic and alpine soils (Reynolds, 1984b; Cavieres and Arroyo, 2001), with seeds maintaining their viability for considerable periods of time (Billings and Mooney, 1968; Heide and Gauslaa, 1999; Wagner and Simons, 2008a).

It is often stated that polyploidy increases with latitude and elevation, being greatest in arctic and alpine regions due to the fact that polyploids are more successful in severe environments (Löve and Löve, 1974). While it seems that in arctic floras the proportion of polyploids is indeed greater than in more southern regions (Murray, 1995), this correlation seems to be weaker in alpine regions, where the higher incidence of autogamy and apomixis—which is correlated with higher ploidy levels—might give the impression of widespread polyploidy (Körner, 1999). Also, it seems that greater success of polyploids in harsher environments is not an intrinsic character of polyploidy, but can be associated with the increased ability of polyploids to perpetuate narrowly adapted, successful genotypes (Bliss, 1971; Murray, 1995), and might be a by-product of selection for increased self-compatibility (Molau, 1993).

1.3. Annual plant species in arctic and alpine environments; the study species, Koenigia islandica

The typical plant of arctic and alpine environments is the small, long-lived, slow growing perennial. Annual plant species are very rare in tundra habitats; merely 1–2% of the flora in most arctic and alpine regions is represented by annuals (Billings and Mooney, 1968). Only 3 out of 183 species are annuals in the flora of Northeast Greenland and 3/340 species are annuals in the Canadian Arctic Archipelago (Bliss, 1971), while the alpine flora of the Beartooth Plateau in Montana and Wyoming, represented by 191 species, has 3 annuals as well (Johnson and Billings, 1962). Apparently, alpine floras from warmer, drier regions have a somewhat larger proportion of annual plants, as in the case of the Sierra Nevada Mountains of California—6 out of 108 are annuals (Chabot and Billings, 1972), or the Himalayas, with 12% of the flora represented by annuals (Körner, 1999). In contrast, the flora of North America has 21.3% annuals (Silvertown, 1983), and the proportion of annual species in particular biomes, such as deserts, is even higher; as much as 50% of the vascular plants of the Sonoran Desert are annuals (Venable et al., 1993).

Clearly, tundra environments, with their low summer temperatures, are adverse habitats to the evolution and persistence of annuals, which typically require considerable heat for their development and seed production (Bliss, 1971). The main drawback of an annual life-history is the absolute requirement of completing a whole life-cycle, from germination to seed production, in a single, short, cold and likely unpredictable growing season (Billings and Mooney, 1968). Seed establishment, a difficult and rare process in arctic and alpine environments (see previous section), becomes a necessity every year for arctic and alpine annuals (Billings and Mooney, 1968). Such is

the pressure for perenniality in arctic and alpine plants that species which are usually annual in below-timberline locations develop in some cases perennial ecotypes in tundra habitats (Billings, 1974).

In arctic and alpine environments, the annual life form demands the ability to metabolize at high rates and at low temperatures, and to quickly use all metabolites in flowering and seed production (Billings and Mooney, 1968; Billings, 1974). These constraints do not allow investment in elaborate vegetative and reproductive structures and deposition of photosynthates in underground storage systems. By necessity, arctic-alpine annuals are extremely small and delicate, with very high shoot:root ratios of 5–7 (Reynolds, 1984a) which contrasts strongly with those of perennials from the same environments (see previous section). They are also very short lived, with life-cycles of only a few weeks, not longer than the short tundra growing season (Reynolds, 1984a; Heide and Gauslaa, 1999; Wagner and Simons, 2008c). Very few annuals were actually able to get through this "filter" of the harsh tundra environment, and many arctic and alpine regions have a single species of annual plant in their flora: *Koenigia islandica.*

Koenigia islandica L. (Figure 1.2) belongs to the Polygonaceae family, and is described as a tiny, glabrous, annual herb (Hedberg, 1997; Aiken et al., 1999 onwards). The stem is decumbent, ascending or erect, filiform, unbranched or with sparse branches. The plants are usually 1–3 cm high; they rarely reach 12–15 cm in height and are often extremely small and delicate, less than 0.5 cm at maturity. The leaves, spatulate-ovate or orbicular, are alternately distributed along the stems; the lower leaves have long petioles while the upper ones are almost sessile. The cotyledons usually persist into plant maturity and in the case of very small individuals are, together with maybe one more pair of leaves, the only photosynthetic structures of the plant.

The inflorescences are terminal or axillary, dense, cymose, with 3–15 small flowers. The flowers are almost sessile, and lack petals, having only a calyx with 3 green, white or translucent sepals (also referred to as tepals). There are usually 3 stamens, and an ovary with 2–3 styles. The dry, indehiscent fruits (hereafter referred to as seeds) are 1–1.5 mm long, ovoid achenes, black or brown at maturity (Hedberg, 1997; Aiken et al., 1999 onwards).

Koenigia islandica sometimes grows in frostboils and other disturbed areas, and occurs mostly in moist habitats, sometimes in running water, along springs and small streams, on pond and lake shores, or near snow fields, which are a water supply for the entire growing season. *Koenigia islandica* is confined to wet areas of tundra because of the increased thermal stability of these habitats. The heat loss by moisture evaporation during the day avoids the overheating of this species (Dahl, 1963), which is intolerant to high temperatures (Löve and Sarkar, 1957b; Reynolds, 1984a; Heide and Gauslaa, 1999), while the temperature buffering of the water allows warmer night temperatures, hence higher growth rates (Billings and Mooney, 1968). It grows in sandy and gravelly substrate, or in organic matter, frequently in moss mats in which the plants are completely embedded (Figure 1.3). *Koenigia islandica* is arguably the most successful and the most widespread annual in the tundra, occurring from sea level in polar regions up to 4000 m in the Rocky Mountains (I. Wagner, personal observation) and at least 4800 m in the Himalayas (Hedberg, 1997). It has a northern circumpolar distribution, occurring almost continuously in the Arctic, from north-eastern Canada, Greenland, Iceland, Svalbard, northern Europe, to northern Siberia and Alaska (Figure 1.4; Hultén, 1971; Hedberg, 1997). It also occurs in the alpine regions of the Himalayas, the high mountains in Western China and Central Asia as well as the Rocky Mountains of North America (Figure 1.4; Hultén, 1971; Hedberg, 1997). *Koenigia islandica* is one of the few arctic-

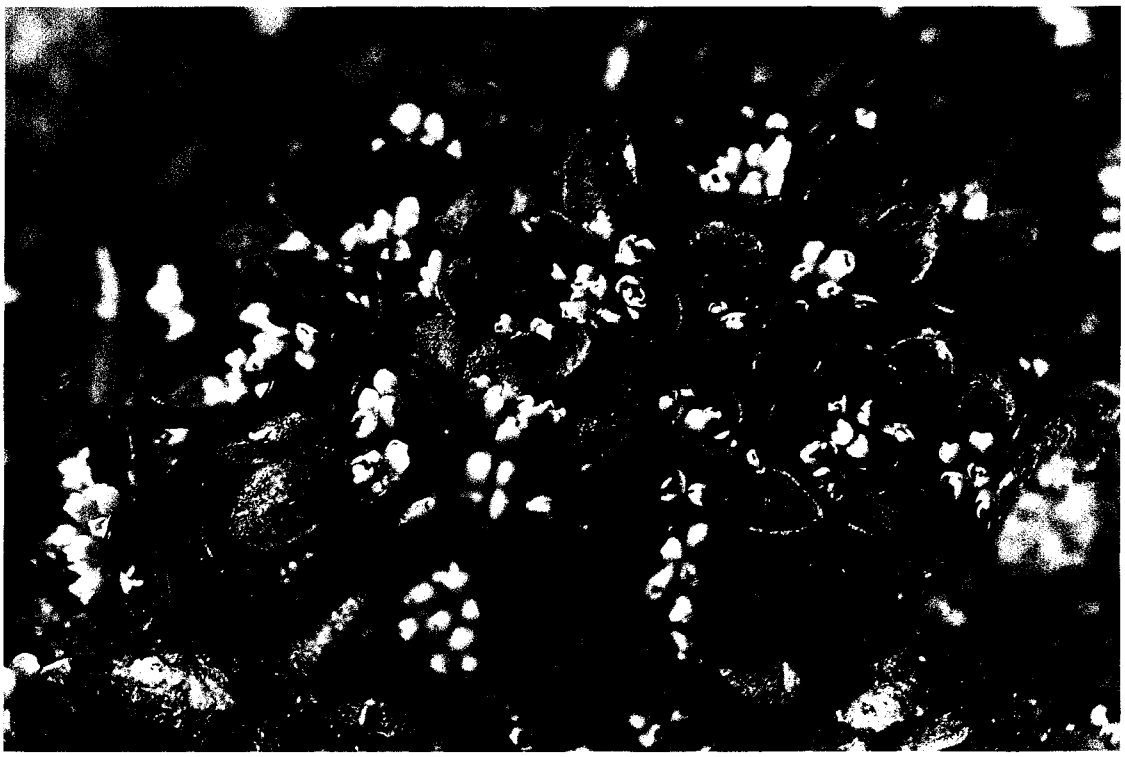

Figure 1.2. *Koenigia islandica* L. at Mount Evans Wilderness, Colorado, USA (Photo I. Wagner).

Figure 1.3. *Koenigia islandica* site at Whistlers Mountain, Jasper National Park, Alberta, Canada (Photo I. Wagner).

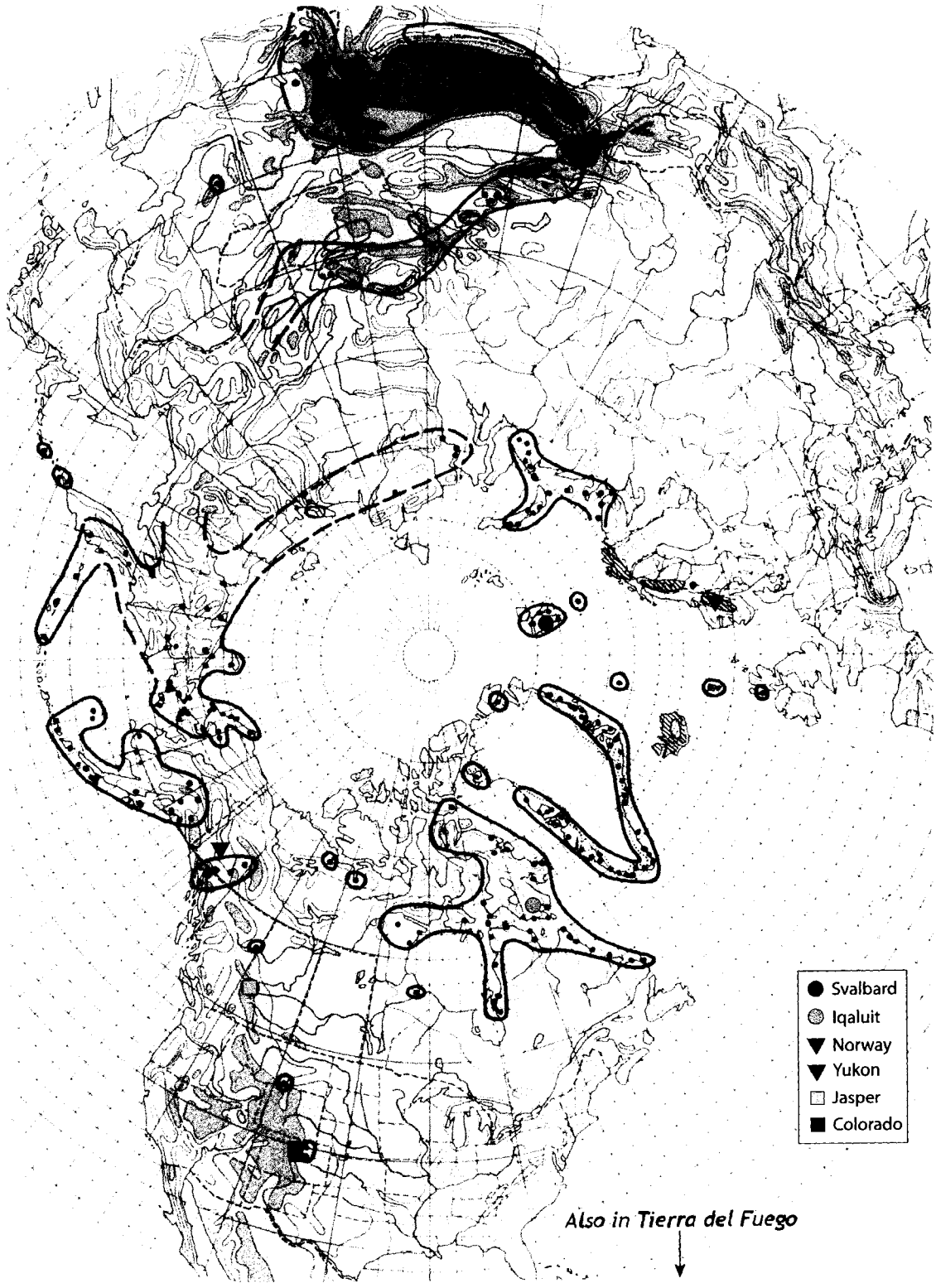

Figure 1.4. Distribution of *Koenigia islandica* (after Hultén, 1971) and the sites of origin of the studied populations.

alpine species that managed to spread to the southern hemisphere, having extreme disjunct populations in southernmost South America, in Tierra del Fuego—formerly regarded as belonging to a distinct, closely related species, *Koenigia fuegiana* Dusén (Zuloaga and Morrone, 1999). In North America, arctic populations of *K. islandica* are present in Northern Quebec, the Canadian Arctic Archipelago, and continental Nunavut and Alaska, while alpine populations occur along the Rocky Mountains, from Colorado northwards to Montana in the United States and in Alberta, British Columbia and Yukon in Canada (Figure 1.4; Hultén, 1971; Hedberg, 1997; Aiken et al., 1999 onwards).

The centre of diversity and likely centre of origin of the genus *Koenigia* lies in the Himalayas and Western China, where besides *K. islandica*, five other species—*K. delicatula, K. nepalensis, K. pilosa, K. forrestii* and *K. nummularifolia*—exist (Hedberg, 1997). From this original high mountain location, *K. islandica* likely spread over the entire Arctic and subsequently colonized other northern hemisphere mountains, as well as its highly disjunct location in South America (Hedberg, 1997). This is supported also by its karyotype; most populations along its arctic and alpine distribution are tetraploid—2n=28 (Löve and Sarkar, 1957a; Hedberg, 1997). Populations from Mongolia, however, were found to have a diploid karyotype—2n=14 (Mesícek and Soják, 1973), which would suggest that the migration routes of *K. islandica* from the Himalayas to the Arctic lead through Central Asian Mountains, prior to the emergence of the tetraploid form. A recent and ongoing study on the molecular phylogeography of *K. islandica* adds more evidence to the Pleistocene history of this species (P. Hollingsworth, personal communication). Levels of diversity in *K. islandica* populations from the recently glaciated regions of Europe were found to be low, whereas the highest diversity was found in populations from Alaska, which is consistent with the refugial hypothesis for

this unglaciated area. Most surprisingly, the disjunct population from Tierra del Fuego was not found to be highly dissimilar; in fact the South American plants showed greater similarities to the European populations than did the Alaskan plants, which would suggest a relatively recent dispersal of *K. islandica* in the southern hemisphere (P. Hollingsworth, personal communication).

1.4. Environmental differences between arctic and alpine habitats

Northern hemisphere tundra can be considered to form a continuum (Webber, 1974), and is thus the only terrestrial biome which, depending on elevation, can be found at any latitude (Körner, 1999). The common dominant environmental factor for all tundra ecosystems is the low summer temperatures. Besides this similarity, the gradients of latitude and elevation along the extensive range of tundra create significant differences in environmental characteristics of arctic and alpine ecosystems (Table 1.1), differences to which arctic-alpine organisms inhabiting both environments must adapt (Bliss, 1956; Billings, 1973).

Photoperiod at a particular location on the globe depends solely on latitude, and day length varies widely along the latitudinal extent of tundra (Table 1.1). There are very long days during the summer in low arctic and subarctic regions. Beyond the Arctic Circle there is a continuous photoperiod for a number of days that increases with latitude; the continuous daylight extends to as much as 157 days in northern Greenland at 83°N, at the northernmost occurrence of arctic tundra and vegetal life. In most arctic regions beyond the Arctic Circle, the photoperiod becomes continuous long before snowmelt and onset of the growing season, and the first sunset occurs at or after the first snowfall. Plants in high arctic locations therefore experience uninterrupted daylight during most of their active period. In alpine regions, however,

Table 1.1. Comparison of environmental characteristics of arctic and northern hemisphere, temperate alpine habitats (after Billings and Mooney, 1968). In brackets, the environmental characteristics of Longyearbyen, Svalbard, and Niwot Ridge, Colorado, as examples.

Component	Arctic tundra	Alpine tundra
Latitude	**usually >67°N**[a] (78°N)	**>35°N, <60°N**[b] (40°N)
Elevation	**sea level or little above**[b] (10 m a.s.l.)	**> 800 m**[b] (3750 m a.s.l.)
Photoperiod (Maximum)	**>19 h or continuous** (127 days)	**< 16 h** (15 h)
Daily total solar radiation (Average July daily total)	**similar** (1782x10⁴ J/m²)[c]	(2075 x10⁴ J/m²)[c]
Solar radiation intensity (Average July intensity)	**low intensity** (12500 J/m²min)[c]	**high intensity** (23400 J/m²min)[c]
Radiation quality (Daily DNA effective UV-B)	**deficient in UV** (~24 J/m²)[d]	**rich in UV** (~107 J/m²)[d]
Temperature (July mean)	**lower means** (5.9°C)[e]	**higher means** (8.23°C)[f]
Soil	**high cryoturbation** permafrost universal	**low cryoturbation** permafrost rare
Precipitation (Annual mean)	**low means** (190 mm)[e]	**high means** (930 mm)[f]
Wind (Annual mean)	**lower wind speeds** (17.6 km/h)[e]	**higher wind speeds** (37 km/h)[g]
Metabolic gases (CO_2 partial pressure) (O_2 partial pressure)	**normal partial pressure** (0.387 mbar) (213 mbar)	**low partial pressure** (0.246 mbar) (135 mbar)

[a] (Barry and Ives, 1974)
[b] (Körner, 1995)
[c] (after Billings and Mooney, 1968)
[d] (Caldwell et al., 1980)
[e] 1961-1990 Longyearbyen Airport, 28 m a.s.l., Norwegian Meteorological Institute
[f] (Greenland, 1989)
[g] (Barry, 1973)

maximum photoperiod is increasingly shorter towards the south, becoming barely longer than 12 hours in the tropical and equatorial alpine regions.

Solar radiation in terms of daily totals is quite similar in arctic and alpine regions. Because of the continuous photoperiod, high arctic regions receive only slightly lower solar energy in 24 hours than temperate alpine regions at about 40°N (Billings and Mooney, 1968). In terms of radiation intensity, however, alpine regions receive solar radiation at about twice that of arctic regions (Table 1.1), because of low incidence angles and high transparency of the atmosphere. This of course is true only for cloudless skies. Because of increasing cloudiness at higher elevation, solar radiation is often not significantly increased (Körner, 1999).

Radiation quality also differs substantially between arctic and alpine environments. Due to low elevations and wide zenith angles of the sun, which lead to increased path lengths through the atmosphere, solar radiation in high latitude regions is deficient in short wavelengths. In contrast, solar radiation in alpine regions is rich in all wavelengths (Billings and Mooney, 1968). To these factors is added also a latitudinal gradient of ozone column thickness in the stratosphere—with the ozone layer thickest at high latitudes—which leads to alpine regions receiving considerably more UV radiation, especially UV-B, than arctic regions. This difference can be as large as seven-fold for total daily effective UV-B radiation (Caldwell et al., 1980), and is further increased by reflection from persistent snow banks and glaciers. These differences between arctic and alpine environments in UV-B radiation, however, may have diminished recently due to increased ozone layer depletions and "ozone holes" at high latitudes (Orsolini et al., 2003). This increase in UV-B is expected to have a significant influence on plant species in arctic regions (Callaghan et al.,

2004; Callaghan et al., 2005).

Temperature, as stated previously, is the main common and limiting factor in tundra habitats; low growth season temperatures define arctic and alpine habitats. Differences in temperatures, however, exist among arctic and alpine environments, as well as along gradients of increasing latitude and elevations within these habitats (Table 1.1). Mean annual, winter and vegetation period temperatures are usually lower in arctic regions. Characteristic of alpine regions are higher mean growth season temperatures and a greater incidence of abnormally high summer temperatures (Billings and Mooney, 1968). Temperature regimes are different in arctic and alpine environments not only in annual and seasonal means, but also in terms of diurnal fluctuations. At one extreme, in the continuously insolated high arctic, the diurnal amplitude of air temperatures is only a few K during the growing season. In contrast, because of the shorter days and increased radiative heat losses due to higher elevation, in mid-latitude alpine regions diurnal temperatures fluctuate widely—in the order of 15 K. At the other extreme, in some tropical and equatorial alpine regions there is "winter and summer every day" (Körner, 1999).

Soils in arctic and alpine environments differ mainly because of differences in temperatures and soil frost. Arctic soil temperatures are lower, and there is considerable soil frost activity as well as nearly universally present permafrost. Soil temperatures are higher in alpine regions, permafrost is rare, and soil frost activity lower and on a smaller scale (Ives, 1974).

Precipitation can differ widely between arctic and alpine regions, as well as within these regions. Annual mean precipitation is low in arctic regions (Table 1.1), and it can be very low in high arctic, polar desert regions, while some alpine regions are among the rainiest on earth (Billings and Mooney,

1968). Because of low evapotranspiration, however, and because arctic soils are underlain by impermeable permafrost, most arctic locations are usually water-saturated for most of the growing season (Rieger, 1974). Water is therefore not a limiting factor in arctic regions, with the notable exception of polar deserts, where severe water stress is common especially towards the end of the growing season, which shortens even further the already very short growing season (Billings and Mooney, 1968). On the other hand, evapotranspiration is higher in alpine regions and soils are usually better drained (Retzer, 1974). In these conditions, many alpine regions offer quite dry conditions for plant life, despite higher precipitation.

Wind is an important environmental factor in arctic and alpine regions. Wind plays an important role in gas exchange and transpiration in plants, with high wind speeds dramatically decreasing leaf water potential in exposed plants, in summer as well as in winter (Neuner et al., 1999a). The stunted growth of arctic and alpine plants is in part an adaptation to the effects of wind. While arctic regions are considerably windier than temperate ones (Billings and Mooney, 1968), alpine regions are even windier (Table 1.1); the highest wind speed ever recorded was measured at the observatory on Mount Washington, New Hampshire.

Concentrations of the metabolic gases CO_2 and O_2 available to arctic and alpine organisms depend on atmospheric pressure, which decreases linearly at a given location with altitude; partial pressures of these gases at the upper altitudinal limits of alpine tundra are about half of those in an arctic tundra at sea level (Körner, 1999). Atmospheric pressure—and therefore concentrations of carbon dioxide and oxygen—at a certain location on earth depends not only on elevation and weather conditions, but also on latitude. The atmosphere is shallower towards the poles, pressure decreases here more

rapidly with elevation than at lower latitudes and the air is more rarified on a polar mountaintop than at the same elevation at the equator. However, because these locations are well beyond the limits of tundra (such as portions of the Alaska Range or of Antarctica), this phenomenon is of little relevance to vegetal life.

1.5. Effects of dissimilar environments on arctic and alpine plants

Environmental components vary greatly along the latitudinal and altitudinal range of tundra environments. Even in the extratropical regions of the northern hemisphere, arctic and alpine ecosystems are dissimilar in many environmental factors (Billings, 1973). Given the differences in environmental components between arctic and alpine regions presented in the previous section, it is expected that arctic-alpine plants inhabiting both regions have adaptations that are not consistent over their entire circumpolar and arctic-alpine range. The expected effect of the dissimilar environmental factors on the morphology, physiology and life-history characters on arctic-alpine plant species are summarized in Table 1.2 and are discussed below in detail.

Day length is an important cue for several life cycle steps in plant development such as germination, and is especially well studied for plant flowering (Keller and Körner, 2003). In many arctic-alpine species there is a clinal increase in photoperiodic requirements for flowering from alpine to arctic populations; plants from lower latitude populations flower in both short and long day photoperiods, while plants from arctic populations require long day, or even continuous photoperiods for initiation of flowering (Mooney and Billings, 1961; Mooney and Johnson, 1965; Heide, 2005). In some species, such as *Saxifraga rivularis,* high arctic populations seem to be rigidly cued to the continuous presence of the sun above the horizon during the summer; not

Table 1.2. Adaptations of arctic and alpine plant species to the dissimilar environmental characteristics in the two habitat types.

Due to	Arctic populations	Alpine populations
Photoperiod	do not flower in short photoperiods	flower in short and long photoperiods
Solar radiation	– photosynthetic light saturation at lower intensities – higher leaf chlorophyll content	– photosynthetic light saturation at high intensities – lower leaf chlorophyll content
Radiation quality	inability to screen out UV	ability to screen out UV
Temperature	– lower optimum temperatures for photosynthesis – high respiration rates – lower lethal summer temperatures	– higher optimum temperatures for photosynthesis – lower respiration rates – higher lethal summer temperatures
Soil	lower germination light requirement	higher germination light requirement
Metabolic gases	lack of adaptation to low partial pressure	adaptations to low partial pressure

only an almost continuous photoperiod, but also a relatively high irradiance is required for initiation of flowering (Teeri, 1976). Although not investigated in Teeri's (1976) study, populations of this widely distributed saxifrage from south of the Arctic Circle could not have such flowering requirements that would make them unable to flower in a less-than-continuous photoperiod. Hence, interpopulation divergence must be involved in this species as well. Interestingly, it seems that there is a relation between photoperiodic requirement in arctic and alpine plants and their global distribution. Species with high divergence in day length requirement, with northern populations requiring long photoperiods for flowering, appear to be restricted to regions north of 30°N (cf. citations above to Hultén, 1958, 1971). Conversely, all investigated species with a bipolar distribution—i.e. with their main area and likely origin in the northern hemisphere, and small outposts in the southern hemisphere—are completely day length neutral across populations in their flowering responses (Heide and Solhaug, 2001; Heide, 2002). This category includes the study species, *Koenigia islandica* (Heide and Gauslaa, 1999). A strict requirement for long days for flowering likely restricts the spread of a species in the alpine zones of tropical and equatorial mountains (Heide, 2005), whereas day length neutrality would allow a mountain-hopping migration across the equator and in the southern hemisphere (Heide, 2002).

Arctic regions are not necessarily lacking in solar energy. The high, and roughly similar daily totals (see section 1.4) in arctic and alpine environments means that plants can photosynthesize at high rates during the growing season in both these habitats. The dissimilar radiation intensities, however (see section 1.4), are expected to have significant effects on the photosynthetic physiology of arctic and alpine plants. Indeed, high altitude populations of arctic-alpine plants reach photosynthetic light saturation at much higher light

intensities than do their arctic counterparts at low elevations (Mooney and Billings, 1961; Mooney and Johnson, 1965). Also, the chlorophyll content seems consistently lower in alpine than in arctic populations (Mooney and Billings, 1961; Mooney and Johnson, 1965). Because of the constraints of rapid growth in annuals, there are differences between tundra annuals and perennials in these respects; alpine annuals have lower compensation points and higher photosynthetic rates than alpine perennials (Reynolds, 1984a).

The differences in received UV-B irradiation between arctic and alpine regions are reflected also in the ability of plants inhabiting these environments to screen out this biologically harmful radiation. Alpine plants display high epidermal reflectivity and low epidermal transmittance as adaptations to higher UV-B radiation, whereas arctic plants are much more sensitive to the effects of high UV-B; these differences can be seen among species (Robberecht et al., 1980; Barnes et al., 1987), as well as among populations (Caldwell, 1968; Caldwell et al., 1982).

Biotic interactions are important in driving trait evolution (Irwin, 2006) in most regions. In arctic and alpine habitats, however, abiotic effects are believed to be of primary importance. Among abiotic factors, temperature in arctic and alpine environments is the most significant. It is not surprising therefore, that divergent adaptations between arctic and alpine populations emerge as a response even to the slight temperature differences between the two habitats (Table 1.1). Arctic populations of the circumpolar, arctic-alpine *Oxyria digyna* and *Thalictrum alpinum* carry out photosynthesis at higher rates at lower temperatures, and attain their maximum photosynthetic rate at lower temperatures than alpine populations (Mooney and Billings, 1961; Mooney and Johnson, 1965). In contrast, alpine populations seem to be more tolerant of high temperatures, although there is little information on this

except a study on *K. islandica* (Löve and Sarkar, 1957b). Alpine annuals were also found to have higher photosynthetic temperature optima than alpine perennials (Reynolds, 1984a).

Dissimilarities in amplitude of diurnal temperature fluctuations between arctic and alpine environments are expected to influence ecotypic variation in germination characteristics related to temperature, particularly in species in which seed germination is promoted by fluctuating temperature. Differences in soil frost activity and soil structure between arctic and alpine environments are also expected to lead to dissimilarities between arctic and alpine plant populations, most notably in seed germination characters—dormancy and germination light requirements. It is, however, unclear how environmental factors related to soil would influence seed germination traits, and comparative studies are scarce. The classical ecotypic comparative study of Mooney and Billings (1961) on *Oxyria digyna* found that a light requirement for germination is more frequent in seeds of alpine than in arctic populations.

Due to the complex nature of the differences between arctic and alpine environments in precipitation, and its biologically relevant consequences—soil moisture—it is hard to predict the direction and extent of divergence between arctic and alpine populations in this respect. Annual precipitation in high arctic regions matches that of deserts—hence the name, polar deserts—and plants growing there are exposed to severe water stress to which they likely evolved adaptations. On the other hand, the soils of many arctic locations are waterlogged the entire growing season, irrespective of the amount of precipitation. Precipitation in alpine regions is considerably higher, and many areas do not experience water shortage for prolonged periods. But wetlands are scarcer here compared to arctic regions, soils are usually better drained and many areas are subjected to regular droughts. The issue of precipitation

might be less important for arctic and alpine annuals; they usually have shallow and weak root systems and are compelled to rapid and intensive growth and are thus confined to moist habitats.

Arctic and alpine plants are well adapted to the effect of strong winds and, except the cases of vegetation on windswept ridges and occasional shrub branches above the snow cover, they are little affected by high wind speeds. It is likely that populations of taller and shrubby perennials in arctic and alpine regions have divergent adaptations to the differences in wind speeds, but information on this is lacking. Because of the small sizes and sheltered habitats of arctic-alpine annuals, however, it is not expected that wind speed is an important environmental factor for them.

Besides photoperiod and solar radiation quality, concentration of the metabolic gases CO_2 and O_2 is one of the most important differences between low elevation, arctic, and high elevation, alpine environments. Plants species from high elevations have a better metabolic efficiency at low CO_2 partial pressures than lowland species (Körner and Diemer, 1987). There is also evidence that alpine populations of *Oxyria digyna* may be able to carry out photosynthesis at lower concentrations than arctic populations (Billings et al., 1961). There are very few studies on the effect of low oxygen concentrations on photosynthesis, but is suggested that low partial pressures of O_2 might compensate for the low concentrations of carbon dioxide at high elevations by reduction of oxygen inhibition on photosynthesis (Sakata and Yokoi, 2002). This would suggest that reduction of photosynthesis with elevation is not proportional to the reduction in atmospheric pressure (Terashima et al., 1995). The gradual increase in atmospheric CO_2 concentration during the last two centuries of industrial activity also contributes to the offset of reduction in photosynthesis at higher elevations; plants living today at 2600 m experience

the same CO_2 partial pressure as plants living at sea level in the year 1800 (Körner, 1999).

1.6. Within-species divergence in plants; existing information

Differentiation among plant populations inhabiting dissimilar environments is not restricted to arctic and alpine species; since Turesson's (1922) study on plant responses to their habitat, considerable evidence has accumulated on variations in morphology, physiology and life-history characters of plants in relation to their environment. Divergence in response to differences in environmental characteristics is substantiated for many traits. Plant populations in dissimilar environments have been shown to differ in seed germination traits—such as temperature requirements (Meyer et al., 1989; Meyer and Monsen, 1991; Schütz and Milberg, 1997), light requirements (Meyer et al., 1990; Milberg and Andersson, 1998), response to wet-cold stratification (Dorne, 1981; Meyer and Monsen, 1991; Cavieres and Arroyo, 2000; Vandvik and Vange, 2003) and dormancy (Meyer et al., 1989; Cavieres and Arroyo, 2000; Vandvik and Vange, 2003)—as well as growth, morphology and reproductive traits (Hickman, 1975; Antonovics and Primack, 1982; Potvin, 1986; Van Tienderen and Van der Toorn, 1991a, 1991b; Gurevitch, 1992a; Winn and Gross, 1993; Li et al., 1998; Joshi et al., 2001; Santamaria et al., 2003; Macel et al., 2007), phenology (Potvin, 1986; Van Tienderen and Van der Toorn, 1991a, 1991b; Winn and Gross, 1993; Li et al., 1998), reproductive allocation (Hickman, 1975, 1977; Primack and Antonovics, 1981, 1982; Potvin, 1986) and physiology (Gurevitch, 1992b). These differences can be found not only among plant populations (see citations above) but also among individuals of the same populations growing in different microenvironments (Linhart, 1974; Waser and Price, 1985; Galen et al., 1991; Miller and Fowler, 1994).

Starting with the comparative study on plant development in arctic and alpine environments of Bliss (1956)—one of the first papers to call attention to the many differences between the two habitats—and the classic ecotype study of Mooney and Billings (1961), divergence among plant populations in arctic and alpine environments has been documented many times (Mooney and Johnson, 1965; Billings et al., 1971; Callaghan, 1974; Chapin, 1981; Chapin and Chapin, 1981; Chapin and Oechel, 1983; Heide and Solhaug, 2001; Heide, 2005; see also Section 1.5). However, the vast majority of the work has been done on perennial plants; comparatively little information exists on arctic and alpine annuals despite the theoretical importance of various aspects of the biology and ecology of these species that manage to be successful in environments where perenniality is strongly favoured. These unusual examples of plant life in extreme environments might serve as "model organisms" which could promote our understanding of adaptive strategies to abiotic factors in general, and to global climate change in particular.

The few studies concerning arctic and alpine annuals have concentrated on the most widely distributed species, *Koenigia islandica* L. Sørensen (1941) conducted seed germination experiments on, among others, a Greenland arctic population of *K. islandica* and found a prompt and nearly 100% germination of the seeds. Löve and Sarkar made observations on the taxonomy and chromosome number of an alpine population of *K. islandica* from Colorado (1957a), and conducted experiments on its heat tolerance (1957b), finding that specimens of *K. islandica* are morphologically and cytologically inseparable throughout their range, and specimens from Colorado can survive and grow at surprisingly high temperatures. A study on the ecology and heat tolerance of *K. islandica* (Dahl, 1963), found that this species is largely confined to wet sites where evaporation keeps plant temperatures below lethal levels. Hedberg (1997)

published a monographic study on the genus *Koenigia* L.

The most detailed and thorough studies on the biology of *K. islandica* were made by Reynolds (1984a; 1984b) and Heide and Gauslaa (1999). Reynolds made detailed observations and conducted experiments related to the phenology, germination, photosynthesis and growth of an alpine population of *K. islandica* from Montana, as well as observations on its population dynamics. Heide and Gauslaa conducted germination, growth and flowering experiments on *K. islandica* from an alpine population from southern Norway, as well as with a small number of plants from a high arctic population from Svalbard. Both studies found that *K. islandica* has special adaptations and ecophysiological characteristics that enhance its survival in severe high-latitude and high-altitude climates. Adaptations include a conservative seed germination strategy, small size, high shoot:root ratio, high vegetative plant survival, low seed production and low optimum photosynthetic temperature.

Without exception, however, previous studies addressed either arctic or alpine populations of *K. islandica*. A direct comparison of individuals from populations in both environments has not yet been made for this or any other annual species. Such a direct comparison is the main contribution of this thesis.

1.7. General goals and approach of research

To help redress the lack of comparative studies among arctic and alpine populations of arctic-alpine annuals, this study aims to focus on dissimilarities between arctic and alpine environments, and on adaptations and differences in morphology and life histories of populations of the study species, *Koenigia islandica*, growing in these superficially similar habitats. For the purpose of this comparative study, seed germination strategies, vegetative growth,

reproductive strategies, and their dependence on temperature, photoperiod and other environmental factors are studied on a latitudinal and altitudinal series of populations of *K. islandica*. Populations from this series cover a wide geographic range of habitats, with elevations ranging from sea level to the upper limits of altitudinal occurrence of the species, and latitudes extending from the southernmost locations of the species to the high arctic.

Previous research reveals a number of prominent ecophysiological characters of *K. islandica,* of great adaptive significance for its survival in high-latitude and high-altitude environments, such as conservative seed germination strategies, minuteness and rapid development as well as low temperature optima for growth and reproduction (Reynolds, 1984a, 1984b; Heide and Gauslaa, 1999). Because of the environmental differences between arctic and alpine regions (see Section 1.4) it is expected that these adaptations might not be consistent over its entire circumpolar and arctic-alpine range. The first goal of this study is to establish whether the analysed populations differ, and to detect possible dissimilarities among populations:

Differences due to photoperiod. Even though *K. islandica* has been found to be completely day-length neutral in germination and flowering requirements (Heide and Gauslaa, 1999), populations inhabiting environments with widely dissimilar photoperiods may still show significant differences in their germination and flowering responses to day length; specifically, northern populations are predicted to have higher germination light requirements, and flowering might be impaired in short photoperiods.

Differences due to temperature. Because of the differences in growth season temperatures between arctic and alpine regions (see Section 1.4), it is expected that seed germination, growth and photosynthetic processes are better adapted to lower temperatures in arctic than alpine populations.

More precisely, arctic populations are predicted to require lower germination temperatures, and are expected to grow better at low temperatures than alpine populations.

Differences due to soil. Mainly traits related to seed germination are expected to differ between arctic and alpine populations of *K. islandica* due to differences in soil texture and frost activity. Due to the higher cryoturbation of arctic soils, arctic populations are predicted to have higher germination light requirements. Because of the somewhat higher prevalence of coarse, rocky soil, seeds of alpine populations might have higher scarification requirements.

Differences due solar radiation intensity and quality. It is expected that arctic and alpine populations of *K. islandica* show different photosynthetic response to light intensity and quality, in the sense presented in Section 1.5. Unfortunately, the small size and the morphology of *K. islandica* prevents the assessment of rates of photosynthesis and respiration with conventional equipment for gas exchange measurements, which is designed to accommodate much larger, single leaves (H. Maherali, personal communication). For this purpose, as well as for the purpose of detecting differences in adaptations to low concentrations of metabolic gases, the construction of a custom-made system would be needed; an undertaking beyond the scope of this research. The same applies for experiments on differences in the ability to screen out UV-B radiation, which would require sophisticated laboratory equipment and a time investment which makes them unfeasible within the famework of this thesis.

For the purpose of answering the question of whether the populations under study differ as stated by the predictions above, responses in germination, growth and phenology are assessed in reciprocal environments; that is, all populations are germinated or grown in all conditions and in all experimental

treatments, and the differences are measured and recorded.

The second goal of the study is to assess if differences exist among populations, as well as the extent and direction of these differences.

The third goal of this study is to establish whether the observed differences reflect local adaptation through selection in the particular local habitats, and to attempt to attribute adaptive explanations to the observed environmental differences. For this purpose, all experiments that test for differences among populations are performed on plant material that has been grown through at least one generation in the laboratory under uniform conditions. This serves to eliminate possible maternal effects and allows inferences that any observed differences are based on genetic rather than environmental effects.

1.8. Koenigia islandica *as a model subject for life-history studies*

For a number of reasons, *Koenigia islandica* is a very suitable subject for testing questions on life-history evolution. Being an annual and therefore necessarily semelparous, *K. islandica* permits the assessment of allocation to reproduction without the additional difficulties of considering development and allocation over several summers to assess lifetime fitness. This permits testing general evolutionary questions also in an environment where the vast majority of plants are long-lived perennials, and where allocation to reproduction and growth can vary widely among growing seasons because flowers and seeds can develop over many years.

In addition, *K. islandica*'s short life cycle and the relative ease with which it can be grown in uniform laboratory conditions through several generations also makes it well suited for experiments investigating adaptive responses in which a separation of genetic and environmental components

due to maternal effects (Roach and Wulff, 1987; Fenner, 1991; Simons and Johnston, 2006) is desired.

Because of its low tolerance to high temperatures (Dahl, 1963; Reynolds, 1984a; Heide and Gauslaa, 1999), the distribution of *K. islandica* is—unlike for most arctic-alpine plants—directly related to maximum growing season temperatures (Dahl, 1951, 1963). As a result, this species might be directly affected by climatic warming (Sætersdal and Birks, 1997), and therefore provides a valuable "early warning" model that has the potential to contribute to the general understanding of biotic responses to environmental change.

K. islandica however, has also some disadvantages in experimental life-history studies, of which the greatest is related to its modest seed yield. Most individuals produce 3-15 seeds, and only in exceptional cases does the number of seeds for one individual exceed 100. This can have a considerable influence on the design and extent of the experiments with this species.

1.9. Summary of the chapters of the thesis

The second chapter of this thesis, after the General Introduction, addresses the sites of origin of the investigated *Koenigia islandica* populations. The locations of seed collections are described and the environmental components of the sites are characterized.

In the third chapter, experiments addressing population divergence in germination patterns of three of the six populations are described. This set of experiments investigates responses in seed germination to wet-cold stratification and scarification, and finds striking differences among the populations. This is discussed in relation to results of an experiment investigating the within-population, genotypic effects on seed germination.

The fourth chapter reports on dissimilar norms of reaction for germination

traits in response to temperature for all six arctic and alpine populations of *K. islandica*. The reaction norms were obtained using a thermogradient incubator, in which the seeds, both untreated and wet-cold stratified, were germinated along a temperature gradient. The statistical analysis of the temperature reaction norms, using the recently introduced Loess smoothing method (Simons and Wagner, 2007), reveals significant divergence among populations. These germination patterns are discussed in relation to the result of field experiments that employed miniature temperature loggers; that is, in relation to temperature regimes experienced by the populations under natural field conditions.

The fifth chapter of the thesis presents the results of an experiment investigating germination light requirements of the six investigated *K. islandica* populations. Once again, significant differences are found among the populations in light requirements for seed germination, as well as in the influence of stratification on light requirement.

The sixth chapter reports on the results of a set of experiments in which differences in morphological, phenological and life-history characters among the six populations of *K. islandica* are analysed. These experiments were reciprocal; all studied populations were grown in two growth chambers, of which one simulated arctic environmental conditions, while the other simulated alpine conditions. The results of the experiments suggest strong ecotypic differentiation among the analysed populations.

The seventh and final chapter of the thesis provides a general discussion of the results of this research, and includes general conclusions to be drawn from the results of the experiments.

CHAPTER 2

POPULATIONS AND SITES

2.1. Seed collections

In order to test the predictions outlined in Chapter 1, seed material of six widely distributed arctic and alpine populations of *Koenigia islandica* was obtained (Figure 1.4; Table 2.1). Because this species usually grows in dense patches, often embedded in moss mats, it is difficult to determine how many individuals were sampled at each location. For some of the populations the seeds were collected from several sites (subpopulations), and care was taken to collect seeds from as many individuals as possible even from the populations represented by more than one subpopulation. The danger of obtaining an unrepresentative sample from a single subpopulation is therefore minimal, because hundreds of seeds were collected, and only 3–15 seeds are produced per individual.

The seeds from Spitsbergen, Svalbard (Svalbard populations) were collected by I. Jónsdóttir in September 2004 from two localities: one just outside Longyearbyen, the other at Hotelneset near the airport. Because the date of collection was late in the season, the seeds were already shed. The dead plants were collected together with the moss and the soil in which they were growing, and the seeds were sorted from the plant material and the substrate in the laboratory by the author. In addition, a small number of seeds were collected in July 2005 by the author from Bjørndalen, near the sea, on the shores of a pond with vegetation intensely grazed by geese.

The seeds from Iqaluit, Nunavut (Iqaluit population) originate from plants growing in moist tundra together with the mosses, *Ranunculus hyperboreus* and *Saxifraga cernua*. The plant material was collected by S. G. Aiken and M. Kotierk in July 2004. At that time the seeds were still unripe and therefore the living plants were collected together with entire portions of turf and

Table 2.1. Origin of the study populations and subpopulations of *Koenigia islandica*

Population name	Subpopulation name	Geographic origin	Latitude	Longitude	Elevation (m a.s.l.)
Svalbard	Longyearbyen	Longyearbyen, Spitsbergen, Svalbard, Norway	78°13'N	15°38'E	10
Svalbard	Hotelneset	Longyearbyen Airport, Spitsbergen, Svalbard, Norway	78°15'N	15°33'E	2
Svalbard	Bjørndalen	Bjørndalen, Spitsbergen, Svalbard, Norway	78°14'N	15°19'E	1
Iqaluit		Iqaluit, Nunavut, Canada	63°44'N	68°34'W	15
Norway		Kongsvoll, Dovre Mountains, Norway	62°18'N	9°36'E	900
Yukon		Rat Lake, Kluane Lake Region, Yukon, Canada	61°10'N	138°25'W	783
Jasper	Whistlers E	The Whistlers, Jasper National Park, Alberta, Canada	52°49'N	118°08'W	2300
Jasper	Whistlers W	The Whistlers, Jasper National Park, Alberta, Canada	52°49'N	118°09'W	2295
Jasper	Edith Cavell	Mount Edith Cavell, Jasper National Park, Alberta, Canada	52°41'N	118°03'W	1825
Jasper	Indian Pass	Indian Pass, Jasper National Park, Alberta, Canada	52°48'N	118°11'W	2413
Colorado	Mount Evans	Summit Lake, Mount Evans Wilderness, Colorado, USA	39°36'N	105°39'W	3910
Colorado	Guanella	Guanella Pass, Mount Evans Wilderness, Colorado, USA	39°37'N	105°44'W	3459
Colorado	Toll Memorial	Toll Memorial, Rocky Mountain National Park, Colorado, USA	40°25'N	105°43'W	3548
Colorado	Sundance	Sundance Mountain, Rocky Mountain National Park, Colorado, USA	40°24'N	105°42'W	3627

transported to Carleton University where they were kept in a growth chamber until seed maturation, after which the seeds were harvested from the plants as well as sorted from the growth substrate.

The seeds from Norway (Norway population) were provided by O. Heide and this population was the subject of an earlier study of this species (Heide and Gauslaa, 1999). The seeds originate from Kongsvoll in the Dovre Mountains (Oppdal, southern Norway) and were collected from a patch of eroded peat of approximately 3 m^2 in area. The field collected seeds had been stored for 23 years at -20°C before being propagated through a generation in the laboratory for this study.

The seeds from south-western Yukon (Yukon population) were collected by M. Vetter in August 2003 from a population at Rat Lake, on the eastern shore of Kluane Lake, from hundreds of plants growing on a marshy lakeshore among small *Scirpus* sp., *Carex* sp. and *Hippuris vulgaris*.

The seeds from Jasper, Alberta (Jasper population), were collected by the author in August 2004 south-west of Jasper, from four localities as follows: one collection on the eastern slope of the ridge connecting Whistlers Mountain and Indian Ridge, from hundreds of plants growing on the shores of a pond in wet sand, gravel and organic material among *Juncus* sp., *Equisetum* sp. and *Salix* sp.; the second from the western slope of the same ridge, from hundreds of individuals growing in wet gravel and organic material along a small stream; a third collection at Mount Edith Cavell, on the Cavell Meadows Path, on the shores of a small pond from plants growing in organic material with *Carex* sp. among boulders; the fourth collection from Indian Pass, from plants growing in organic material along a small creek which runs from the pond below the pass.

The seeds from Colorado (Colorado population) were collected by the author in August 2003 from several subpopulations from the Front Range: two subpopulations were from the Mount Evans Wilderness; the first from plants growing in wet gravel on the shores of Summit Lake near Mount Evans and the other from plants on the shores of a small stream in wet gravel near Guanella Pass Campground. The other two subpopulations were from Rocky Mountain National Park; the first was situated on the north side of the drainage of the saddle between Toll Memorial and Sundance Mountain, and collections were made from plants growing on peat in a marshy area. Seeds from the final subpopulation were collected from the south-eastern slope of Sundance Mountain from plants growing in a small spring in gravel and organic material. Collections were made also from a third subpopulation in the Rocky Mountain National Park, from Fall River Pass Cirque at 3434 m a.s.l. However, the seeds of this subpopulation germinated very poorly and material propagated through one generation in the laboratory was insufficient. This subpopulation was therefore excluded from the study.

After collection, all seeds were dried at room temperature and stored for 2–3 months at –18 °C until used, with the exception of the Norway population, as mentioned above.

2.2. Environmental characteristics of the sites of origin

Latitude and elevation of a particular tundra location can offer only a rough estimate of its environmental characteristics and whether it belongs to the arctic or alpine life zone (Figure 2.1). The great variety of local climates, the position in relation to continental land masses or oceans and their moderating effect can have as much influence on the environmental particularities of an arctic or alpine site as can latitude. For example, in

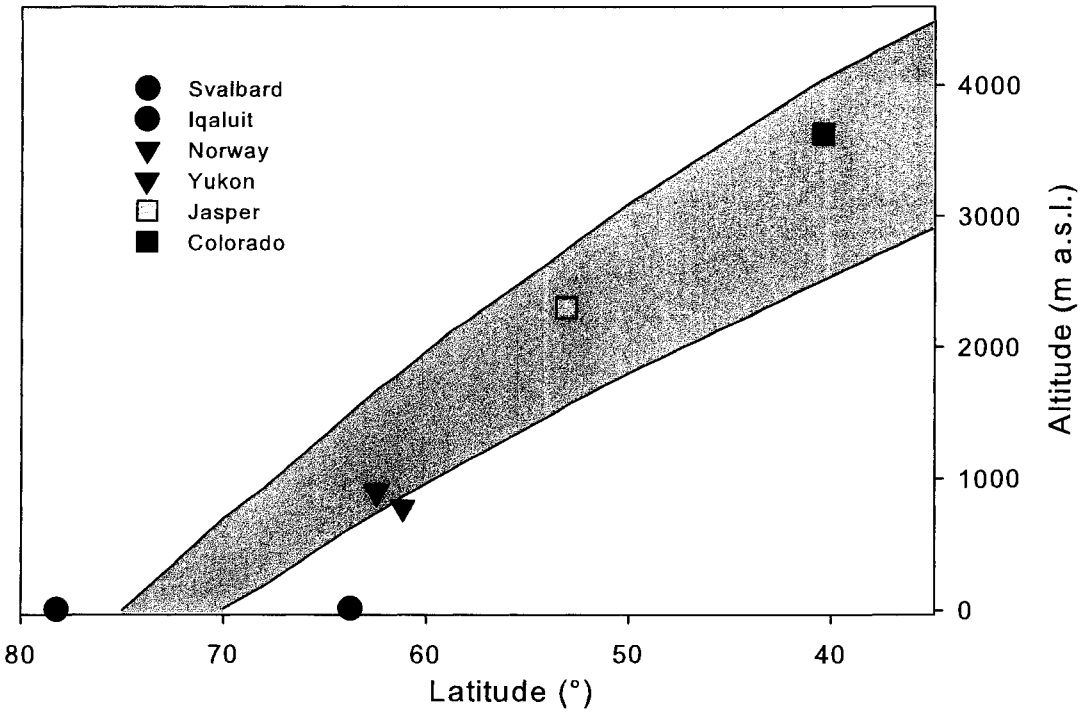

Figure 2.1. The position of the *Koenigia islandica* sites in relationship to the altitudinal range of alpine zone at arctic and temperate latitudes of the northern hemisphere. Mean upper and lower altitude limits of the alpine zone after Körner (1995).

eastern North America, in a region with a considerable negative temperature anomaly, arctic tundra can be encountered on the southern shores of Hudson Bay, at the 55th parallel—its southernmost location anywhere in the world (Figure 1.1). In contrast, northern Europe—warmed by the Gulf Stream and therefore a region with a strong positive temperature anomaly—has boreal forests extending well beyond the Arctic Circle, at latitudes at which in North America climate as well as vegetation is arctic (Figure 1.1).

Therefore, the great variety in climates and environmental characteristics of the study sites (Table 2.2) makes their simple classification as arctic and alpine difficult. Although the two extremes—the high arctic site in Svalbard and the low latitude alpine site in Colorado—are easy to categorize as such, the intermediary sites pose more problems for classification. The Jasper site, despite its relatively high latitude, still falls in the alpine category, while Iqaluit, in spite of its relatively southern location, is obviously an arctic site (because of its low elevation and location in north-eastern North America in the above-mentioned negative temperature anomaly). The Norway and the Yukon sites however, even though they are located at roughly similar latitudes as the Iqaluit site, have to be considered alpine locations. Being both situated in the western extremes of continental landmasses in the northern hemisphere, they enjoy milder climates with oceanic influences. While the Yukon site, on the eastern shore of Kluane Lake, is blocked by high mountain ranges from the direct influence of the ocean and has therefore a more extreme climate, the Norway site, situated in southern Scandinavia, is more directly exposed to the oceanic influences and has the mildest climate of all the studied sites. The most relevant environmental characteristics for comparison of the *K. islandica* populations are summer photoperiod—ranging from continuous or nearly so in

Table 2.2. Environmental characteristics of the sites of origin of the *Koenigia islandica* populations.

Population name	Svalbard[a]	Iqaluit[b]	Norway[c]	Yukon[d]	Jasper[e]	Colorado[f]
Population category	high arctic	arctic	high latitude alpine	high latitude alpine	alpine	alpine
Maximum photoperiod (h)	24	20.8	19.9	19.3	16.9	15
Summer photoperiod (Jun–Aug, h)	24–19.1	19.9–14.6	19.1–14.5	18.7–14.3	16.5–13.7	14.8–13.1
Mean annual temperature (°C)	**–6.7**	**–9.8**	**–0.1**	**–3.8**	-	**–3.7**
Mean summer temp. (Jun–Aug, °C)	4.2	6.0	9.1	11.4	7.5	6.6
Extreme maximum summer temp. (°C)	21.3	25.8	26.8	31.7	29.0	19.0[g]
Extreme minimum summer temp. (°C)	–8.4	–10.2	–6.0	–8.5	–12.2	–12.0[g]
Days / year with maximum temp.>20°C	0.06	1.19	9.90	28.10	5.65	0[g]
Mean January temperature (°C)	–15.3	–26.6	–8.8	–22.0	-	–13.1
Extreme maximum winter temp. (°C)	7.7	4.4	8.8	13.5	-	5.0[g]
Extreme minimum winter temp. (°C)	–46.3	–45.6	–32.4	–55.0	-	–37.0[g]
Mean annual precipitation (mm)	190	412.1	435	279.7	-	930
Mean summer precipit. (Jun–Aug, mm)	51	160.1	180	158.2	256.3	172.1
Days with snow cover (>=1cm)	238	247.3	203.4	176.8	-	-
Average winter snow depth (Dec–Mar, cm)	14.1	13	39.4	15.2	-	66.8[h]

[a] 1961–1990 Longyearbyen Airport, 28 m a.s.l., Norwegian Meteorological Institute
[b] 1971–2000 Iqaluit Airport, 33.5 m a.s.l., Environment Canada
[c] 1961–1990 Fokstugu Station, 972 m a.s.l., Norwegian Meteorological Institute
[d] 1971–2000 Burwash Airport, 807 m a.s.l., Environment Canada
[e] 1963–2003 Adams Creek, 2210 m a.s.l., Environment Canada. Dash indicates data not available
[f] 1951–1985 Niwot Ridge D1 Station, 3749 m a.s.l. (Greenland, 1989). Dash indicates data not available
[g] 1952–1970 Niwot Ridge D1 Station, 3749 m a.s.l. (Barry, 1973)
[h] 1982–1990 Niwot Ridge climate station. Data were provided by the Niwot Ridge Long-Term Ecological Research project (NSF DEB 0423662)

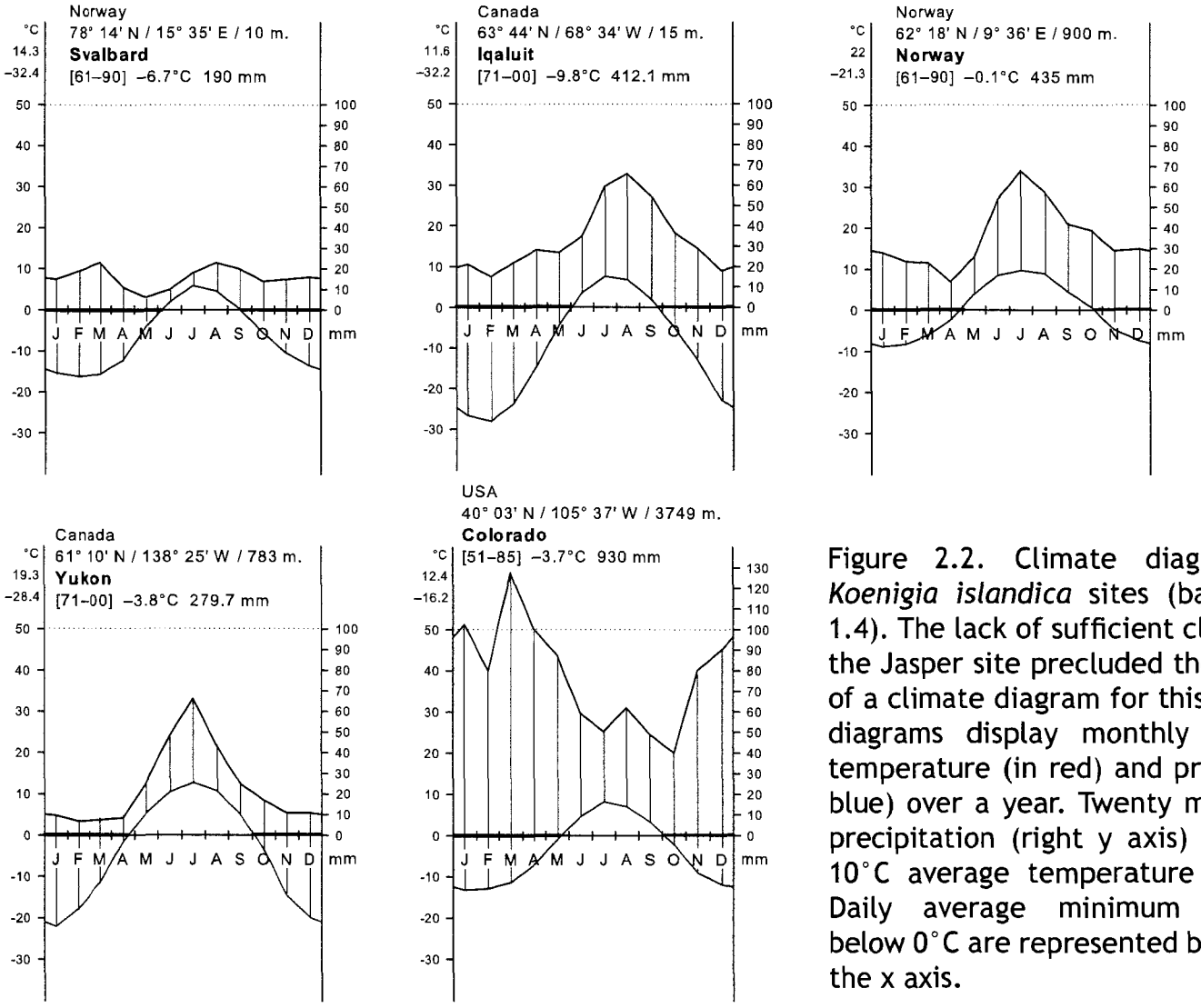

Figure 2.2. Climate diagrams of the *Koenigia islandica* sites (based on Table 1.4). The lack of sufficient climate data on the Jasper site precluded the construction of a climate diagram for this location. The diagrams display monthly averages for temperature (in red) and precipitation (in blue) over a year. Twenty mm of monthly precipitation (right y axis) correspond to 10°C average temperature (left y axis). Daily average minimum temperatures below 0°C are represented by bold lines on the x axis.

the two arctic locations to 15–17 hours in the two southernmost alpine sites (Table 2.2)—as well as mean summer temperatures and length of snow cover (Figure 2.2; Table 2.2).

Chapter 3

DIVERGENCE IN SEED GERMINATION TRAITS

3.1. Introduction

The timing of germination is of major importance in seedling survival of plant populations inhabiting seasonal environments (Baskin and Baskin, 1988; Simons and Johnston, 2006), and is crucial for plant establishment in arctic and alpine tundra environments, characterized by low temperatures and a short and sometimes unpredictable growing season (Billings and Mooney, 1968). In these environments, dominated by slow-growing perennials reproducing predominantly by vegetative means (Billings and Mooney, 1968; Bliss, 1971), the germination characteristics of the few annual species, which reproduce exclusively by seeds, must be under particularly strong selective pressure. Despite similarities between arctic and alpine environments, the gradients of latitude and elevation along this extensive range create important differences in environmental characteristics of tundra (see Section 1.4), differences to which arctic-alpine plant species, including arctic-alpine annuals, germinating in these different environments should have adapted (Bliss, 1956). Differences in germination characteristics of populations of the same species inhabiting dissimilar environments are a widespread feature (Milberg and Andersson, 1998), and adaptations of seed germination to latitudinal and altitudinal climatic differences are well documented in polycarpic perennials (Mooney and Billings, 1961; Meyer et al., 1989; Meyer and Monsen, 1991; Schütz and Milberg, 1997; Vandvik and Vange, 2003; Gimenez-Benavides et al., 2005).

However, in spite of the theoretical importance of arctic and alpine annuals, there has been little research on the germination responses of these interesting species, and virtually none on intraspecific differences in germination of arctic-alpine annuals. Some of the few studies on germination of arctic and alpine annuals have either addressed only interspecific

differences (Reynolds, 1984a), or have studied a single population (Heide and Gauslaa, 1999). Moreover, most of the previous studies on interpopulation germination differences were conducted on seeds collected directly from natural populations from the field, not allowing for the separation of genetic and environmental components in germination responses (Fenner, 1991). A comparative study on interpopulation variation of germination responses of an arctic-alpine annual, conducted on seeds produced in the laboratory under uniform conditions (Quinn and Colosi, 1977) appeared therefore desirable.

In these experiments we investigate differences in germination characteristics in three of the six populations of *Koenigia islandica*: in Norway, Yukon and Colorado populations. At the time these experiments were conducted, seeds of only these three populations were available. Although all three habitats can be considered alpine (see Section 2.2), they occur at vastly different latitudes, and there are significant differences in their environmental characteristics (Table 2.2). Previous studies have found that populations from more severe climates have deeper dormancies that require longer periods of cold stratification to overcome it (Meyer et al., 1989; Meyer and Monsen, 1991; Cavieres and Arroyo, 2000, 2001). The experiments aimed to test this hypothesis and to characterize differences in germination pattern in these *K. islandica* populations, test the effect of scarification and different length of cold stratification on seed germination and to advance preliminary adaptive explanations for these differences. An additional experiment performed on the Norway and Yukon populations aimed to test the within-population variation in germination. More specifically, to test whether differences in germination percentages observed at the population level are consistent, or due to variable germination rates at the individual plant level within populations. Seeds obtained under uniform laboratory conditions are used for the experiment to

allow detection of genetically based differences.

3.2. Materials and Methods

3.2.1. Plant material and laboratory seed propagation

Only seeds of three populations were available for these sets of experiments: the Norway and the Yukon populations, as well as the four subpopulations from Colorado (see Section 2.1). In order to eliminate possible maternal effects (Quinn and Colosi, 1977; Simons and Johnston, 2006), the populations were grown for one generation in the laboratory under uniform conditions. The seeds were germinated in germination chambers (Enconair SG-30, Biochambers Inc., Winnipeg, Manitoba, Canada) under long day (LD) photoperiod (15:9) at 24°C day and 17°C night temperatures on wet filter paper in Petri dishes. The germinating seedlings were transplanted to pots in a mixture of sand and peat-based standard growing medium and grown in growth chambers (Enconair GC-40) under LD (15:9) photoperiod at 15°C day and 10°C night temperatures. The resulting seeds were harvested, dried at room temperature and stored at –18°C for further use.

3.2.2. Germination experiments

Two replicate germination experiments—with some minor differences outlined below—were conducted using seeds produced in the laboratory; one in July 2004 (trial 1), and the second in January 2005 (trial 2). In both germination trials 400 seeds for each population were split between the following four treatments: 100 seeds were scarified by slitting open the seed coat (actually the pericarp) with a scalpel, 100 were stratified in the dark at 4°C on wet filter paper in Petri dishes wrapped in two layers of aluminum foil, 100 seeds per population received both scarification and stratification treatments, and the final 100 seeds remained untreated. To eliminate the possible confounding

influence of positional or Petri dish effects, the 100 seeds for each treatment were divided among 4 Petri dishes. Because seeds from Colorado were derived from four subpopulations, 25 seeds of each of the subpopulations were used in each treatment and were each split between two of the four Petri dishes. The two germination experiments differed only in that the stratification period for the first trial was 6 weeks, whereas it was 20 weeks for the second trial. This allows for at least a crude appraisal of the additional effect of stratification duration on germination characteristics. The seeds were germinated in an SG-30 germination chamber under a LD (15:9) photoperiod (ca. 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density) and 24°C day and 17°C night temperatures. The choice of 24°C as daytime germination temperature follows Heide and Gauslaa's finding (1999) that this temperature is optimal for the germination of *K. islandica*. Time from imbibition to germination (time to germination) for individual seeds was determined by counting germinated seeds daily over a 28-day period, and cumulative final germination percentages (germination fraction) were recorded at the end of the experiment.

3.2.3. Experiment on within-population variation in germination

In order to test within-population variation in germination—i.e., to test the prediction that the fractions observed in the germination experiments are uniformly distributed among individual plants of the population—an additional germination experiment was performed in the summer of 2006 on the Norway and Yukon populations. Thirty–32 laboratory-obtained seeds per individual plant were germinated and the germination percentages of the individuals—26 for the Norway population and 25 for the Yukon population—were compared. The seeds from Norway were previously stratified for 48 days in the dark at 4°C, and the germination temperatures of 24°C (day) and 18°C (night), chosen for the experiment, were suboptimal for both stratified Norway seeds and

unstratified Yukon seeds, in order to keep the mean germination percentages of both populations at about 70%. Because of the low seed yield per individual of the Colorado population—not enough individuals with at least 30 seeds were available—this population was not included in the experiment. The seeds of each individual plant were split among three Petri dishes, each dish containing the seeds of three different individuals. The seeds of the Norway population that germinated during the stratification period were counted towards the germination percentage of that particular individual, and the remaining ungerminated seeds were split among three Petri dishes as for the rest of the individuals. The seeds were germinated in an SG-30 germination chamber at the above-mentioned temperatures under a LD (15:9) photoperiod. The germinated seeds were counted and removed daily over a 30-day period and germination fraction was recorded.

3.2.4. Statistical analysis

We used a mixed model factorial analysis of variance (ANOVA) to test the dependence of germination fraction on population, seed treatment, germination trial, and their interaction. Because we are interested in the independent effects of scarification and stratification (as well as their interaction), these were considered two factors, each with two levels, in the analysis. To account for possible differences between the two germination experiments, experimental trial (length of stratification period) was included as an additional factor along with its interaction terms. Experimental trial was considered to be a random effect, whereas all other effects were fixed. Because germination fractions (p) are proportional measurements and cannot be measured at the individual level, analyses were performed at the level of replicated Petri plates (see Section 2.2.2., above). Germination fractions were transformed as p'=arcsine ($p^{0.5}$) prior to all analyses. When ANOVA indicated

significant effects ($P \leq 0.05$), post-hoc Tukey HSD-tests were performed to further examine the differences detected. Additional ANOVA and Tukey HSD analyses were performed in an identical manner to ask whether germination characters differ among the Colorado subpopulations.

Data from individual seeds (days from imbibition to germination) were used in the assessment of time to germination. Although seeds that do not germinate are valid data for analyses of germination fractions, they constitute missing data in analyses of time to germination. Germination time constitutes time-to-event data which are appropriately treated with time-series or failure-rate analysis (Vange et al., 2004) in which nongerminated seeds are missing or "censored" data. However, for numerous population and treatment combinations, germination fraction was low. Although survival analysis is designed to handle censored data that occur randomly among treatments or groups, the extreme inequality of proportion of censored data among treatments and populations in the present dataset would lead to systematic bias in results. Thus, no statistical tests were applied to time to germination data, and the assessment of trends in time to germination should be interpreted with due caution.

To assess within-population variation in germination, we used a similar ANOVA on arcsine-transformed germination fractions (see above), for each of the two populations separately, with individual plant considered a random effect.

3.3. Results

Analysis of variance reveals general differences in overall germination fractions among populations as well as significant effects of the various seed treatments. It also shows that response to these seed treatments is population-

dependent, as indicated by significant population by stratification, and population by scarification interaction terms (Table 3.1). The post-hoc Tukey tests provide more specific insight into where these significant differences occur (Table 3.2).

Scarification had a positive general effect on germination fraction, but the germination pattern of untreated seeds revealed strong differences among populations (Figure 3.1). For example, a significantly greater fraction of untreated seeds of the Norway population germinated than those of other populations (Table 3.2). Untreated seeds from the other two populations that germinated in a lower percentage also appeared to reach their maximum germination percentage later (Figure 3.2), although this difference in time to germination was not tested statistically (see Section 2.2.4).

A small number of the seeds germinated during the stratification treatment at 4°C in the dark: 5% of the seeds of the Norway population, only 0.5% of the seeds from Yukon, and 0.25% of the seeds from Colorado. Cold stratification alone had very different effects on the germination fraction of the seeds of the three populations (Figure 3.1). Neither stratification nor its duration had a significant effect on the germination of the Colorado seeds, whereas cold stratification of the Yukon seeds significantly improved their germination (Table 3.2). Cold stratification significantly reduced the germinability of the seeds from the Norway population (Table 3.2), and also appeared to increase the time to germination for the seeds from Norway (Figure 3.2). Overall, germination patterns of the two experimental trials conducted six months apart were consistent, with no significant differences between the trials (Figure 3.1).

Scarification of the seed coat resulted in significantly higher germination fractions for all population trials except those for Norway (Figure 3.1). Again,

Table 3.1. Analysis of variance of germination fraction for three populations of *Koenigia islandica*. Experimental trial was considered a random effect; all other effects were considered fixed.

Source	MS	*df*	*F*	*P*
Scarification	14.100	1	5739.841	0.008
Stratification	0.240	1	31.144	0.112
Population	4.335	2	1081.096	0.000
Trial	0.005	1	.	.
Scarification*Trial	0.002	1	0.079	0.834
Stratification*Trial	0.007	1	0.431	0.720
Population*Trial	0.004	2	0.077	0.929
Scarification*Population	1.144	2	25.983	0.037
Stratification*Population	1.466	2	47.823	0.020
Scarification*Stratification	0.042	1	4.089	0.292
Scarification*Stratification*Population	1.108	2	48.858	0.020
Scarification*Stratification*Trial	0.010	1	0.445	0.566
Scarification*Population*Trial	0.044	2	1.942	0.339
Stratification*Population*Trial	0.030	2	1.352	0.425
Scarification*Stratification*Population*Trial	0.022	2	0.699	0.499

Table 3.2. Germination fraction of seeds of three populations of *Koenigia islandica* subjected to various treatments. Trial 1—July 2004; Trial 2—January 2005. Values with different letters indicate table-wide significant differences according to post-hoc Tukey HSD-tests (see Table 3.1).

Treatment	Norway		Yukon		Colorado	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
untreated	97.00^{A}	98.00^{A}	24.00^{CDE}	7.00^{EF}	2.00^{F}	6.00^{EF}
stratified	49.00^{BC}	46.00^{CD}	85.00^{AB}	93.00^{A}	10.00^{EF}	17.00^{DEF}
scarified	100.00^{A}	100.00^{A}	94.00^{A}	96.00^{A}	85.00^{A}	86.00^{A}
stratified and scarified	100.00^{A}	100.00^{A}	96.00^{A}	99.00^{A}	90.00^{A}	88.00^{A}

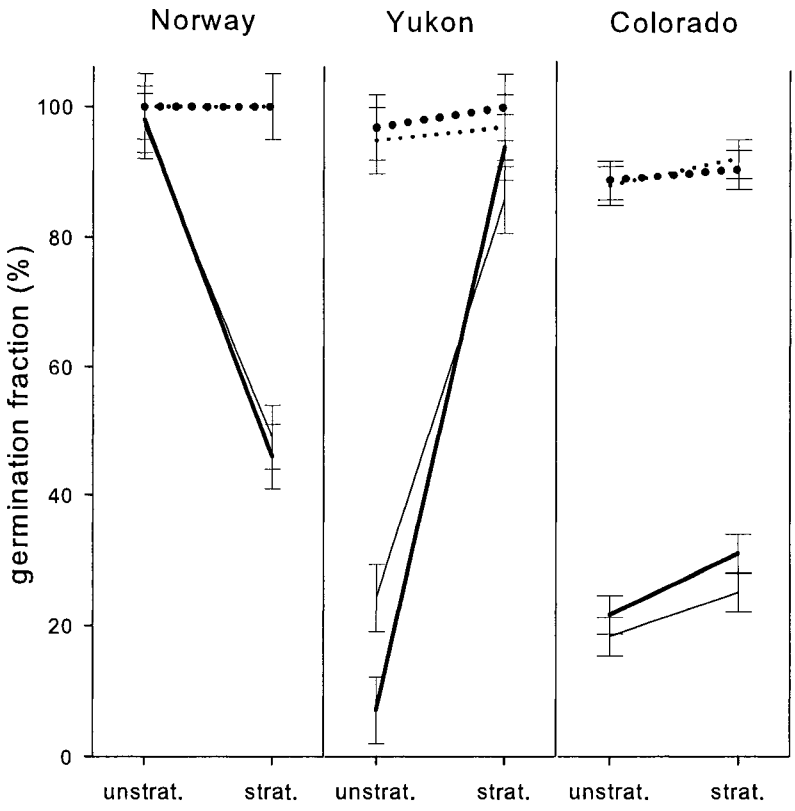

Figure 3.1. Germination fraction for unstratified and stratified seeds in three populations of *Koenigia islandica* at 24°C. Broken lines represent germination of scarified seeds; solid lines represent germination of unscarified seeds. Thin lines represent trial 1 (July 2004 — stratification of 6 weeks); bold lines represent trial 2 (January 2005 — stratification of 20 weeks). Error bars indicate ±1SD.

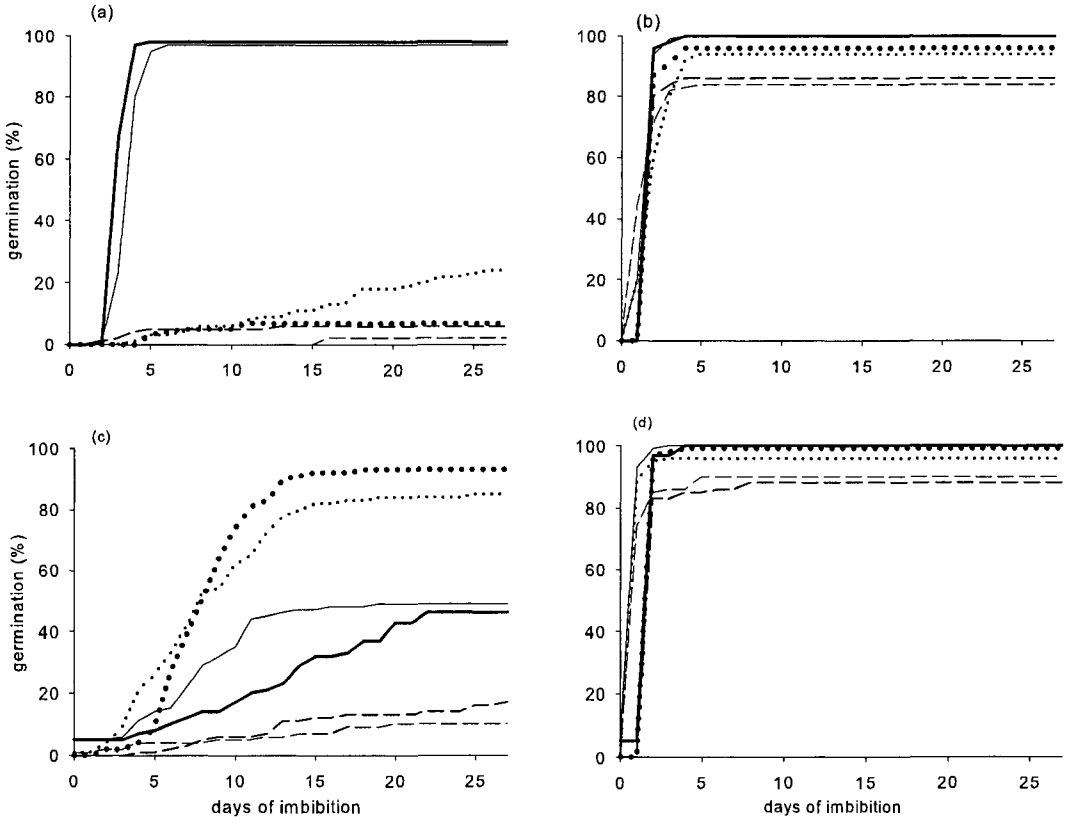

Figure 3.2. Trends in time to germination for seeds of three populations of *Koenigia islandica* at 24°C. a. untreated seeds, b. scarified seeds, c. stratified seeds, d. scarified and stratified seeds. Solid line: Norway, dotted line: Yukon, broken line: Colorado. Thin lines represent trial 1 (July 2004—stratification of 6 weeks); bold lines represent trial 2 (January 2005—stratification of 20 weeks). Because statistical comparisons among populations and treatments could not be performed (see section 3.2.4), results are intended to represent general trends only.

although time to germination was not compared statistically, the trends suggest that treatments that tend to increase germination fraction also shorten times to germination (Figure 3.2). There is no clear or simple relationship between germination fraction and time to germination; this relationship—even within treatments—is highly dependent on population (Figure 3.3).

The analysis of the four subpopulations from Colorado revealed a consistent germination pattern among subpopulations (Table 3.3), with only slight differences among them (Table 3.4). The only statistically significant differences were observed among seed treatment main effects. All seeds of the Colorado subpopulations germinated in very low percentages if untreated; stratification did not result in a significantly higher germination percentage in any of the subpopulations, and germination in all subpopulations was strongly promoted by scarification (Figure 3.4). The time to germination of seeds from all subpopulations was also consistent, with seeds germinating slowly if untreated or stratified, reaching the germination maximum toward the end of the experiment, and germinating promptly in the first 5 days if scarified (Figure 3.5).

The results of the ANOVA performed on the germination fractions of individual plants indicate that highly significant differences in germination percentages among different genotypes (individuals) exist in the case of the Norway population (Figure 3.6 a), but not in the case of the Yukon population (Figure 3.6 b). The differences between the two populations are striking. There is a high variability in seed germination within the Norway population, where as much as 50% of the seeds of some individual plants germinated at 4°C in the dark during the stratification period and the rest shortly after being placed in the germination chamber, while only 15–30% of the seeds of other individual plants (genotypes) germinated at all (Figure 3.6. a). The seeds of

the Yukon populations germinated much more uniformly among individuals; the seeds of none of the individuals germinated below 40% and very few of them germinated above 90%. These differences among the individual germination fractions are statistically nonsignificant (Figure 3.6. b). Possible significant differences among individual plants from Yukon were not hidden by significant differences among Petri dishes; the "Plate" effect, when introduced as a factor in the ANOVA, is highly non-significant ($P = 0.232$, $P = 0.833$ for Norway and Yukon population, respectively).

3.4. Discussion

Likely because the growing season is short, annual plants are rare in arctic and alpine habitats. Those annual species that do persist, then, are expected to show phenological adaptations associated with the seasonal constraints imposed. In particular, seed germination is expected to have evolved to respond to available cues such that the effective length of the potential growing season is maximized and, at the same time, the risk of germination outside the narrow window appropriate for seedling survival and growth is minimized. In this set of experiments, we ask whether genetically based differentiation exists in germination traits of three populations of the arctic-alpine annual, *Koenigia islandica* originating from widely divergent latitudes and altitudes.

The results of the germination experiments indicate that important differences in germination characteristics exist among the investigated populations of *K. islandica* in overall germination fraction despite evidence for significant within-population variability of germination fraction. The fact that these differences are maintained through a generation grown under a common garden environment in the laboratory indicates that these are

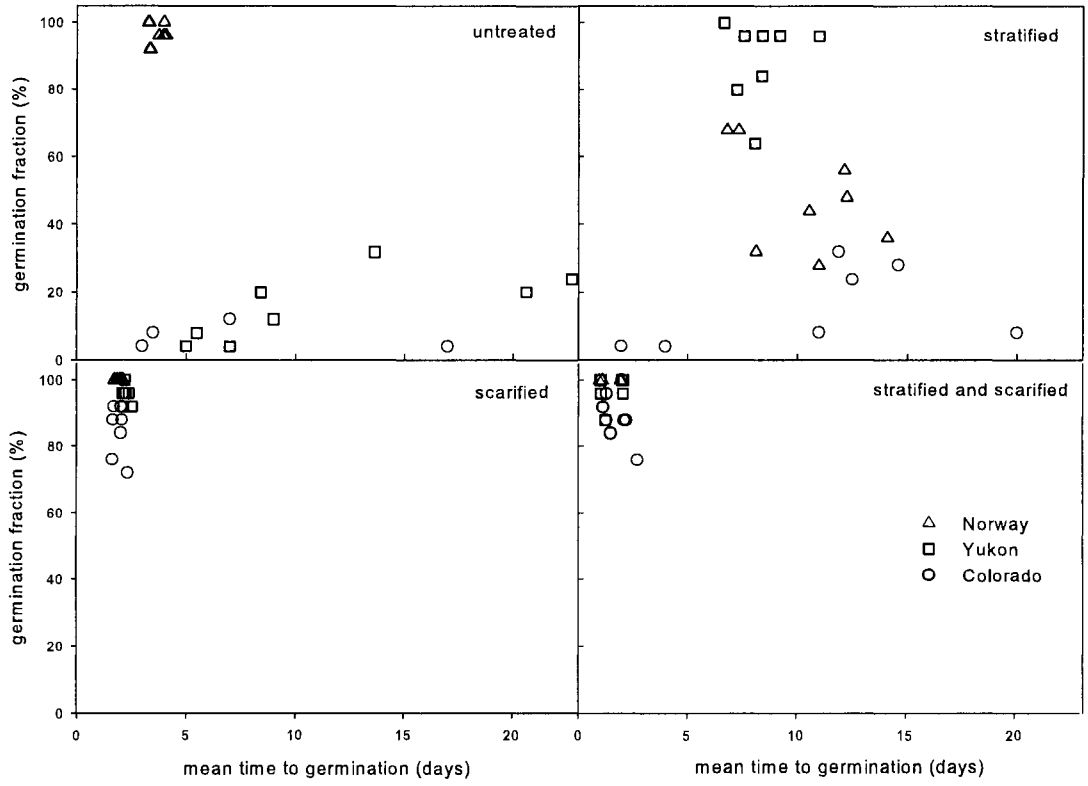

Figure 3.3. The relationship between germination fraction and time to germination in seeds of three populations of *Koenigia islandica* under the four seed treatments (see text). Data points represent each of the four Petri dishes per treatment, per population and per experimental trial.

Table 3.3. Analysis of variance of germination fraction for four subpopulations of *Koenigia islandica* from Colorado. Experimental trial was considered a random effect; all other effects were considered fixed.

Source	MS	*df*	*F*	*P*
Scarification	17.685	1	573.364	0.026
Stratification	0.230	1	201.486	0.044
Subpopulation	0.135	3	4.488	0.124
Trial	0.018	1	.	.
Scarification*Trial	0.030	1	0.639	0.547
Stratification*Trial	0.001	1	0.054	0.874
Subpopulation*Trial	0.030	3	0.332	0.804
Scarification*Subpopulation	0.015	3	0.217	0.879
Stratification*Subpopulation	0.082	3	1.895	0.306
Scarification*Stratification	0.087	1	98.714	0.063
Scarification*Stratification*Subpopulation	0.019	3	0.855	0.549
Scarification*Stratification*Trial	0.000	1	0.037	0.858
Scarification*Subpopulation*Trial	0.070	3	3.028	0.193
Stratification*Subpopulation*Trial	0.043	3	1.853	0.312
Scarification*Stratification*Subpopulation*Trial	0.023	3	0.543	0.655

Table 3.4. Germination fraction of seeds of four subpopulations of *Koenigia islandica* from Colorado, subjected to various treatments. Trial 1—July 2004; Trial 2—January 2005. Values with different letters indicate table-wide significant differences according to post-hoc Tukey HSD-tests (see Table 3.3).

Treatment	Mt. Evans		Guanella		Toll Memorial		Sundance	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
untreated	4.00^{E}	8.00^{DE}	0.00^{E}	12.00^{DE}	0.00^{E}	0.00^{E}	4.00^{E}	4.00^{E}
stratified	4.00^{E}	8.00^{CDE}	12.00^{BCDE}	52.00^{ABCDE}	16.00^{CDE}	4.00^{E}	8.00^{CDE}	4.00^{E}
scarified	82.14^{ABC}	82.14^{A}	96.00^{A}	80.00^{ABC}	76.00^{ABCD}	75.00^{ABCD}	86.36^{A}	92.30^{A}
stratified and scarified	84.00^{A}	92.00^{A}	92.00^{A}	96.00^{A}	84.00^{A}	88.00^{AB}	92.00^{A}	84.00^{AB}

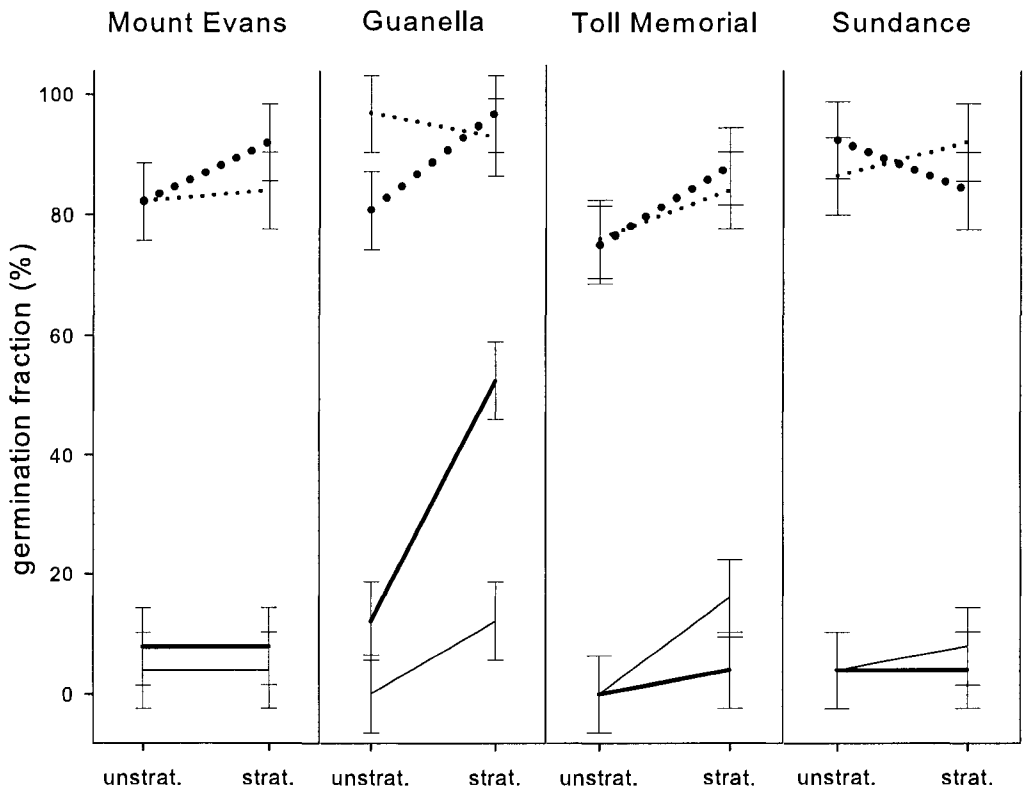

Figure 3.4. Germination fraction for unstratified and stratified seeds in four subpopulations of *Koenigia islandica* from Colorado at 24°C. Broken lines represent germination of scarified seeds; solid lines represent germination of unscarified seeds. Thin lines represent trial 1 (July 2004—stratification of 6 weeks); bold lines represent trial 2 (January 2005—stratification of 20 weeks). Error bars indicate ±1SD.

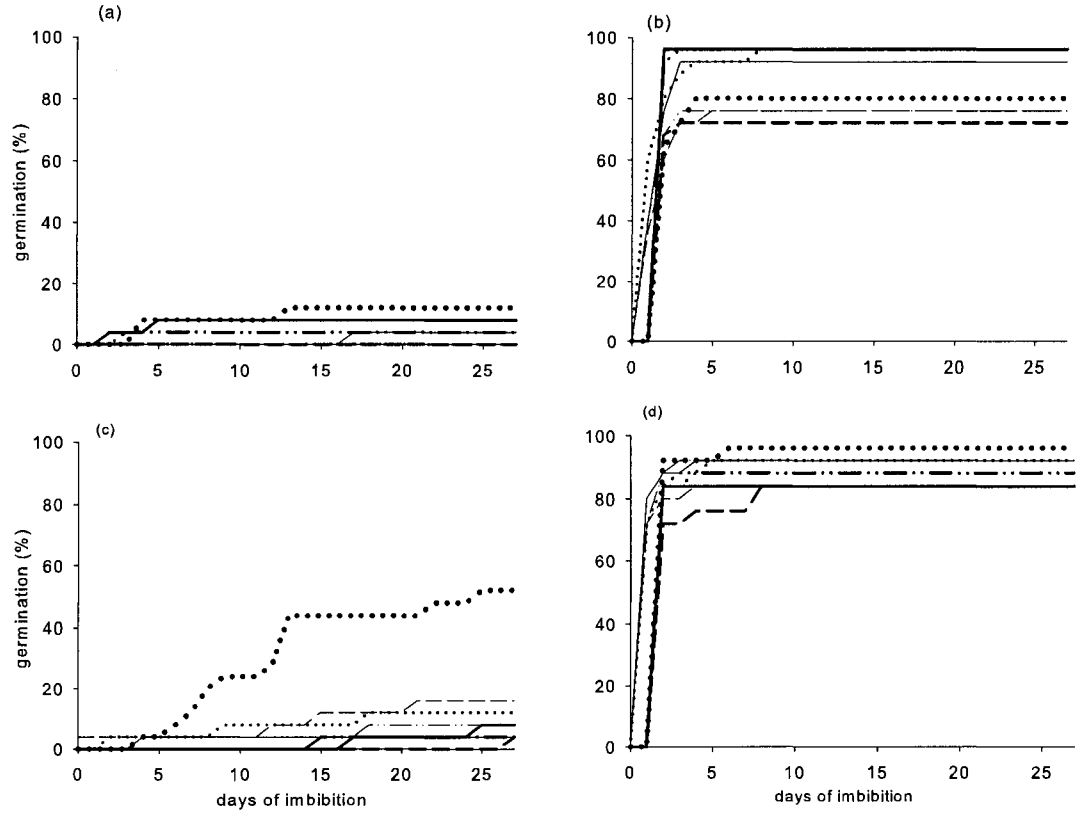

Figure 3.5. Trends in time to germination for seeds of four subpopulations of *Koenigia islandica* from Colorado at 24°C. a. untreated seeds, b. scarified seeds, c. stratified seeds, d. scarified and stratified seeds. Solid line: Mount Evans, dotted line: Guanella, broken line: Toll Memorial, dash-dot-dot line: Sundance. Thin lines represent trial 1 (July 2004—stratification of 6 weeks); bold lines represent trial 2 (January 2005—stratification of 20 weeks). Because statistical comparisons among populations and treatments could not be performed (see section 3.2.4), results are intended to represent general trends only.

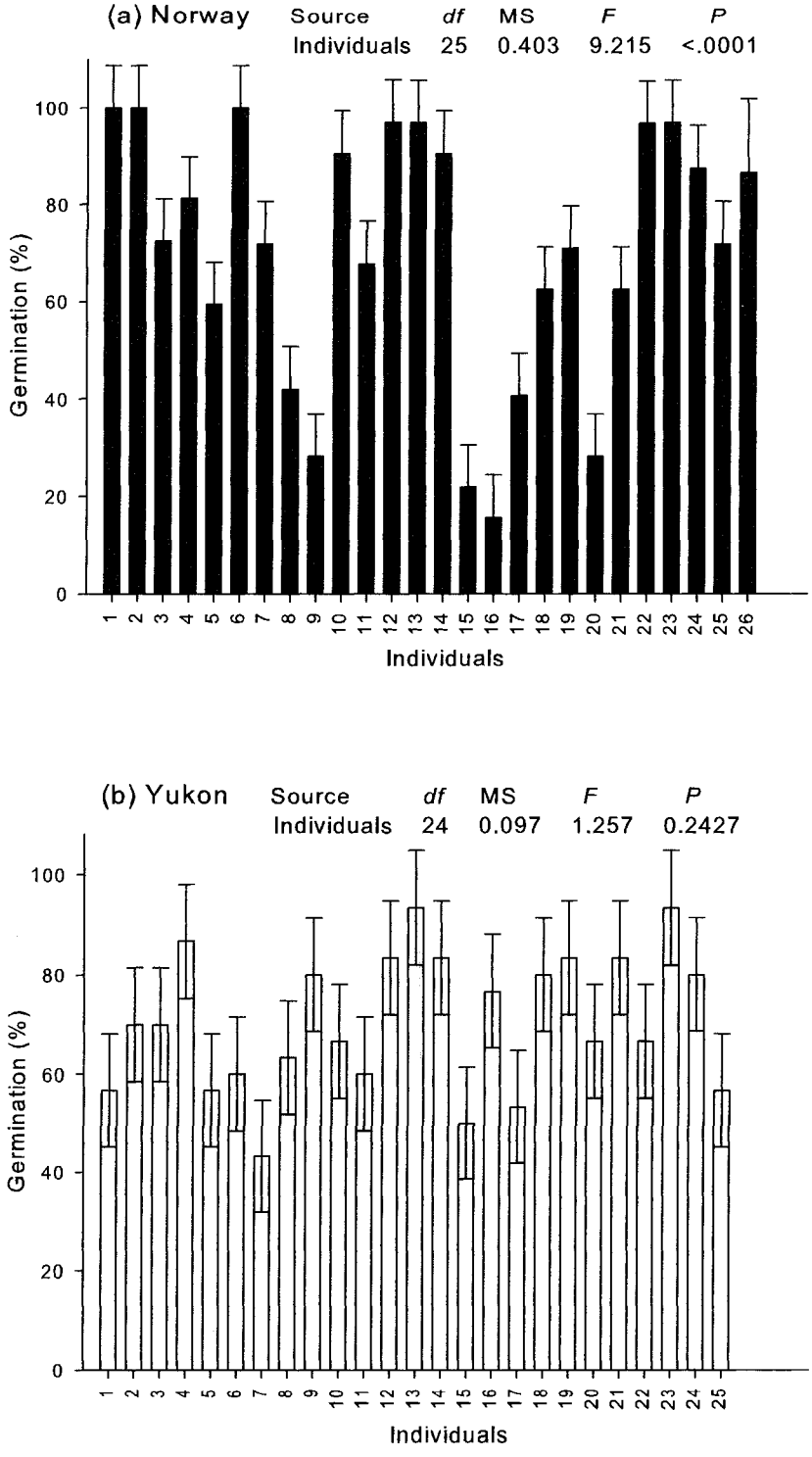

Figure 3.6. Germination fraction at 24°C day and 18°C night temperatures, of seeds of individual *Koenigia islandica* plants belonging to the (a) Norway and (b) Yukon populations. The seeds from Norway were stratified for 48 days at 4°C.

evolved differences. Also, the consistency of the observed patterns over two independent germination trials lends confidence to the reliability of the tests. Furthermore, the fact that little difference is observed among the four geographically close subpopulations from Colorado suggests that the germination patterns are locally adapted.

The germination of seeds not subjected to a period of cold stratification simulates the germination of freshly matured seeds late in the same growing season. At 24°C, at which the unstratified, seemingly non-dormant seeds of the Norway populations readily germinate in high percentage, the unstratified seeds from Yukon and Colorado reach only a modest germination fraction in both trials, indicating at least a conditional dormancy (Shimono and Kudo, 2005) that could be overcome by cold stratification in the Yukon population, and to a much lesser extent in the Colorado population (Figure 3.1). The finding that cold stratification improves the germinability of seeds from tundra environments is consistent with results of previous work on arctic and alpine species (Bell and Bliss, 1980; Marchand and Roach, 1980; Reynolds, 1984a; Densmore, 1997; Cavieres and Arroyo, 2000), and confirms the initial hypothesis that seeds of populations from severe winter sites are less likely to germinate under autumn and early winter thaw conditions than seeds from regions with milder winters (Meyer and Monsen, 1991).

The relative inhibition of germination of unstratified seeds from Yukon—which is uniformly distributed among individual genotypes (Figure 3.6 b)—might be an important adaptation to the severe and unpredictable climate there, with extreme negative winter temperatures alternating with short periods of positive temperatures and thaw that might induce the germination of seeds lacking dormancy. This observation is similar to the finding of Vandvik and Vange (2003), that populations from an environment with higher probabilities

of repeated freeze-thaw events have deeper dormancies. The increase in germination percentage following wet-cold stratification in the Yukon population is consistent with a summer annual's germination pattern in which seed dispersal occurs in the first summer/fall, a seed overwinters under snow cover, and germinates, grows and sets seed in late summer or autumn (Baskin and Baskin, 1988). The Colorado population, on the other hand, originating from a high-altitude environment with the most severe growing season of the studied populations, is characterized by germination that is little improved by cold stratification of any length. This observation is similar to those of Amen (1966) and Dorne (1981): seed germination fractions generally decrease with increasing latitude or elevation of origin; that is, with the increasing severity of the climate.

As in the study of Dorne (1981), the relative dormancy of the Colorado seeds might be due to the seed coat: their dormancy was broken by seed scarification and their germination fraction greatly improved and time to germination decreased following this treatment (Figures 3.1, 3.2). The breaking of dormancy through mechanical seed coat scarification is likely a treatment that the seeds of the Colorado population receive in their natural habitat when they are rolled in coarse sand and gravel of the small streams and ponds they usually inhabit. Seed germination fractions were increased by scarification in the Norway and Yukon populations as well (Figure 3.1), consistent with the finding that seed dormancy of alpine plants is overcome by scarification (Pelton, 1956). Likewise, germination fraction was improved and time to germination of *K. islandica* decreased by scarification in Heide and Gauslaa's study (1999).

Previous work on germination variation among populations along an

elevation gradient found that populations normally encountering long periods of snow cover and adverse winter conditions would require longer periods of cold stratification for germination than those exposed to milder winters (Meyer and Monsen, 1991; Cavieres and Arroyo, 2000). Our observations that the longer stratification period failed to significantly improve the germinability of the seeds from Colorado (Figure 3.1), do not seem to reinforce these previous findings and are similar to the results of Schütz and Milberg (1997), that longer cold stratification did not improve the seed germination of populations from a harsher climate.

The most divergent results were provided by the germination of the Norway population of *K. islandica*. Virtually all of the seeds of this population germinated readily if untreated, which supports our hypothesis that seeds of populations from milder climates are less dormant. Following cold stratification, however, the Norway seeds experienced a significant reduction in germination fraction and an increase in time to germination. This reduction is however not evenly spread among individual genotypes; the seeds of some individual plants still germinated quickly and completely, while in other individuals seed germination is almost completely inhibited (Figure 3.6. a). A reduction in germination fraction following a longer stratification period, together with the ability of unstratified seeds to germinate at low temperatures (including during the stratification treatment itself) is consistent with the germination characteristics of some winter annuals, but is a surprising trait for an arctic-alpine annual. However, this finding is reinforced by the observation that the cold stratified seeds of this population also show a decreased temperature range for germination (Chapter 4; Simons and Wagner, 2007; Wagner and Simons, 2008b), and an increased light requirement for germination (see Chapter 5), which are again typical traits of most winter annuals (Milberg and

Andersson, 1998). A winter annual is a plant which germinates in the autumn, passes the winter in vegetative state under the snow cover, and flowers, sets seed and dies in the following spring or summer (Baskin and Baskin, 1988). In the case of *K. islandica* from Dovre Mountains in southern Norway, however, it is difficult to imagine how seedlings that had germinated in the previous season would persist for more than six months under the snow cover in this alpine region. On the other hand, the flora of southern Scandinavia, in a mild oceanic climate, is known to have many winter annual plant species (Milberg and Andersson, 1998). Further field observations are required to shed light on this question.

It is beyond the scope of this experiment to unequivocally assess the adaptive significance of the particular germination patterns found. However, this work implies that important differences in the selective forces exist among habitats of conspecific populations which have resulted in strong and genetically based population differentiation in life-history characters.

CHAPTER 4

DIVERGENCE IN NORMS OF REACTION FOR GERMINATION IN RESPONSE TO TEMPERATURE

4.1. Introduction

Temperature is the main limiting factor for plant life in the short and unpredictable growing season which characterizes arctic and alpine tundra habitats (Billings and Mooney, 1968; Wielgolaski and Karlsen, 2007). Therefore, germination responses to temperature, the primary environmental factor regulating seed germination in temperate regions (Baskin and Baskin, 1988), are expected to be of critical importance for plant establishment in arctic and alpine environments, in which the severity and shortness of the growing season might hinder successful seed reproduction in some years (Billings, 1974; Körner, 1999). Because climate change is predicted to be disproportionately pronounced in these environments (Callaghan et al., 2005), local adaptation to the historical temperature regime provides a particularly pertinent demonstration of the relevance of both basic evolutionary and ecological knowledge in making inferences about potential effects of environmental change.

Differences in environmental characteristics among arctic and alpine habitats, created by the gradients of latitude and elevation along the extensive range of tundra in the northern hemisphere, are expected to lead to divergent adaptations in plant species germinating in dissimilar tundra environments. This must be particularly true in the case of the few exclusively seed-reproducing annual species inhabiting these environments (Bliss, 1956; Wagner and Simons, 2008a) in which perenniality and vegetative reproduction are dominant features of the floras (Billings and Mooney, 1968; Bliss, 1971). Because seed germination is a critical life-history trait in arctic-alpine annuals, local adaptation might place these species among the most sensitive to environmental change. The evaluation of germination reaction norms to

temperature is thus of both theoretical and applied interest in such species.

Interpopulation differences in germination characteristics, including germination responses to temperature, are common in plant species with wide geographic distributions (Milberg and Andersson, 1998), and adaptations of seed germination responses to different thermal regimes have been the subject of many studies (Meyer et al., 1989; Meyer and Monsen, 1991; Schütz and Milberg, 1997). In arctic and alpine environments, however, most studies on plastic responses of germination to temperature have been done on polycarpic perennials (Mooney and Billings, 1961; Cavieres and Arroyo, 2000). In spite of the theoretical importance of arctic and alpine annuals, virtually none have addressed intraspecific differences in germination responses of these unique and interesting species. The few studies on germination responses to temperature in arctic and alpine annuals were either conducted on a single population (Heide and Gauslaa, 1999), or addressed differences on an interspecific level, under a single temperature environment (Reynolds, 1984a). Furthermore, most studies on germination differences among populations have employed seeds obtained in the field from natural populations, and do not allow for separation of genetic and environmental components in germination responses (Fenner, 1991; Wagner and Simons, 2008a). Also, studies on the effect of temperature on germination assume linear or other simple relationships for profiles of plastic responses to temperature—the temperature norms of reaction (Trudgill et al., 2000)—an approach with considerable limitations in analysing the shape of complex norms of reaction (Izem and Kingsolver, 2005; Simons and Wagner, 2007).

In this experiment we analyze among-population genetic variation in norms of reaction of germination traits of *Koenigia islandica*, using seeds obtained in the laboratory under uniform conditions to eliminate

environmental effects (Quinn and Colosi, 1977; Simons and Johnston, 2006). Loess smoothing techniques, used previously for other purposes (e.g. Shipley and Hunt, 1996), were recently introduced for the characterization of complex norms of reaction (Simons and Wagner, 2007) and are used here for the characterization of continuous temperature norms of reaction. We investigate differentiation in germination responses to temperature among all six available populations of *K. islandica*. The present experiment aimed to detect differences in temperature norms of reaction of the *K. islandica* populations and to test the effect of cold stratification on plastic germination responses to temperature. An additional experiment was conducted to test the prediction that fluctuating temperatures promote germination in *K. islandica*. Finally, to obtain information on field conditions, soil surface temperatures at three sites were monitored continuously throughout one year. Adaptive explanations for the observed population differences are discussed.

4.2. Materials and Methods

4.2.1. Plant material and laboratory seed propagation

All six arctic and alpine *Koenigia islandica* populations were tested in this experiment. In order to minimize possible maternal effects (Quinn and Colosi, 1977; Schmitt et al., 1992), and the effects of different lengths of storage time of the field collected seeds (see Section 2.1), the six studied populations were grown through one generation in the laboratory. Exceptions are the Yukon and the Colorado populations. For these two populations, second generation seeds were used for the reaction norm experiment because insufficient first generation seeds were available. The seeds collected from the field were germinated in germination chambers (Enconair SG-30, BioChambers Inc., Winnipeg, Manitoba) under long day (LD) photoperiod (15:9) at 24°C day

and 17°C night temperatures on wet filter paper in Petri dishes. The resulting seedlings were transplanted to pots in a 1:8 mixture of sand and peat-based standard growing medium and grown in growth chambers (Enconair GC-40). Because of being in short supply, the seeds of each population were in equal proportion from plants that had been grown under a continuous photoperiod at 14°C day and 5°C night temperatures and from plants grown under a LD (15:9) photoperiod at 18°C day and 6°C night temperatures. The seeds were harvested, dried at room temperature and stored for up to two years at –18°C until further use.

4.2.2. Experimental characterization of norms of reaction

Norms of reaction for seed germination for the six *Koenigia islandica* populations were tested using a thermogradient plate. Preliminary experiments conducted to test the Loess method on seeds from an earlier generation using three populations and fewer temperatures, as well as an additional species, shows that the Loess method can distinguish among norms of reaction of conspecifics (Simons and Wagner, 2007). The plate was lined with filter paper moistened with distilled water, and placed in continuous fluorescent light (ca. 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density). Twenty–25 of both unstratified and stratified seeds of each of the six populations were placed on the plate at 12 discrete temperature positions; 10 positions with 3°C spacing from 6°C to 33°C along the temperature gradient, with two additional positions at 28°C and 31°C (Figure 4.1). The stratified seeds were previously kept for 45 days in the dark at 4°C on wet filter paper in Petri dishes wrapped in two layers of aluminum foil. Within each temperature position groups of 5–7 seeds for each population and treatment were placed in randomly assigned positions, and a new randomization was used for each temperature (Figure 4.1). Germinated seeds were counted and removed every day over a 60-day

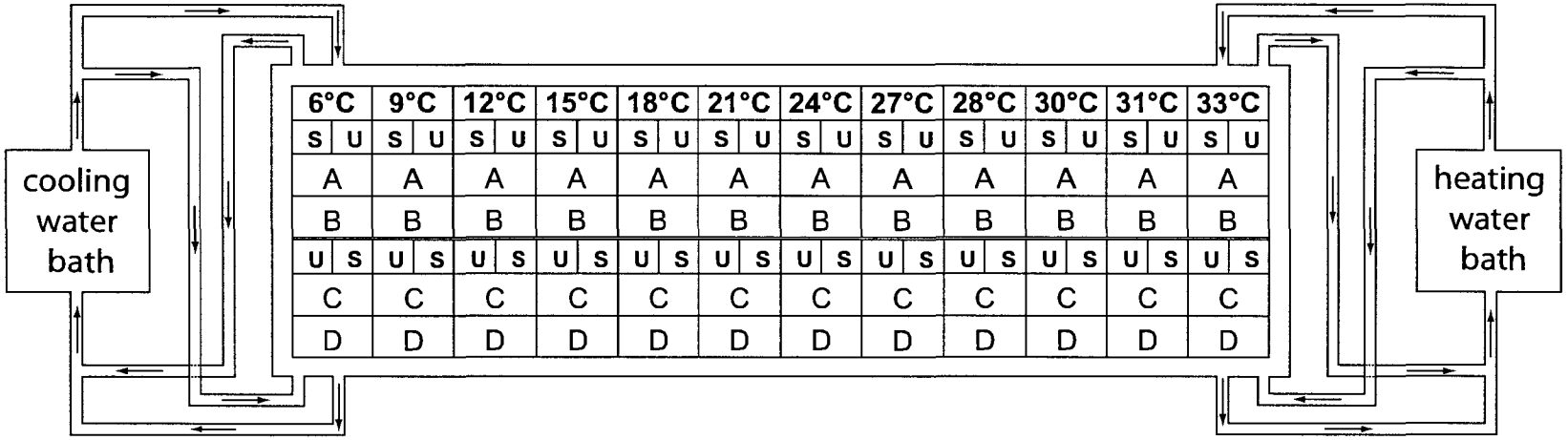

Figure 4.1. Experimental design of thermogradient plate. In each of the blocks (A,B,C,D) at each temperature, the position of all populations (Svalbard, Iqaluit, Norway, Yukon, Jasper and Colorado) were randomized and represented by 5-7 seeds for both stratified (S) and unstratified (U) treatments. In the lower half of the thermogradient plate the position of unstratified and stratified seeds were reversed.

period and germination, as well as time from imbibition to germination (time to germination) was recorded for individual seeds.

4.2.3. Seed viability tests

To determine whether the seeds not germinating after the 60-day period were viable, each seed was subjected to a triphenyl tetrazolium chloride (TTC) viability test (Poulsen et al., 2006). No tetrazolium staining technique has been described for *Koenigia islandica*; therefore viability trials on reserve seeds were first performed. Seeds that were subjected to one of three techniques: (1) seed coat intact; (2) seed sectioned longitudinally; and (3) seed coat slit open with a scalpel, were placed on filter paper on Petri dishes moistened with a 1% TTC solution. The Petri dishes were placed in growth chambers. After both 4 hours and 24 hours the seeds were observed, and deemed viable if the embryo of a dissected seed or its radicle appeared obviously red. Showing the highest viability, the technique of slitting open the seed coat and allowing 24 hours for the staining reaction was selected as being the most appropriate. All remaining ungerminated seeds from the germination temperature experiment were moved from the thermogradient plate in Petri dishes and were tested for viability using the technique described above.

4.2.4. Experiment on germination in fluctuating temperatures

In order to test the effect of fluctuating temperatures on seed germination of *Koenigia islandica*—i.e. to determine if the strong temperature fluctuations experienced by *K. islandica* seeds in the field after snowmelt (see Results in Section 4.3.) promote germination—a seed germination experiment in fluctuating vs. near-constant temperatures was performed in March 2007. A total of 360 seeds for each population were used; half of the seeds were stratified in the dark for 45 days at 4°C, the other half remained untreated. The seeds of each stratification treatment of each population were split among

6 Petri dishes, by placing 30 seeds in each dish on wet filter paper. Three of these 6 dishes were placed in an SG-30 germination chamber with 20°C day and 4°C night temperatures (fluctuating temperatures treatment) while the remaining 3 dishes were placed in an SG-30 germination chamber with 20°C day and 17°C night temperatures (near-constant temperatures treatment). Both germination chambers were set on a LD (15:9) photoperiod (ca. 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density). The germinated seeds were counted and removed every day over a 35-day period, and final germination percentages (germination fraction) were recorded.

4.2.5. Soil surface temperature measurements

It has been suggested that the distribution and biology of *Koenigia islandica* is related to the thermal balance and microclimatic conditions of its growing sites as much as it is related to the macroclimate (Dahl, 1963). In order to obtain more precise information about the thermal regimes experienced by *K. islandica* plants in field than can be provided by the nearest weather stations, monitoring experiments of soil surface temperatures in three of the *K. islandica* sites were performed in 2005-2006. A total of 13 miniature temperature data loggers (iButtons, Maxim Integrated Products Inc., Sunnyvale, California) were placed at the soil surface among *K. islandica* plants in Colorado, Yukon, and Svalbard sites. For accessibility reasons, in Svalbard the temperature loggers were placed in a *K. islandica* population in Adventdalen, approximately ten kilometres from the Svalbard populations described in Section 2.1. The iButtons recorded soil surface temperatures with a precision of 0.5°C every 4 hours for about 11 months, from midsummer 2005 until midsummer 2006. Most of the data loggers—at least one from each region—were successfully retrieved after the experiment, and the downloaded temperature records were used to infer the time of snowmelt and the temperatures to which *K. islandica* seeds are

subjected at the time of germination.

4.2.6. Statistical analysis

Plastic responses in seed germination along the temperature gradient for the six *Koenigia islandica* populations were analysed using the Loess nonparametric smoothing function (Cleveland, 1979). This procedure allows the assessment of the shape of complex norms of reaction and therefore permits testing for differences among populations (Simons and Wagner, 2007). Each population and each treatment was analysed in two steps; in the first the smoothing parameter based on the unbiased selection criterion AIC_{C1} (SAS Institute Inc., 2003) was independently obtained (Hurvich et al., 1998); in the second a SAS PROC LOESS analysis was performed using the single smoothing parameter selected in the first step (SAS Institute Inc., 2003). The final output of the analysis contained the predicted smoothed surface for the population and treatment, as well as its 95% confidence limits. Although a conservative test (Simons and Wagner, 2007), non-overlap of the 95% confidence limits allows for statistical inferences on significant differences between norms of reaction of populations and treatments across temperatures.

The Loess predictor was used also to assess the degree of plasticity to temperature expressed by each population and treatment (Simons and Wagner, 2007). Total plasticity (P_T) was estimated as the absolute value of the cumulative change in the Loess function over the complete range of temperatures. This also allowed the estimation of plasticity per °C in germination fraction ($P°C^{-1}$).

The effect of temperature fluctuations on seed germination in *K. islandica* was tested using a factorial analysis of variance (ANOVA), with all effects—i.e., population, stratification treatment and temperature regime—considered as fixed. The analysis was performed at the level of replicated Petri dishes (see

previous section), and germination fractions were transformed as p'=arcsine ($p^{0.5}$) prior to the analysis. Post-hoc Tukey HSD-tests were also performed to examine in more detail the observed differences among populations and treatments.

4.3. Results

The shapes of reaction norms indicate a distinct germination temperature resulting in the highest germination for most populations, below and above which germination is markedly inhibited (Figures 4.2, 4.3). The Loess assessments using the unbiased smoothing selection criterion reveal dissimilarities in temperature reaction norms among populations for both germination fraction (Figure 4.2) and time to germination (Figure 4.3). When the different populations are compared, the shapes of the reaction norms for germination fraction are significantly different in most cases (Figure 4.4). Moreover, cold stratification of the seeds induced significantly different germination responses to temperature in four out of the six populations analyzed (Figure 4.5).

With the exception of the Norway population, the untreated *K. islandica* seeds germinated with high percentages in a relatively narrow range of high temperatures from 27–30°C. Temperatures below 21°C seemed to inhibit the germination of unstratified seeds for all populations except the one from Norway, and at 33°C all six populations exhibited poor germination (Figure 4.2).

A small percentage of the seeds germinated during the stratification treatment at 4°C in the dark: 0.74% of the seeds belonging to the Svalbard population, 3.33% of the seeds from Colorado, 5.92% of the Norway seeds, 6.66% of the Iqaluit seeds and 12.96% of the seeds from the Jasper population.

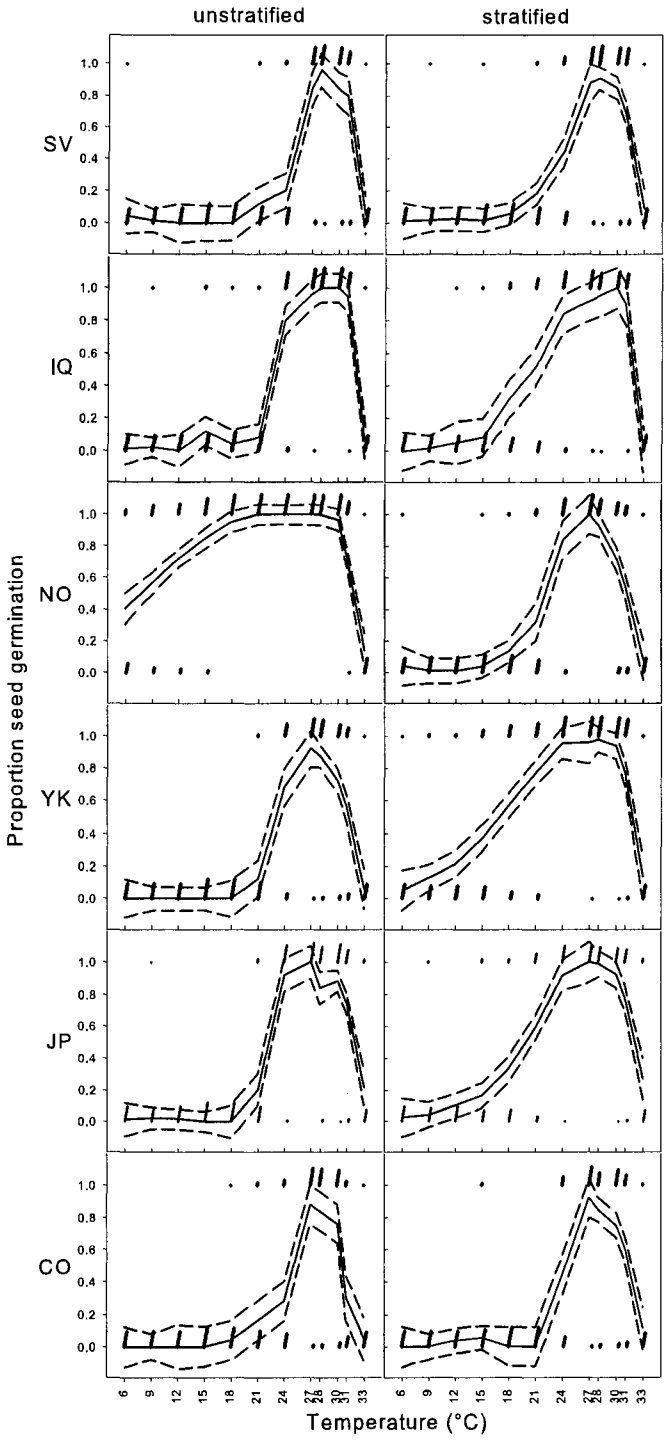

Figure 4.2. Norms of reaction for germination fraction for six populations of *Koenigia islandica* in response to temperature as predicted by the Loess smoothing procedure. The populations are from Svalbard (SV), Iqaluit (IQ), Norway (NO), Yukon (YK), Jasper (JP) and Colorado (CO). Germination data (diamonds) for unstratified (left) and stratified (right) seeds are offset so as to reveal all individual germination and nongermination events at each of the twelve temperatures. The predicted Loess function (solid line) is fitted using an objectively selected smoothing parameter. Broken lines are the 95% confidence limits.

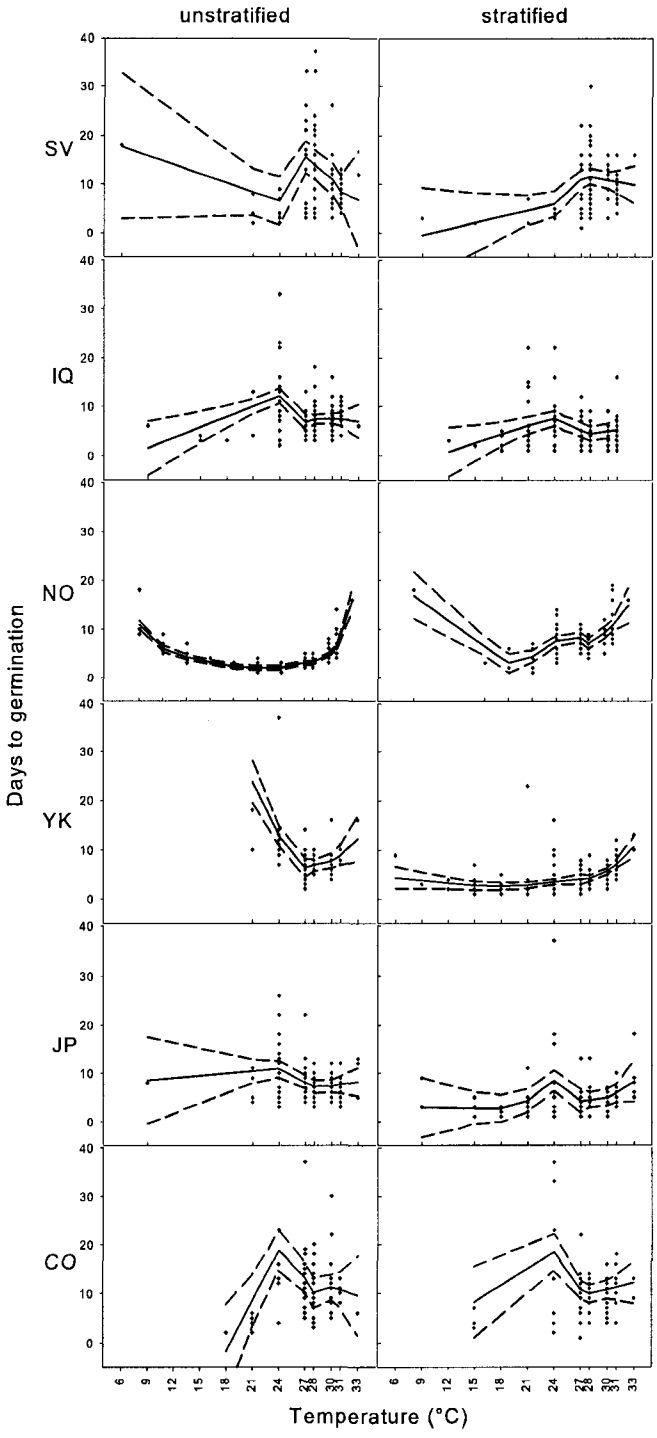

Figure 4.3. Norms of reaction for time to germination for six populations of *Koenigia islandica* in response to temperature as predicted by the Loess smoothing procedure. The populations are from Svalbard (SV), Iqaluit (IQ), Norway (NO), Yukon (YK), Jasper (JP) and Colorado (CO). Diamonds are for individual germination events of unstratified (left) and stratified (right) seeds at each of the twelve temperatures. The predicted Loess function (solid line) is fitted using an objectively selected smoothing parameter. Broken lines are the 95% confidence limits.

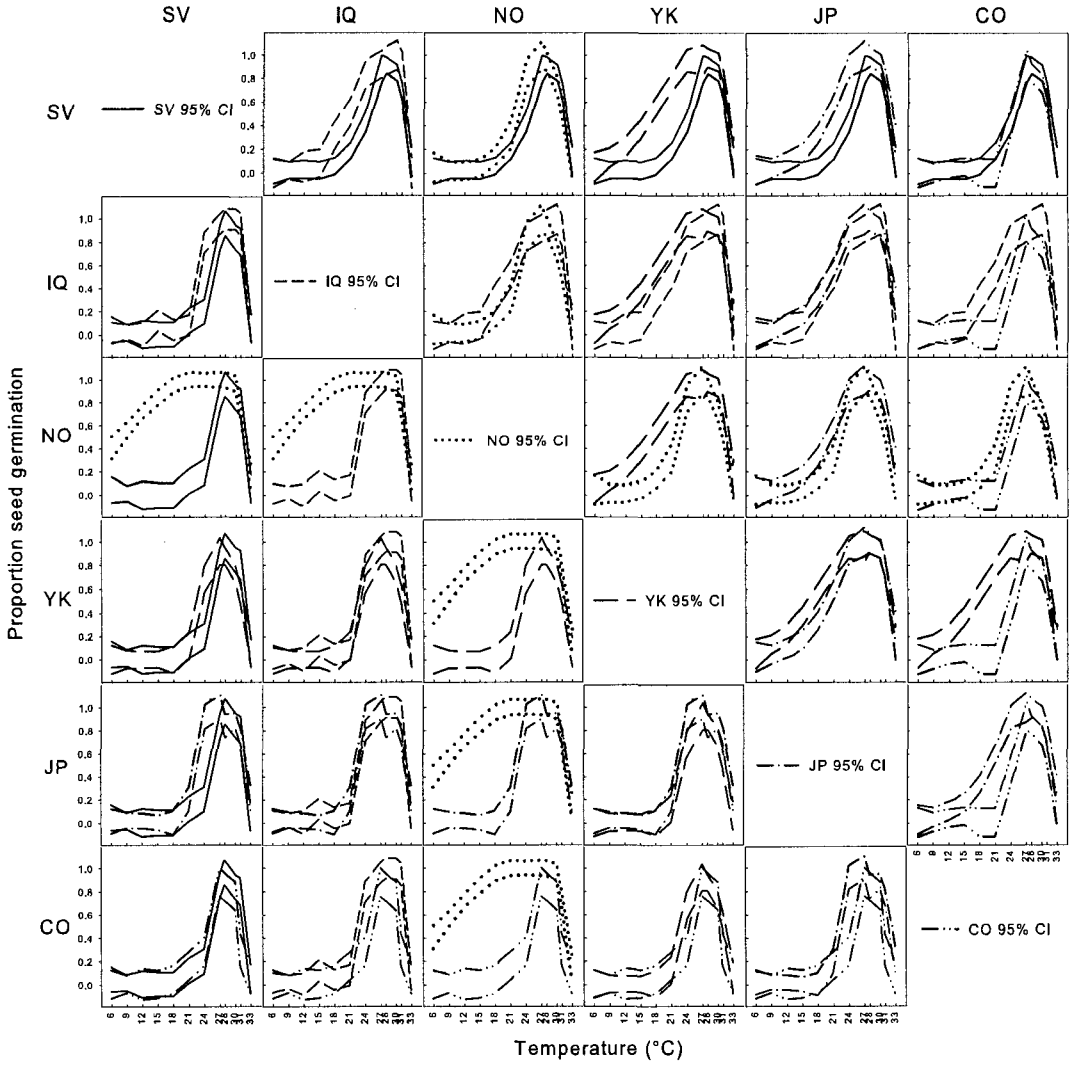

Figure 4.4. Among-population comparison of the 95% confidence intervals for germination fraction for seeds of six *Koenigia islandica* populations. SV = Svalbard; IQ = Iqaluit; NO = Norway; YK = Yukon; JP = Jasper; CO = Colorado. Stratified seeds are shown above the diagonal; unstratified, below.

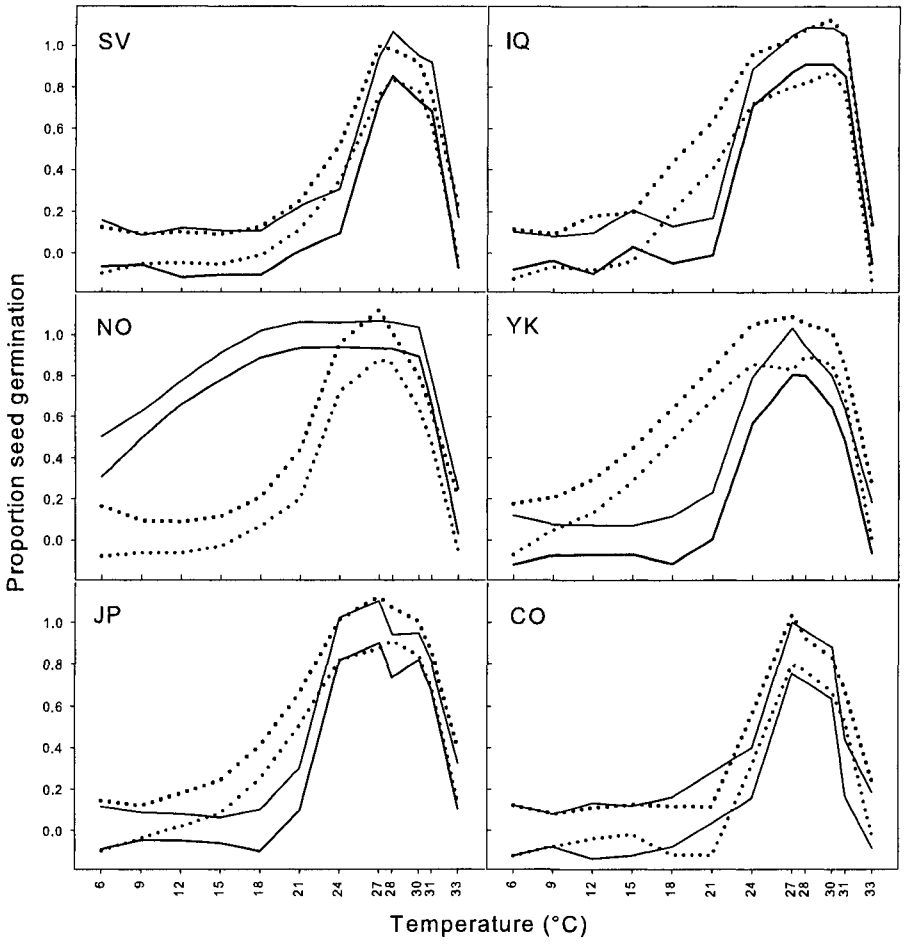

Figure 4.5. Comparison of the 95% confidence intervals for germination fraction between unstratified (solid lines) and stratified (dotted lines) seeds of *Koenigia islandica* populations from Svalbard (SV), Iqaluit (IQ), Norway (NO), Yukon (YK), Jasper (JP) and Colorado (CO).

Cold stratification significantly increased the seed germinability of the Yukon population (Figure 4.5), and greatly reduced the temperature requirement for germination (Figure 4.2); stratified seeds of this population germinated at temperatures as low as 6°C, whereas the unstratified seeds did not germinate at all below 18°C. Cold stratification lowered the minimum germination temperature also in the Iqaluit and Jasper populations, but had little influence on seed germination of the Svalbard population, and virtually no effect in the case of the Colorado population (Figure 4.2). Unexpectedly, cold stratification had a negative effect on seed germination of the Norway population, producing a significant decrease in the seed germinability at lower temperatures (Figure 4.6), and increasing the time to germination at any temperature (Figure 4.5). The germination responses to temperature observed in the present experiment are in overall concordance with independent results of Simons and Wagner (2007), in which seed material from a previous generation, and from only three of the six *K. islandica* populations along with another plant species were tested under eight temperature environments for the purpose of introducing Loess analysis for characterising complex continuous norms of reaction.

In the case of unstratified seeds, the 95% confidence limits of Loess predictors for the six populations overlap at and above 27°C, and also at temperatures below 21°C, with the notable exception of the Norway population. At intermediate temperatures, however, significant differences can be observed in the seed germination of some of the populations, with the Iqaluit, Yukon and Jasper populations showing similar reaction norms, while the populations from the two extremes, the high arctic population from Svalbard and the low latitude alpine population from Colorado, exhibited similar germination responses to temperature (Figure 4.4). Cold stratification of the seeds increased the differences among the populations, with nonoverlapping

confidence limits at most temperatures outside the 27–28°C range for many populations (Figure 4.4).

A separate comparison of the reaction norms of unstratified and stratified seeds of each population indicate that seed stratification had almost no effect on the seed germinability of the Svalbard and Colorado populations, while significantly promoting the germination of the Iqaluit, Yukon and Jasper populations (Figure 4.5). This effect was especially marked in the case of the Yukon population; in contrast, the germination of the Norway population was strongly inhibited by cold stratification.

In contrast to the marked differences observed in norms of reaction, the assessment of the total plasticity expressed by *K. islandica*—the absolute value of the cumulative change in Loess predictor over the entire temperature range—showed little difference among the studied populations (Table 4.1).

The results of the TTC viability test indicated that over 97% of the ungerminated seeds of both unstratified and stratified treatments of all six populations kept their viability, with the exception of all seeds subjected to 33°C, from which none remained viable, having probably been killed by the abnormally high germination temperature to which they were subjected for two months.

The ANOVA performed on germination fractions of seeds of the six populations in different temperature fluctuation regimes indicate that the germination of *K. islandica* seeds is not promoted by fluctuating temperatures in any of the analysed populations (Figure 4.6); most seeds germinated significantly better in the near-constant, higher temperatures (Tables 4.2, 4.3).

The temperature records of the miniature temperature loggers (iButtons)

Table 4.1. Total plasticity (PT) and plasticity per °C ($P°C^{-1}$) in germination fraction in *Koenigia islandica*. Values are calculated from the cumulative change in Loess predictor across 12 temperature environments between 6 and 33°C.

Population	Treatment	P_T	$P°C^{-1}$
Svalbard	unstratified	1.706318	0.070889
	stratified	1.730265	0.064084
Iqaluit	unstratified	2.140761	0.079287
	stratified	2.005989	0.074296
Norway	unstratified	1.455833	0.05392
	stratified	1.940263	0.071862
Yukon	unstratified	1.778623	0.065875
	stratified	1.767206	0.065452
Jasper	unstratified	1.904595	0.070541
	stratified	1.706318	0.063197
Colorado	unstratified	1.71	0.063333
	stratified	1.836111	0.068004

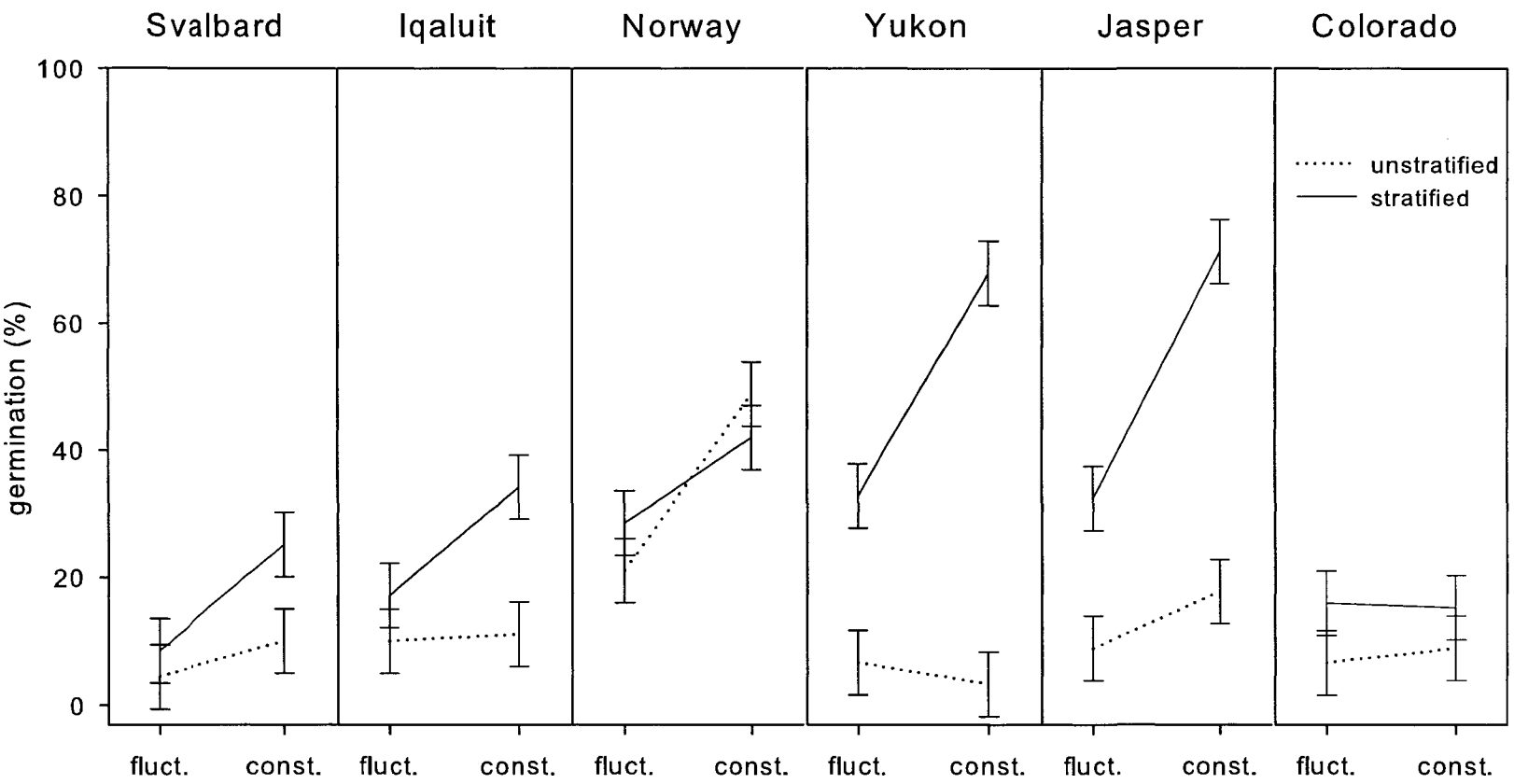

Figure 4.6. Germination fraction in six populations of *Koenigia islandica* at fluctuating temperatures (fluct. =4-20°C) and near-constant temperatures (const.=17-20°C). Solid lines represent germination of unstratified seeds; dotted lines represent germination of stratified seeds. Error bars indicate ±1SD.

Table 4.2. Analysis of variance of germination fraction under two temperature fluctuation regimes of unstratified and stratified seeds of six populations of *Koenigia islandica*, from Svalbard, Iqaluit, Norway, Yukon, Jasper and Colorado.

Source	*df*	MS	*F*	*P*
Population	5	0.199	12.245	<.0001
Stratification	1	1.236	75.921	<.0001
Temperature regime	1	0.396	24.315	<.0001
Population*Stratification	5	0.138	8.487	<.0001
Population*Temperature regime	5	0.029	1.798	0.1311
Stratification*Temperature regime	1	0.107	6.587	0.0134
Population*Stratification*Temperature regime	5	0.037	2.289	0.0604

Table 4.3. Germination fraction of unstratified and stratified seeds of six populations of *Koenigia islandica* under two temperature fluctuation regimes. Values with different letters indicate table-wide significant differences according to post-hoc Tukey HSD-tests (see Table 4.3).

Populations	Fluctuating temperatures		Near-constant temperatures	
	Unstratified	Stratified	Unstratified	Stratified
Svalbard	4.44^{FG}	8.51^{EFG}	10.00^{DEFG}	25.27^{CDEFG}
Iqaluit	10.00^{DEFG}	18.30^{CDEFG}	11.11^{EFG}	35.61^{ABCDE}
Norway	21.11^{CDEFG}	28.73^{CDEF}	48.88^{ABC}	41.86^{ABCD}
Yukon	6.66^{EFG}	32.53^{BCDE}	3.33^{G}	67.77^{AB}
Jasper	8.88^{EFG}	32.58^{BCDE}	17.77^{CDEFG}	71.59^{A}
Colorado	6.66^{EFG}	15.47^{CDEFG}	8.88^{EFG}	15.11^{CDEFG}

allowed assessments of the temperature regimes experienced by *Koenigia islandica* plants and seeds, from summer 2005 until summer 2006 (Figure 4.7) and permitted inferences on the date of snowmelt as well as the germination conditions of *K. islandica* in the field (Table 4.4). At the Colorado site the temperatures registered the greatest diurnal oscillations during the snow-free period, with maxima exceeding 20°C and minima at or a few degrees above 0°C for most of the growing season (Figure 4.7). In the northern sites of Yukon and Svalbard the daily temperature fluctuations during the snow-free period were less pronounced, and a decrease in amplitude with increasing latitude is apparent across the three locations. In all three sites the soil surface temperatures were very constant, and remained near 0°C under the snow cover (Figure 4.7). Immediately following snowmelt, the temperatures again registered strong oscillations, with daily maxima exceeding 20°C within a few days at the Colorado site (Table 4.4).

4.4. Discussion

In an environment in which vegetative reproduction and distribution of flower and seed formation over more than one year are dominant reproductive strategies (Heide, 1992), arctic and alpine annuals must complete their life cycle from seed to seed in one short, cool and sometimes unpredictable growing season (Billings, 1974). In order to do so, annuals in arctic and alpine environments must both maximize the effective length of the potential growing season and minimize the risk of germinating too early or too late in the season, when the chances of seedling survival are low. From the available cues to which germination timing is expected to have evolved to respond, temperature is among the most important (Baskin and Baskin, 1988). The existence of population differentiation as opposed to a generalist germination

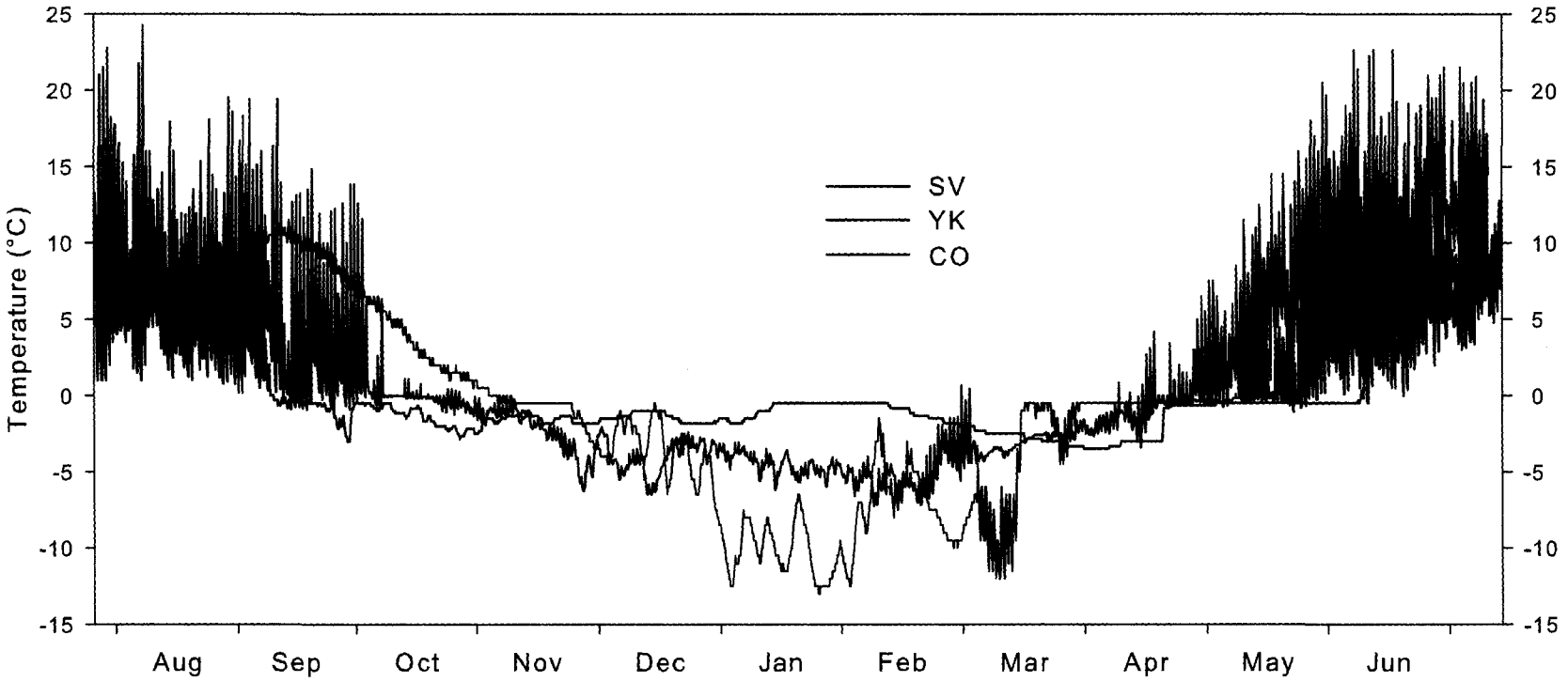

Figure 4.7. Soil surface temperatures between July 2005 and July 2006 at the three *Koenigia islandica* sites, as recorded by miniature temperature loggers (iButtons). SV (blue) = Svalbard; YK (green) = Yukon; CO (black) = Colorado.

Table 4.4. Soil surface temperature regimes after snowmelt in three *Koenigia islandica* sites for May–July 2006 as recorded by miniature temperature loggers (iButtons). The number of days elapsed from the time of final snowmelt until temperatures reach 15°, 20° and 25°C are given, except in cases where this temperature is never reached. Max is the maximum temperature observed for the entire growing season.

Site	Date of snowmelt	Days to T>15°C	Days to T >20°C	Days to T >25°C	Max
Svalbard	June 16	26	-	-	16.5°C
Yukon	May 05	27	60	-	21.5°C
Colorado	May 27	7	11	11	25.5°C

strategy would suggest a mechanism underlying vulnerability of arctic-alpine annuals to rapid environmental change.

In these experiments we test for genetic differentiation in germination responses to a gradient of temperature environments in six populations of the arctic-alpine annual *Koenigia islandica*, originating from a geographically wide range of arctic and alpine habitats. Populations of *K. islandica* were found to differ in their norms of reaction to temperature environments. The fact that these differences are maintained in seeds produced under laboratory conditions indicates that norms of reaction are genetically divergent.

Seed germination without a prior period of cold stratification can be considered equivalent to the germination of freshly matured seeds late in the autumn of the same growing season. With the exception of the Norway population seeds, which seem to germinate rapidly and with high percentages at almost any of the tested temperatures, the seeds of the *K. islandica* populations not subjected to cold stratification require high temperatures (27–28°C) for germination (Figure 4.2), a characteristic commonly encountered in arctic and alpine species (Mooney and Billings, 1961; Sayers and Ward, 1966; Chabot and Billings, 1972; Olson and Richards, 1979; Acharya, 1989; Kibe and Masuzawa, 1994; Nishitani and Masuzawa, 1996; Heide and Gauslaa, 1999; Gimenez-Benavides et al., 2005). The germination fractions obtained here are consistent with those observed in previous germination experiments (Wagner and Simons, 2008a), in which unstratified seeds of the Norway, Yukon and Colorado populations exhibited similar patterns at a single germination temperature. The temperature at which greatest germination occurs for *K. islandica* is among the highest observed in plant species from arctic and alpine environments, and is in sharp contrast with the low temperatures required for optimum growth for this species (Reynolds, 1984a; Heide and

Gauslaa, 1999). This discrepancy between the conditions resulting in highest rates of germination and those optimal for plant growth (Amen, 1966), together with the almost complete lack of seed germination at temperatures below 21°C indicate that all populations, except the one from Norway, have a conditional, or temperature-dependent dormancy (Meyer et al., 1989; Shimono and Kudo, 2005). This is also supported by the high viability of over 97% for the ungerminated seeds, and is considered an important mechanism for avoiding mortality caused by late spring frost (Amen, 1966; Billings and Mooney, 1968; Chabot and Billings, 1972).

Differences among population reaction norms for unstratified seeds (Figure 4.4) are apparent. At high temperatures, at which all populations germinate well, and at low temperatures, at which most populations germinate poorly or not at all, the 95% confidence intervals of the predictors almost completely overlap (Figure 4.4). At intermediate temperatures, however, differences suggestive of adaptive germination responses emerge. The high latitude alpine population from Norway—originating from the mildest climate—germinated with high percentages at almost any temperature. The temperature at which the seeds most rapidly reached 100% germination was also lower (Figure 4.3). The populations from Jasper, Yukon and Iqaluit—with intermediary severity of their climates of origin—each had similar germination responses. They all had a sharp temperature threshold at 24°C, below which seed germination decreased drastically (Figure 4.2). Surprisingly, the two populations from the extremes, the high arctic population from Svalbard and the low latitude, alpine population from Colorado, had very similar temperature reaction norms. The comparatively high temperature needed to attain maximum germination fraction, and the protracted germination process (Figure 4.3) might be explained by the fact that these two populations originate

from the most severe climates; the Svalbard site having a high arctic climate (moderated in part by the warming effect of the Gulf Stream), and the Colorado site, despite being at relatively low latitude, being at high elevation with relatively low summer temperatures. These observed germination patterns are consistent with previous findings that seeds from warmer sites germinate rapidly over a broader temperature range, while seeds from higher elevation and colder climates require higher germination temperatures and germinate more slowly (Meyer et al., 1989; Cavieres and Arroyo, 2000). Both Svalbard and Colorado populations also failed to achieve 100% germination, this being consistent with previous observations that seed germination fractions generally decrease with increasing latitude or elevation of origin (Amen, 1966; Dorne, 1981).

Germination in seeds subjected to cold stratification, which simulates spring or early summer germination of seeds that have spent the winter under snow cover, was significantly increased compared to unstratified seeds in three out of the six *K. islandica* populations. Increased germinability following stratification was also manifested through a decrease in the minimum temperature required to elicit germination especially in the Yukon population, in which the stratified seeds germinated at temperatures as low as 6°C, whereas the unstratified seeds did not germinate at all below 18°C and germinated poorly below 24°C (Figures 4.2, 4.5). Furthermore, germination occurred more rapidly following stratification in this population (Figure 4.3). This germination pattern was observed to a lesser extent also in the Iqaluit and Jasper populations (Figure 4.5), and is consistent with the results of previous germination studies on arctic and alpine plants (Bell and Bliss, 1980; Densmore, 1997; Shimono and Kudo, 2005). An increased germination percentage and a decrease in light requirement for germination when stratified (see Chapter 5)

are typical of summer annuals, which germinate in the spring after spending the winter as seeds under the snow cover and set seed in late summer or autumn (Baskin and Baskin, 1988).

In both populations from the most extreme environments, Svalbard and Colorado, stratification had little effect on germination; the stratified seeds germinating in high percentages only in the same narrow range of high temperatures (Figures 4.2, 4.5). This apparent lack of response to cold stratification might indicate that the seeds of these two populations, from climates with longer winters, may need longer stratification periods to break their conditional dormancy (Meyer et al., 1989; Meyer and Monsen, 1991; Cavieres and Arroyo, 2000). However, previous experiments indicated that at least in the case of the Colorado population, a longer stratification period, of 20 weeks, failed to improve the germination fractions (Wagner and Simons, 2008a). Regardless, the requirement of high temperatures and light for germination (see Chapter 5), together with the platykurtic distribution of time to germination—seeds of these two populations were still germinating after over a month from imbibition (Figure 4.3)—indicate that the populations from Svalbard and Colorado may have evolved cautious germination (Schütz, 2002) through a diversification bet-hedging strategy (Simons and Johnston, 2006). It also confirms the observations of Amen (1966) and Dorne (1981) that germination generally decreases with increasing severity of the climate.

Germination traits of the Norway population were anomalous in that the untreated seeds germinated very quickly (Figure 4.3) and completely (Figure 4.2) under most temperature environments. This is consistent with the observation that seeds from milder sites germinate rapidly and over a broader temperature range (Meyer et al., 1989). The decrease in germinability (Figure 4.5) and increase in time to germination (Figure 4.3)

at low temperatures, together with the strong light requirement induced by stratification (see Chapter 5) is consistent with germination characteristics of some winter annuals—plants which germinate in autumn, pass the winter in vegetative state under the snow cover, and flower, set seed and die the following spring or summer (Milberg and Andersson, 1998)—but is a most surprising trait for an arctic-alpine annual.

Population differentiation in germination responses to temperature was further increased by cold stratification (Figure 4.4). That reaction norms overlapped only in the high temperature range for stratified seeds (Figure 4.4) does not support the observation of Schütz and Milberg (1997) that cold stratification decreases germination variability among geographically different populations. Rather, it supports the finding that in some cases stratification increases the differences in germination responses of different populations (Milberg and Andersson, 1998).

In contrast to population differentiation in norms of reaction, the comparison of total plasticity—measured as the change in Loess predictor—shows little difference among populations (Table 4.1). Lack of replication for this measure of total plasticity, however, precludes a quantitative statistical analysis.

The results of the experiment testing the effect of fluctuating temperatures on germination in *K. islandica* suggest that in none of the analysed populations seed germination is promoted by fluctuating temperatures (Figure 4.6, Table 4.2). This contradicts the findings that fluctuating temperatures generally improve germination in arctic and alpine (Mooney and Billings, 1961; Sayers and Ward, 1966), and boreal (Schütz and Rave, 1999) species, but confirms Heide and Gauslaa's (1999) results that *K. islandica* does not germinate better in fluctuating temperatures.

The high germination temperature optima observed in most investigated *K. islandica* populations begs the question of how the seeds germinate in the field in these sites, where even the extreme maximum summer air temperatures never, or only seldom, reach the temperatures needed by some of the populations for seed germination in laboratory conditions (Table 2.1). Soil surface temperatures, under the effect of solar radiation on clear days in early summer, can greatly exceed the air temperatures, especially in more southern, alpine locations (Dahl, 1963). This has been substantiated by the soil surface temperature monitoring experiment (Figure 4.7). The iButtons placed at the soil surface among the *K. islandica* plants indicated that temperatures at the soil level reached temperatures sufficient for seed germination in only a few days after snow melt in the Colorado site (Table 4.4). Also in the Yukon site, the soil surface warmed in the first few weeks of the growth season to temperatures at which about half of the overwintered (stratified) seeds of this population would germinate. In the Svalbard site, however, the maximum recorded soil temperature during the entire growing season was only 16.5°C (Table 4.4), at which even the cold-stratified seeds germinate at less than 20% in laboratory conditions (Figure 4.2). Although it is believed that pronounced fluctuations in temperature as observed at all sites following snow melt might represent a signal for germination, temperature fluctuations in the laboratory experiment failed to promote germination (Figure 4.6). Another possibility is that an opportunistic germination behaviour of the Svalbard population causes germination only in the rare occasions when the relatively high temperatures are reached. However, this is contradicted by the observation that *K. islandica* is plentiful in this location on a yearly basis. A third explanation is that the 45 days tested were insufficient to break dormancy. Fourth, the iButton data were gathered during a single season, and may be atypical. Finally, the

possibility exists that large soil seed banks are carried over many years in these populations, from which only a small fraction germinates each season. Reynolds (1984b) found only a small residual bank of viable seeds in a *K. islandica* population from Montana, but populations in more severe climates might have larger and more persistent seed banks (Cavieres and Arroyo, 2001). Further investigation might shed light on this aspect.

The Loess procedure used in this study has provided evidence of strong population differentiation in norms of reaction to temperature in germinating seeds of arctic-alpine annual species. Although we can conclude that these differences are genetically based, the adaptive nature of this differentiation remains tentative until specifically tested. Local adaptation of a critical life-history trait suggests that the implications of environmental change for arctic-alpine plants merit further study.

CHAPTER 5

INTRASPECIFIC DIVERGENCE IN LIGHT REQUIREMENTS FOR GERMINATION

5.1. Introduction

Successful seed reproduction is closely associated with the seasonal timing of germination (Baskin and Baskin, 1988; Simons and Johnston, 2006), and light is among the most important environmental factors influencing the timing of seed germination (Grime et al., 1981; Meyer et al., 1990; Baskin and Baskin, 1998). Specifically, the presence of a light requirement is one of the main elements of conservative germination strategies (Schütz, 2002), and in the ability of seeds to postpone germination, i.e., to stay dormant in the soil and form seed banks (Pons, 1991; Milberg and Andersson, 1997; Kettenring et al., 2006).

Conservative germination strategies are obviously of great importance in arctic and alpine environments, where the conditions for germination and seedling establishment might not be favourable every growth season (Bell and Bliss, 1980; Körner, 1999; see also section 1.2), and in response, some species develop large and persistent seed banks (Cavieres and Arroyo, 2001). Environmental conditions, however, are far from homogeneous over the considerable range of tundra habitats (see section 1.4), and plant species growing in both arctic and alpine environments are expected to have diverged in germination traits, including light requirements (see section 1.5). In an environment which is so adverse to seed reproduction—most tundra plants reproduce vegetatively—the few arctic and annual species are compelled to reproduce by seed every year. It is expected therefore that the germination responses of these annuals are especially highly tuned to the environmental cues they encounter, including light, and to the sometimes subtle differences between arctic and alpine environments.

Considering the high theoretical interest of adaptive germination response to harsh environments, light requirements in arctic and alpine seeds are little studied. There is no integrated concept of germination light requirements in arctic and alpine plant species (Shimono and Kudo, 2005), and studies that investigate this topic are also scarce (Acharya, 1989; Densmore, 1997; Schütz, 2002; Shimono and Kudo, 2005). Although interpopulation differences in light requirements for seed germination in temperate species make the subject of several studies (Meyer et al., 1990; Schütz and Milberg, 1997; Milberg and Andersson, 1998), Mooney and Billings' (1961) seminal analysis on *Oxyria digyna*—a perennial species—is the only work that compares germination responses to light of arctic and alpine populations of a tundra plant. There are very few studies that investigate germination light responses in arctic-alpine annual species (Reynolds, 1984a; Heide and Gauslaa, 1999), and none that investigate within-species divergence in light requirements.

This experiment investigates differences in germination light requirements, as well as the effect of wet-cold stratification on germination responses to light in six arctic and alpine *Koenigia islandica* populations (Table 2.1). The aim of the study is to test the prediction that arctic populations have stronger light requirements than alpine populations (see section 1.7). A second prediction that this experiment attempts to test is whether populations that experience more severe climates at their location of origin have stronger light requirements than populations from sites with milder climates (Meyer et al., 1990). By using laboratory-obtained seeds, the experiment can detect if the observed differences are genetically, rather than environmentally based.

5.2. Materials and Methods

5.2.1. Plant material and laboratory seed propagation

Seeds of all six analysed arctic and alpine *Koenigia islandica* populations (see section 2.1) were tested in this experiment for germination light requirements. In order to minimize maternal effects (Fenner, 1991), only laboratory-obtained seeds were used. The tested seeds of the Svalbard, Iqaluit, Norway and Jasper populations were from plants grown through one generation in the laboratory. Because insufficient seeds from first generation plants were available for the Yukon and Colorado populations, seeds from plants grown through two laboratory generations were used for these populations. The field-collected seeds were germinated in SG-30 Enconair germination chambers under long day photoperiod (15:9) at 24°C day and 17°C night temperatures on wet filter paper in Petri dishes. The resulting seedlings were transplanted to pots in a 1:8 mixture of sand and peat-based standard growing medium and grown in growth chambers (Enconair GC-40). The seeds were harvested, dried at room temperature and stored for up to two years at –18°C until further use.

5.2.2. Experimental procedures

The experiment testing germination light requirements of seeds of the six studied populations was performed in the summer of 2006. Six Petri dishes for each population were prepared by placing either 20 unstratified seeds or 15 seeds stratified in the dark for 45 days at 4°C on wet filter paper in the dish. Half the dishes for each population and stratification treatment (the dark treatment) were wrapped in two layers of aluminum foil, and the remainder of the dishes were wrapped in a layer of transparent plastic foil (the light treatment), in order to avoid humidity differences between the two

treatments. All dishes were placed in a germination chamber at continuous light and constant 27°C. The germinated seeds were counted and removed once every two days over a period of 60 days. Percent of germinated seeds (germination fraction) and the time from imbibition to germination (time to germination) were recorded. A small number of seeds germinated in the Petri dishes during the stratification treatment; they were counted towards the germination fraction of that particular plate.

The preparation of dishes and the counting of seeds for the dark treatment were performed in a darkroom under a dim green darkroom light. Because germination light requirements of seeds of various species may be fulfilled by exposure to light of less than a minute (Milberg et al., 1996), seeds of the dark treatment do not receive any photosythetically active light after imbibition during the experiment. Also, tests with temperature loggers placed in Petri dishes indicated that there are no discernible differences in temperatures inside dishes wrapped in aluminum vs. transparent plastic foil due to a greenhouse effect.

To determine whether the remaining seeds did not germinate in the dark because of lack of light or other factors, after the end of the experiment the Petri dishes of the dark treatment were placed into light conditions for several weeks in the germination chamber.

5.2.3. Statistical analysis

To test the dependence of germination fraction on population, stratification treatment, light treatment and their interaction, a factorial analysis of variance (ANOVA) was used. The analysis was performed at the level of replicate Petri dishes, and germination fractions were transformed as p'=arcsine ($p^{0.5}$) prior to the analysis. Because none of the populations and treatments germinated in very low percentages (see chapter 3), an analysis

of time to germination was possible with an ANOVA performed at the level of individual seeds. For both germination fraction and time to germination, post-hoc Tukey HSD-tests were also performed to examine the differences detected by the ANOVAs among populations and treatments.

5.3. Results

The analyses of variance indicate that significant differences in germination light requirements exist among the six populations of *Koenigia islandica*. The effect of light, as well as its interactions with the population effect, is highly significant for both germination fraction (Table 5.1), and time to germination (Table 5.2). Stratification, however, has no effect on the light requirements of most populations; the Light*Stratification interaction term is nonsignificant for both germination fraction (Table 5.1) and time to germination (Table 5.2). The post-hoc Tukey test reveals that only one population out of the six, the Norway population, is significantly influenced by the stratification treatment: for this population, this is true for both germination fraction (Table 5.3) and time to germination (Table 5.4).

The germination responses to light were found to be widely divergent among the six arctic and alpine populations in germination fraction (Figure 5.1) and time to germination (Figure 5.2). Light had no influence on the germination of seeds from Jasper, while the Iqaluit and Yukon populations germinated in light slightly better (Figure 5.1) and faster (Figure 5.2). Statistically, however, only the decrease in light of time to germination of the Yukon population is significant (Table 5.4). As in the previous germination tests (see chapters 3 and 4), the populations from the two extreme tundra environments, the high arctic Svalbard and the low latitude alpine population from Colorado, had very similar germination responses. Light strongly promoted germination in

seeds of both populations, while stratification did not decrease their light requirement (Figure 5.1). As before (chapters 3 and 4), the most divergent germination responses were exhibited by the Norway population. Unstratified seeds in both light and dark, and stratified seeds in light germinated readily (Figure 5.2) and in high percentage (Figure 5.1). Cold stratification, however, induced a strong light requirement in the Norway seeds; only 11% of the stratified seeds of this populations germinated in the dark (Figure 5.1).

The lack of germination in dark conditions in some populations cannot be attributed to dead or deeply dormant seeds; over 70% of the ungerminated seeds of the dark treatment germinated 2 weeks after being moved into light conditions in the germination chamber (Table 5.5).

5.4. Discussion

Arctic and alpine annual plant species must reproduce by seed every year in a strongly limiting environment with short and unpredictable growing seasons, or otherwise develop strategies, such as seed banks, that would ensure their persistence over unfavourable years. Either way, seed germination responses are expected to play a major role in the adaptive strategies of arctic and alpine annuals, with light being one of the major cues to which germination timing is expected to have evolved to respond (see chapter 1). This experiment tests whether genetically based divergence exists in the germination light responses among the six, widely distributed arctic and alpine populations of *Koenigia islandica*.

The results of the experiment indicate that significant differences exist among the six populations in the germination responses of their seeds to light, as well as in the effect of stratification on germination light requirement. These differences were maintained over one, or several generations of plants

Table 5.1. Analysis of variance of germination fraction in light and dark conditions of unstratified and stratified seeds of six populations of *Koenigia islandica*, from Svalbard, Iqaluit, Norway, Yukon, Jasper and Colorado.

Source	*df*	MS	*F*	*P*
Population	5	0.424	18.989	<.0001
Light	1	3.348	149.686	<.0001
Stratification	1	0.008	0.397	0.5314
Population*Light	5	0.386	17.287	<.0001
Population*Stratification	5	0.181	8.130	<.0001
Light*Stratification	1	0.041	1.854	0.1796
Population*Light*Stratification	5	0.142	6.352	0.0001

Table 5.2. Analysis of variance of time to germination in light and dark conditions of unstratified and stratified seeds of six populations of *Koenigia islandica*, from Svalbard, Iqaluit, Norway, Yukon, Jasper and Colorado.

Source	*df*	MS	*F*	*P*
Population	5	5818.762	103.650	<.0001
Light	1	6794.84	121.037	<.0001
Stratification	1	0.00798	0.0001	0.9905
Population*Light	5	1383.828	24.650	<.0001
Population*Stratification	5	575.991	10.260	<.0001
Light*Stratification	1	55.361	0.986	0.3209
Population*Light*Stratification	5	270.383	4.816	0.0002

Table 5.3. Germination fraction of unstratified and stratified seeds of six populations of *Koenigia islandica* in light and dark conditions. Values with different letters indicate table-wide significant differences according to post-hoc Tukey HSD-tests (see Table 5.1).

Populations	Dark		Light	
	Unstratified	Stratified	Unstratified	Stratified
Svalbard	50.00^{CDE}	34.72^{DE}	98.55^{A}	100.00^{A}
Iqaluit	68.33^{BCD}	95.81^{AB}	93.33^{AB}	97.77^{A}
Norway	86.66^{ABC}	11.11^{E}	100.00^{A}	100.00^{A}
Yukon	80.00^{ABC}	95.55^{AB}	96.66^{A}	97.77^{A}
Jasper	96.66^{A}	93.33^{AB}	96.66^{AB}	93.33^{AB}
Colorado	35.00^{DE}	31.11^{DE}	83.33^{ABC}	82.22^{ABC}

Table 5.4. Mean time to germination (days) of unstratified and stratified seeds of six populations of *Koenigia islandica* in light and dark conditions. Values with different letters indicate table-wide significant differences according to post-hoc Tukey HSD-tests (see Table 5.2).

Populations	Dark		Light	
	Unstratified	Stratified	Unstratified	Stratified
Svalbard	28.76^{AB}	35.81^{A}	15.19^{C}	13.42^{CD}
Iqaluit	11.34^{CDEFG}	5.93^{FGHI}	5.46^{HI}	2.72^{I}
Norway	5.67^{GHI}	19.00^{BCDE}	3.63^{I}	8.11^{DEFGHI}
Yukon	15.64^{C}	9.16^{DEFGH}	5.48^{HI}	3.22^{I}
Jasper	6.28^{EFGHI}	5.40^{HI}	5.25^{HI}	3.28^{HI}
Colorado	13.00^{CDEF}	$8.71^{CDEFGHI}$	11.94^{CD}	12.81^{CD}

Table 5.5. Germination fractions of *Koenigia islandica* seeds of the dark treatment remaining ungerminated (see Table 5.3), two weeks after being moved into light conditions.

	Svalbard	Iqaluit	Norway	Colorado
unstratified	80	57.14	62.50	44.44
stratified	86.66	-	79.48	68.42

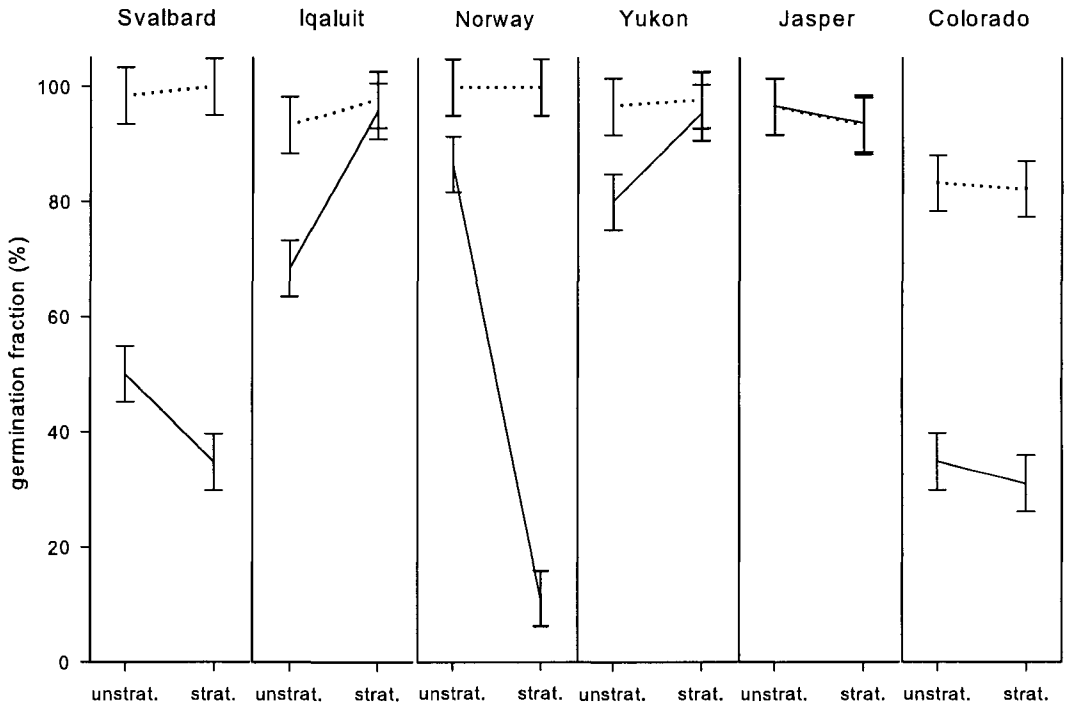

Figure 5.1. Germination fraction for unstratified and stratified seeds in six populations of *Koenigia islandica* at 27°C. Solid lines represent germination in dark conditions; dotted lines represent germination under light. Error bars indicate ±1SD.

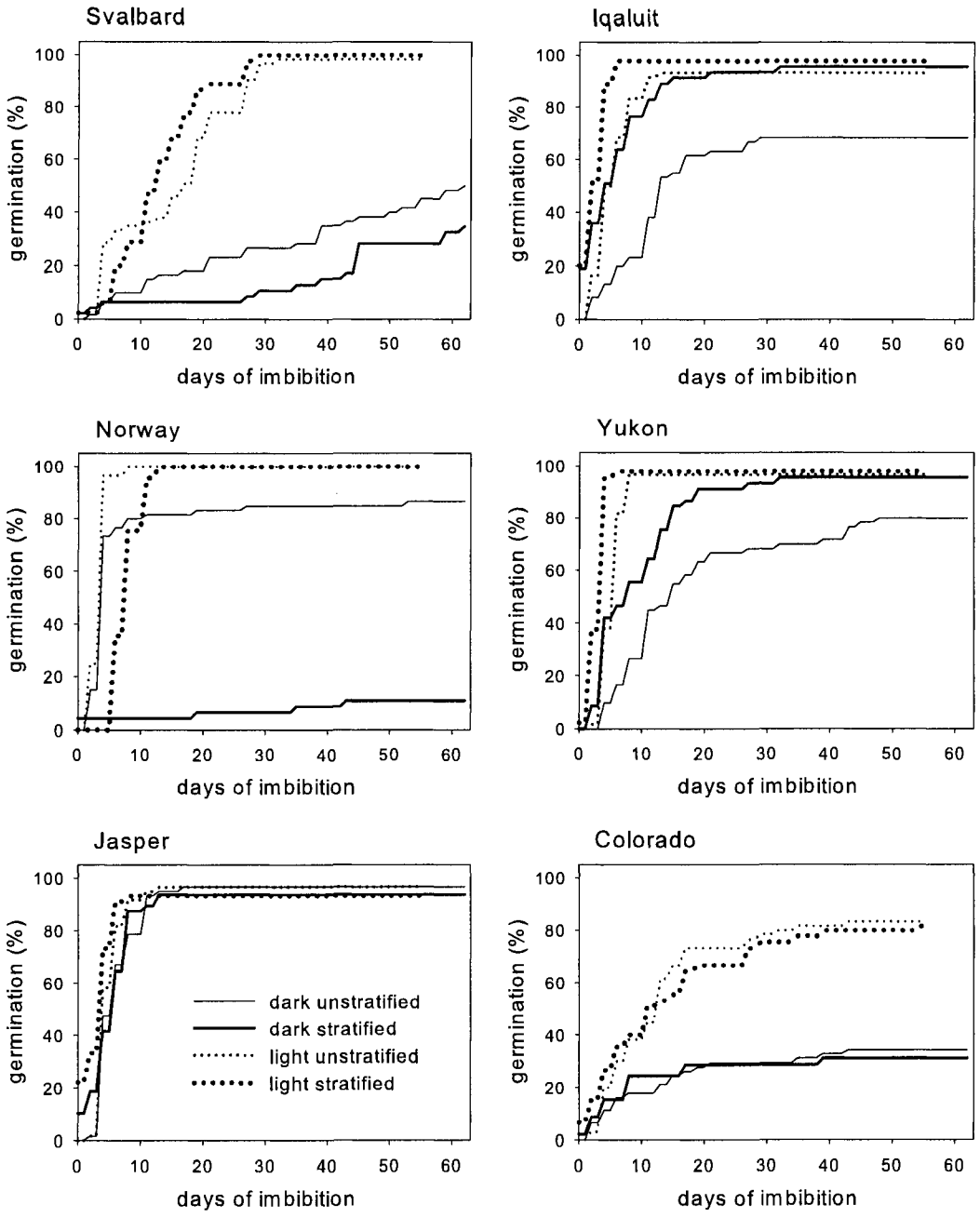

Figure 5.2. Trends in time to germination for unstratified and stratified seeds of six populations of *Koenigia islandica* at 27°C, in dark and light conditions. Solid lines represent germination in dark, dotted lines germination in light. Thin lines are for unstratified seeds, bold lines for stratified seeds.

grown under laboratory conditions; this indicates that the observed differences are genetically based.

The initial prediction, that arctic populations have stronger light requirements, was not confirmed; moreover, the observed differences are not consistent with the arctic or alpine origin of the populations, as expected (Table 2.2). In fact, the observed light germination responses seem to relate more to the geographic proximity of the populations. For example, the Jasper population, with no germination requirement for light (Figure 5.1), is geographically close to a *K. islandica* population from Montana, for which the same result was reported (Reynolds, 1984a).

The observed light germination responses appear also to be related to similarities in environmental characteristics, such as growth season temperatures. Despite originating from study sites situated at the extremes of the tundra gradient, the high arctic Svalbard population and the southern alpine population from Colorado have very similar germination responses to light and stratification (Figure 5.1). The two populations, however, are from sites with the coldest climates among those studied (Table 2.2). Both populations have the strictest light requirements among those studied, undiminished by the stratification treatment (Figure 5.1). This confirms the second prediction, that populations from sites with colder climates have stronger light requirements than populations from warmer climates; a finding reinforced by the results of other studies as well (Meyer et al., 1990).

The strong light requirement of the Svalbard and Colorado populations, even at the temperature at which their seeds normally reach their highest germination fraction (27°C; see chapter 4), leads to another interesting consequence. Germination light requirement is considered one of the main factors that promote the formation of large seed banks in the soil (Pons, 1991;

Milberg and Andersson, 1997; Shimono and Kudo, 2005; Kettenring et al., 2006). It can be therefore hypothesized that these two *K. islandica* populations form important seed banks in the soil. This hypothesis is suggested also by the fact that the climates these populations experience are marginal for the high germination temperature requirements of their seeds, especially in the case of the Svalbard populations (see section 4.4). Unfortunately, there is very little information on *K. islandica* soil seed banks. The only study on this reports only a small residual seed bank for an alpine *K. islandica* population from Montana (Reynolds, 1984b). However, this population was also found not to have a germination light requirement (Reynolds, 1984a). This is consistent with the observation that a light requirement for germination is needed for the formation of large seed banks (see citations above). Further work is needed to investigate whether *K. islandica* populations with strong light requirements have important residual seed banks.

Previous studies reported that stratification diminishes the light requirement of seeds of spring germinators from temperate (Milberg and Andersson, 1997; Schütz and Milberg, 1997; Schütz and Rave, 1999), as well as arctic and alpine regions (Densmore, 1997; Schütz, 2002; Shimono and Kudo, 2005). The results of this experiment seem to contradict these findings; the seeds of none of the analysed populations germinated significantly higher after stratification. It needs to be noted, however, that the temperature of 27°C used in this experiment is that at which the seeds of most of the investigated populations, including unstratified seeds, germinate best. The possibility exists that potential significant differences among stratification treatments in light and dark were hidden by the very high percentages of seed germination at this temperature. If tested for light requirements at suboptimal temperatures of, for example, 24°C, seeds of the Iqaluit and Yukon populations might reveal

significant effects of stratification on light requirement.

The most divergent germination patterns were provided, once again, by the population from Norway. Unstratified seeds of this population had no light requirement for germination, whereas stratification induced a strong light requirement in these seeds; very few of the stratified seeds of this population germinated in the dark (Figure 5.1). This germination characteristic, together with the observed decrease in germinability at low temperatures following stratification (see chapter 4) matches the germination syndrome of some autumn germinators (Milberg and Andersson, 1998). This is a very surprising trait in a plant from an environment where all species are supposedly spring germinators. However, the possibility also exists that the low germination of the seeds, i.e. their dormancy in the dark, was induced not by the stratification treatment, but by the dark condition itself (Pons, 1991). Since light requirement induced by burial and dark conditions is another important mechanism of seed bank formation (Pons, 1991; Milberg and Andersson, 1997), it can be again hypothesized that the Norway population too forms important seed banks in the soil. Shedding light on these issues would require further field investigations.

This experiment presents evidence for divergence in yet another life-history trait among the six arctic and alpine *K. islandica* populations, divergence that is genetically based. Because of the complex differences among the environmental characteristics of the sites of origin, however, the precise adaptive significance of the observed differences are not known; clarifying the exact nature of local adaptation would require specific testing and further examination.

CHAPTER 6

DIVERGENCE IN LIFE-HISTORY, MORPHOLOGY AND PHENOLOGY

6.1. Introduction

Arctic and alpine tundra are inhospitable to most organisms. Adaptation to these harsh conditions is thus of interest not only from an evolutionary standpoint, but also because specialized arctic and alpine organisms would be vulnerable to climate change, which appears to be exaggerated in these habitats. As outlined in Chapter 1, however, major differences exist among tundra ecosystems: arctic and alpine tundra habitats are among the most complex examples of environmental gradients. Although divergence in morphology, phenology and life-history traits among plant populations as adaptive responses to environmental gradients in general has received much attention (e.g. Turesson, 1922; Primack and Antonovics, 1981, 1982; Waser and Price, 1985; Potvin, 1986; Van Tienderen and Van der Toorn, 1991a, 1991b; Li et al., 1998; Santamaria et al., 2003; Macel et al., 2007), little is known about local adaptation in arctic-alpine annual plants.

There is evidence that perennial plant species inhabiting both arctic and alpine tundra respond to these vast and complex differences through the evolution of genetically differentiated ecological races (Mooney and Billings, 1961; Mooney and Johnson, 1965; Chapin and Chapin, 1981; Galen et al., 1991; Gurevitch, 1992a; Heide, 2005). The very few arctic and alpine annuals have the additional constraint of having to complete their entire life cycle in one short and unpredictable growing season, and are therefore expected to show especially marked local adaptations to the particular environment they inhabit. Studies on arctic-alpine annuals (Löve and Sarkar, 1957b; Dahl, 1963; Reynolds, 1984a, 1984b; Wagner and Simons, 2008a), however, are scarce. A notable exception is a comparative study on growth, phenology and life-history characters (Heide and Gauslaa, 1999), but this study employs a limited

number of populations and individuals.

In this set of experiments, differentiation in morphology, phenology and life-history characters among the six arctic and alpine populations of *Koenigia islandica* is investigated. The experiments used plants resulting from seeds produced under laboratory conditions, and were performed in growth chambers. A reciprocal transplant design was used, in which all populations were grown in both simulated arctic and alpine conditions. Reciprocal transplant studies predict that the relative performance of populations will be enhanced in their environment of origin which, when found, is interpreted as evidence for local adaptation (Waser and Price, 1985; Potvin, 1986; Van Tienderen and Van der Toorn, 1991a; Joshi et al., 2001; Macel et al., 2007). I aimed to test this prediction, as well as the predictions that more northern populations have smaller statures and decreased fecundity, and that populations from northern latitudes develop and flower earlier (Chapin and Chapin, 1981; Potvin, 1986; Winn and Gross, 1993; Li et al., 1998). I also sought to determine whether the different responses are due to ecological races rather than to phenotypic plasticity, and to suggest possible adaptive explanations for the observed differences. These adaptive explanations, however, require further test.

6.2. Materials and Methods

6.2.1. Plant material and laboratory seed propagation

For the purpose of both multiplying the number of available seeds and reducing maternal effects (Quinn and Colosi, 1977; Roach and Wulff, 1987), the investigated populations were grown through one (Svalbard, Iqaluit and Jasper) or two generations (Norway, Yukon and Colorado) in the laboratory. Seeds were germinated on wet filter paper in Petri dishes in germination chambers (Enconair SG-30, Biochambers Inc., Winnipeg, Manitoba, Canada).

The seed germination conditions for the Norway, Yukon and Colorado populations were of long day (LD) photoperiod (15:9) at 24°C day and 17°C night temperatures, while for the seed germination of the Svalbard, Iqaluit and Colorado populations a continuous 24h photoperiod and constant 27°C were used. The resulting seedlings in each case were moved to Enconair GC-40 growth chambers in pots with a 1:8 mixture of sand and peat-based standard growing medium. The first generation of laboratory-reared plants of the Norway, Yukon and Colorado populations were grown under LD (15:9) photoperiod at 15°C day and 10°C night temperatures, while the second generation of these populations, as well as those from Svalbard, Iqaluit and Jasper were grown in both a continuous photoperiod at 14°C day and 5°C night temperatures and a LD (15:9) photoperiod at 18°C day and 6°C night temperatures. In every case the resulting seeds were harvested, dried at room temperature and stored at –18°C until used.

6.2.2. Experimental procedures

Experiments testing for differences in vegetative and generative growth, as well as in phenology among populations, were performed in two growth chambers (Enconair GC-40). One chamber was used to simulate a high arctic environment above the Arctic Circle (hereafter referred to as the arctic chamber). The day temperature was set to 14°C for 18 h and the night temperature to 5°C for 6 h. The chamber, constantly lighted, was illuminated for 18 h/day by both fluorescent and incandescent bulbs delivering a photosynthetic photon flux density of about 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Daylight was extended for the remaining 6 hours only by incandescent bulbs (~40 $\mu mol \cdot m^{-2} \cdot s^{-1}$). A second growth chamber was used to simulate growing conditions in an alpine environment at approximately 40°N latitude (alpine chamber). Day and night temperatures were kept at 18°C and 6°C, respectively, for 12 h each. A 15:9, LD photoperiod

was maintained, with 12 hours of 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density lighting, prolonged to 15 hours by incandescent bulbs. Plants were grown in plastic trays in individual cells in a 1:8 mixture of sand and peat-based standard growing medium. The experiments were reciprocal transplants, in which both arctic and alpine populations were grown in both growth chambers (Mooney and Billings, 1961).

Trial 1. This initial experiment was performed on the Norway, Yukon and Colorado populations, the only ones with available plant material at the time the experiment was conducted. Because two growth chambers were not available at the same time, an arctic chamber experiment was performed starting in July 2004 and an alpine chamber experiment starting January 2005. Because of the reciprocal nature of the design, any differences in function of the chamber over time would result in differences in performance among arctic and alpine treatments, but not in the relative performance of arctic and alpine populations within these treatments. The seeds for this trial were germinated in an SG-30 germination chamber under a LD (15:9) photoperiod (approx. 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density) and 24°C day and 17°C night temperatures. Untreated seeds, scarified seeds, seeds stratified for 45 days at 4°C in the dark and seeds that received both treatments were used (see Chapter 3). After the completion of their life cycle, i.e. seed maturation, the plants were harvested and the following vegetative and generative traits were measured and recorded: height, branch length, average leaf length (as a mean of two measured leaves), number of leaves, number of seeds, seeds per infructescence, infructescences per individual, total seed mass as well as above-ground dry plant mass (after drying to constant weight at 50°C). From these measurements the total aboveground plant mass (as the sum of seed mass and aboveground plant mass), the average mass of one seed and reproductive

allocation of each plant (total mass of the seeds/total plant weight) were also inferred. The very high shoot/root ratio of 5–7 for *K. islandica* plants (Reynolds, 1984a) makes the above-ground reproductive allocation a good approximation of the actual reproductive allocation that would include the root mass as well (Heide and Gauslaa, 1999).

Trial 2. This experiment, initiated in March 2006, was performed with all six available populations concomitantly using an arctic and an alpine chamber. The seeds for this experiment were germinated in a continuously lighted SG-30 germination chamber (photosynthetic photon flux density as above) at constant 27°C prior to transfer to the arctic and alpine chambers. Sixteen trays were placed in each chamber, with each tray containing all six populations, in blocks of six individual cells with a randomized position within trays. A new randomization was used for each tray, and the randomization was identical in the two chambers. The individual plants were monitored every second day throughout their life cycle for the presence of 11 vegetative and 11 generative phenological stages (Table 6.1). After most plants reached their last phenological stage, they were harvested, and the same vegetative and generative traits as in trial 1 were measured and recorded.

6.2.3. Statistical analysis

Factorial analyses of variance (ANOVA) were used to test for the effects of chamber, population and their interaction on the measured vegetative and generative traits. The Chamber*Population interaction is of particular importance in a reciprocal environment design, and tests the relative performance of populations across the environments after controlling for environmental response common to all populations. ANOVAs were performed independently on several traits. We use a recent method, known as the false discovery rate (FDR) to minimize the frequency of significant results that are

Table 6.1. Phenological stages of *Koenigia islandica* individuals.

Vegetative stages	Generative stages
0 — cotyledons only	0 — no buds/flowers
1 — 1st leaf	1 — 1st buds visible
2 — 1st pair of leaves	2 — buds just before opening
3 — 2nd pair of leaves	3 — 1–30% of flowers open
4 — 3rd pair of leaves	4 — 31–60% of flowers open
5 — 1st branch	5 — full flowering
6 — 2nd branch	6 — flowers wilted
7 — 1–30% of leaves dry	7 — 1st achenes visible
8 — 31–60% of leaves dry	8 — achenes mostly green
9 — 61–99% of leaves dry	9 — achenes mostly brown
10 — plants dead	10 — achenes fully ripe and dispersing

type-1 errors (Benjamini and Hochberg, 1995; Garcia, 2003); a method that does not suffer from loss of power as do methods such as Bonferroni correction. Because of the differences in experimental design, the results of the two experimental trials were analysed separately, with all the effects considered fixed. When the analysis of variance indicated significant effects ($P > 0.05$), post-hoc Tukey HSD-tests were performed to further examine differences detected. Additional ANOVA and Tukey HSD analyses were performed on the subpopulations of the Colorado population in the first trial.

The phenological data resulting from the second trial was expressed as the number of days from germination until the occurrence of the observed phenological stage. Because multiple measures are taken within subjects over time, we performed a multivariate analysis of variance (MANOVA) for repeated measures, testing for the effect of chamber, population, the within-subjects effect of stage, and interaction terms. The null hypothesis of no difference among populations in time taken to reach different stages is tested with the Stage*Population interaction term. *F* tests and Wilk's Lambda (Wilk's λ) tests were used. Because of the large number of stages measured, insufficient degrees of freedom preclude analysis of the whole set of phenological stages. Therefore, the nine most relevant vegetative, and the eight most relevant generative stages of the total of eleven stages were included in the analysis. Stage 0 for both the vegetative and generative phenology—attained right after germination—is identical for all populations and was therefore not included. Vegetative stage 6—appearance of a 2^{nd} branch—was reached only by a relatively small percentage of individuals, and has been excluded as well. Similarly, the generative stages 2 and 5, with low percentages of occurrence, were not included in the analysis.

6.3. Results

Trial 1. Vegetative and reproductive traits differ significantly among the three *K. islandica* populations (Table 6.2), while among the four subpopulations from Colorado the differences are much less marked. After correcting for FDR, only 4 of 12 traits, all vegetative, are significantly different among the Colorado subpopulations (Table 6.3), while 11 out of 12 traits are significantly different among the three studied populations (Table 6.2). The two high latitude populations, Norway and Yukon, grew significantly better in the simulated arctic environment, reaching larger sizes and producing more seeds in the cooler, continuously lighted conditions (Figure 6.1). The significant Chamber*Population interactions, after controlling for false discovery rate (see Section 6.2.3) show that, despite the fact that the mean values for the Colorado population show no significant differences across environments, the relative performance of this population is higher in the alpine chamber for important characters including total seed mass and seed number, although the increased value for total aboveground biomass is nonsignificant. For average mass of a seed no differences between the growing environments could be detected, but the Norway population had significantly larger seeds overall (Figure 6.1). Reproductive allocation (total mass of the seeds/total plant weight), differs between environments, and the significant interaction shows that Norway alters its reproductive allocation relative to the other populations across the two chamber environments, showing increased allocation in the arctic chamber.

Trial 2. On the whole, the six analysed *K. islandica* populations grew worse in this experiment compared to trial 1 (Figure 6.2). However, the trends of the analysed morphological and life-history characters are similar

Table 6.2. Analysis of variance for vegetative, reproductive and life-history traits of three populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions (trial 1). No false rejections were found through calculation of false discovery rate (at α=0.05) for multiple comparisons (see section 6.2.3).

Source	*df*	MS	*F*	*P*
Vegetative traits				
Plant mass:				
Chamber	1	8117.12	346.67	<.0001
Population	2	238.23	10.17	<.0001
Chamber*Population	2	27.54	1.17	0.30
Height:				
Chamber	1	155.65	10.16	0.0015
Population	2	678.49	44.31	<.0001
Chamber*Population	2	0.22	0.01	0.98
Branch length:				
Chamber	1	1061.21	26.71	<.0001
Population	2	1212.87	30.53	<.0001
Chamber*Population	2	701.11	17.64	<.0001
Average leaf length:				
Chamber	1	21.22	13.31	0.0003
Population	2	41.39	25.97	<.0001
Chamber*Population	2	12.31	7.72	0.0005
Number of leaves:				
Chamber	1	1679.11	34.28	<.0001
Population	2	345.98	7.06	0.0009
Chamber*Population	2	957.00	19.53	<.0001
Reproductive traits				
Total seed mass:				
Chamber	1	6137.87	73.83	<.0001
Population	2	654.32	7.87	0.0004
Chamber*Population	2	1431.61	17.22	<.0001

Table 6.2. continued

Source	*df*	MS	*F*	*P*
Number of seeds:				
Chamber	1	37245.43	57.64	<.0001
Population	2	8624.09	13.34	<.0001
Chamber*Population	2	14365.58	22.23	<.0001
Seeds/infructescence:				
Chamber	1	991.53	61.37	<.0001
Population	2	225.33	13.94	<.0001
Chamber*Population	2	265.87	16.45	<.0001
Infructescences/individ.:				
Chamber	1	163.24	51.53	<.0001
Population	2	40.17	12.68	<.0001
Chamber*Population	2	76.02	24.00	<.0001
Average mass of a seed:				
Chamber	1	0.04	1.04	0.30
Population	2	3.64	99.18	<.0001
Chamber*Population	2	0.02	0.66	0.51
Life-history traits				
Total aboveground mass:				
Chamber	1	54974.20	382.99	<.0001
Population	2	349.75	2.43	0.08
Chamber*Population	2	335.76	2.33	0.09
Reproductive allocation:				
Chamber	1	0.15	15.44	<.0001
Population	2	0.17	17.61	<.0001
Chamber*Population	2	0.04	3.59	0.02

Table 6.3. Analysis of variance for vegetative, reproductive and life-history traits of four subpopulations of the Colorado population of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions (trial 1). † NS indicate nonsignificant result after controlling for false discovery rate (see section 6.2.3).

Source	*df*	MS	*F*	*P*
Vegetative traits				
Plant mass:				
Chamber	1	1069.50	47.06	<.0001
Subpopulation	3	83.06	3.65	0.01
Chamber*Subpopulation	3	70.14	3.08	0.03 NS†
Height:				
Chamber	1	34.25	2.41	0.12
Subpopulation	3	85.81	6.05	0.0006
Chamber*Subpopulation	3	29.46	2.08	0.10
Branch length:				
Chamber	1	0.62	0.04	0.83
Subpopulation	3	56.70	3.96	0.0092
Chamber*Subpopulation	3	29.46	2.05	0.10
Average leaf length:				
Chamber	1	30.65	14.45	0.0002
Subpopulation	3	9.31	4.39	0.0053
Chamber*Subpopulation	3	2.13	1.00	0.39
Number of leaves:				
Chamber	1	25.60	0.59	0.44
Subpopulation	3	125.80	2.91	0.03 NS†
Chamber*Subpopulation	3	18.70	0.43	0.72
Reproductive traits				
Total seed mass:				
Chamber	1	0.41	0.006	0.93
Subpopulation	3	88.98	1.31	0.27
Chamber*Subpopulation	3	46.09	0.67	0.56

Table 6.3. continued

Source	*df*	MS	*F*	*P*
Number of seeds:				
Chamber	1	34.20	0.07	0.77
Subpopulation	3	445.89	1.03	0.37
Chamber*Subpopulation	3	266.71	0.62	0.60
Seeds/infructescence:				
Chamber	1	0.96	0.06	0.79
Subpopulation	3	6.40	0.43	0.72
Chamber*Subpopulation	3	5.89	0.40	0.74
Infructescences/individual				
Chamber	1	0.12	0.04	0.82
Subpopulation	3	6.61	2.54	0.05 NS†
Chamber*Subpopulation	3	4.41	1.69	0.17
Average mass of a seed:				
Chamber	1	0.04	0.31	0.57
Subpopulation	3	0.03	0.22	0.88
Chamber*Subpopulation	3	0.01	0.11	0.94
Life-history traits				
Total aboveground mass:				
Chamber	1	6842.76	51.63	<.0001
Subpopulation	3	440.11	3.32	0.02 NS†
Chamber*Subpopulation	3	400.00	3.01	0.03 NS†
Reproductive allocation:				
Chamber	1	0.01	1.73	0.19
Subpopulation	3	0.01	1.64	0.18
Chamber*Subpopulation	3	0.01	1.85	0.14

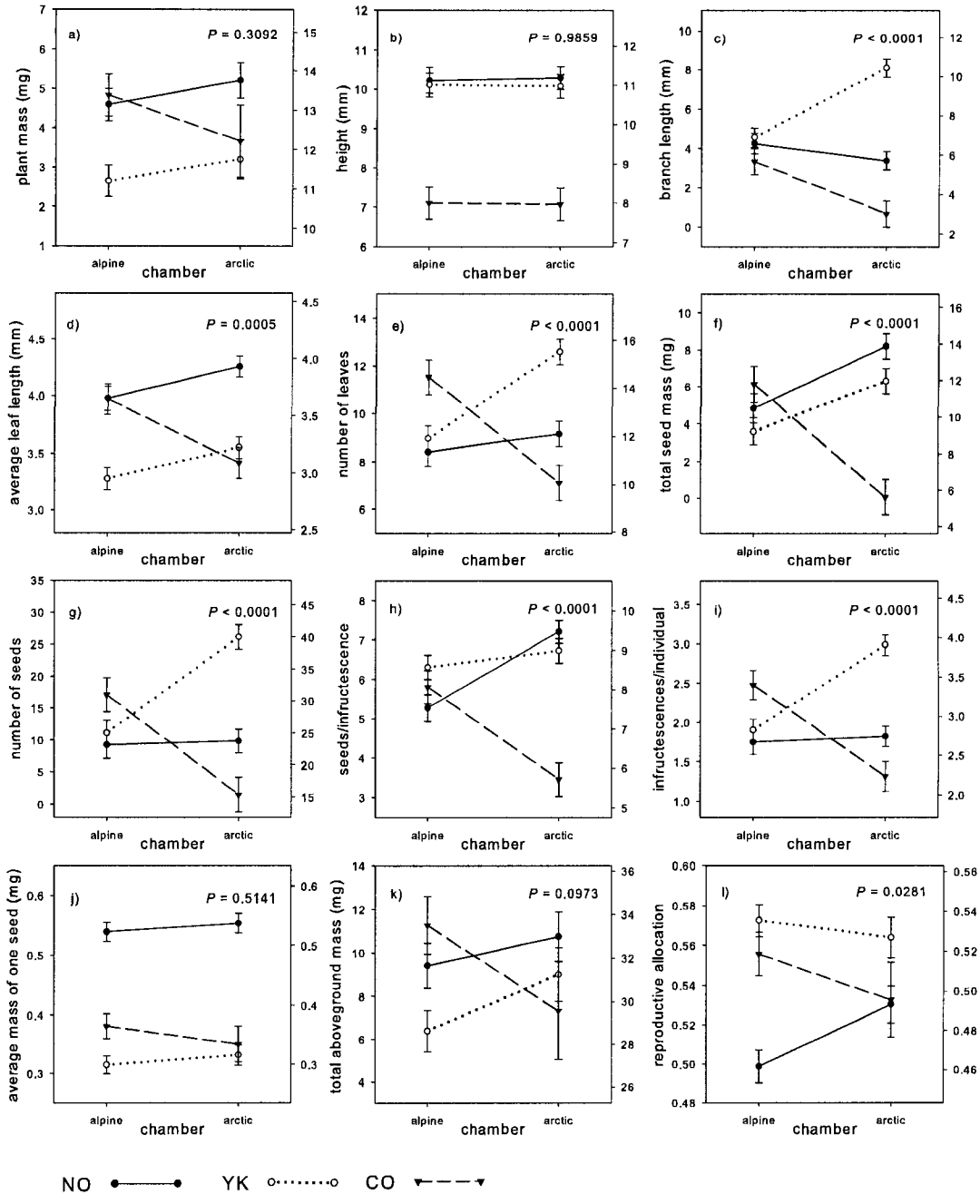

Figure 6.1. Vegetative (a–e), reproductive (f–j) and life-history traits (k–l) of three populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions. Trial 1. NO (solid line)—Norway; YK (dotted line)—Yukon; CO (short dashed line)—Colorado. To represent Chamber*Population interactions rather than simple differences in trait means across environments, means for each trait are shifted to coincide along the two y-axes (see Methods). Left y-axis is for alpine chamber values; right y-axis for arctic chamber values. P-values are for Chamber*Population interaction. No false rejections (type-I errors) at α=0.05 were detected after controlling for false discovery rate. Error bars indicate ±1SD.

to trial 1; the Chamber*Population interactions, after controlling for false discovery rate, were significant for all traits except those related to leaves, indicating significant ecotypic differentiation among populations (Table 6.4). Again, post-hoc Tukey HSD-tests show that the high latitude populations from Iqaluit, Norway and Yukon accumulated significantly more biomass in the simulated arctic conditions, a tendency which was found, unexpectedly, also in the Jasper population (analyses not shown). However, the significant Chamber*Population interaction for aboveground biomass is primarily a result of comparatively high positive slopes across the alpine to arctic chambers for Norway and Yukon (Figure 6.2). Although mean values for traits do not indicate enhanced growth of the plants from Colorado under alpine conditions, such an effect is suggested by the consistently negative slopes across the alpine to arctic chamber environments for this population (Figure 6.2). Very similar growth was, surprisingly, exhibited by the high arctic Svalbard plants (Figure 6.2); the post-hoc Tukey HSD-tests also showed that only one out of the twelve analysed traits—seeds per infructescence—was significantly larger in the arctic chamber for this population.

In the analysis of phenology of the six *K. islandica* populations, the between-subjects "Population" effect reveals significant differences among the populations in both the time taken for vegetative development (Table 6.5) and in the overall generative development (Table 6.6). Some of the populations tended to develop and flower earlier in the arctic chamber, and several within-subjects interactions with chamber effect were significant for both vegetative and generative phenology (Tables 6.5, 6.6). For vegetative phenology, the within-subjects Stage*Population effect is marginally nonsignificant; the time taken for *K. islandica* to reach different vegetative phenological stages shows little difference among populations (Table 6.5). Vegetative traits of the arctic

populations from Svalbard and Iqaluit developed earlier than these traits in alpine populations from Jasper and Colorado (Figure 6.3). The analysis of the generative phenology, however, indicates a more marked differentiation among the populations (Figure 6.4), as confirmed by the MANOVA (Table 6.6). The Stage*Population effect is highly significant in this case (Table 6.6). Again, the plants of the arctic populations from Svalbard and Iqaluit form buds and flowers several days earlier than the plants of the alpine populations from Jasper and Colorado (Figure 6.4). The plants from Norway and Yukon have again a somewhat intermediate position: at the beginning of their life cycle the Yukon plants develop more like the arctic populations and the Norway plants like the alpine populations, while at the end of their life cycle they form a category on their own (Figure 6.4). These trends are more evident when the populations are collapsed in one of the three categories: arctic, high latitude alpine and alpine (Table 2.2). The MANOVA indicates in this case that the Stage*Population Category interaction is now significant for both vegetative and generative phenologies (Tables 6.7, 6.8) and the differentiation among these categories is more marked (Figures 6.5, 6.6). The three-way within-subjects interaction term provides further evidence for greater differentiation among populations in generative compared to vegetative phenology: differences in stage timing expressed between the two chamber environments are strongly dependent on population for generative (Table 6.6), but not for vegetative (Table 6.5) phenology.

Table 6.4. Analysis of variance for vegetative, reproductive and life-history traits of six populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions (trial 2). No false rejections were found through calculation of false discovery rate (at α=0.05) for multiple comparisons (see section 6.2.3).

Source	*df*	MS	*F*	*P*
Vegetative traits				
Plant mass:				
Chamber	1	189.74	115.07	<.0001
Population	5	52.06	31.57	<.0001
Chamber*Population	5	4.78	2.89	0.01
Height:				
Chamber	1	69.31	7.03	0.008
Population	5	864.81	87.76	<.0001
Chamber*Population	5	52.72	5.34	<.0001
Branch length:				
Chamber	1	799.72	86.79	<.0001
Population	5	468.73	50.87	<.0001
Chamber*Population	5	95.90	10.40	<.0001
Average leaf length:				
Chamber	1	28.09	40.17	<.0001
Population	5	54.47	77.90	<.0001
Chamber*Population	5	1.41	2.01	0.07
Number of leaves:				
Chamber	1	5995.66	3.36	0.06
Population	5	2066.43	1.15	0.32
Chamber*Population	5	1584.25	0.88	0.48
Reproductive traits				
Total seed mass:				
Chamber	1	830.33	157.06	<.0001
Population	5	174.46	33.00	<.0001
Chamber*Population	5	43.90	8.30	<.0001

Table 6.4. continued

Source	*df*	MS	*F*	*P*
Number of seeds:				
Chamber	1	9289.34	203.06	<.0001
Population	5	1664.77	36.39	<.0001
Chamber*Population	5	786.28	17.18	<.0001
Seeds/infructescence:				
Chamber	1	490.34	75.09	<.0001
Population	5	372.57	57.06	<.0001
Chamber*Population	5	26.52	4.06	0.0012
Infructescences/individ.:				
Chamber	1	117.05	126.35	<.0001
Population	5	29.22	31.54	<.0001
Chamber*Population	5	8.57	9.25	<.0001
Average mass of a seed:				
Chamber	1	0.01	0.51	0.47
Population	5	1.63	83.82	<.0001
Chamber*Population	5	0.05	2.47	0.03
Life-history traits				
Total aboveground mass:				
Chamber	1	1813.92	153.85	<.0001
Population	5	405.59	34.40	<.0001
Chamber*Population	5	62.50	5.30	<.0001
Reproductive allocation:				
Chamber	1	1.15	30.60	<.0001
Population	5	0.74	19.87	<.0001
Chamber*Population	5	0.43	11.47	<.0001

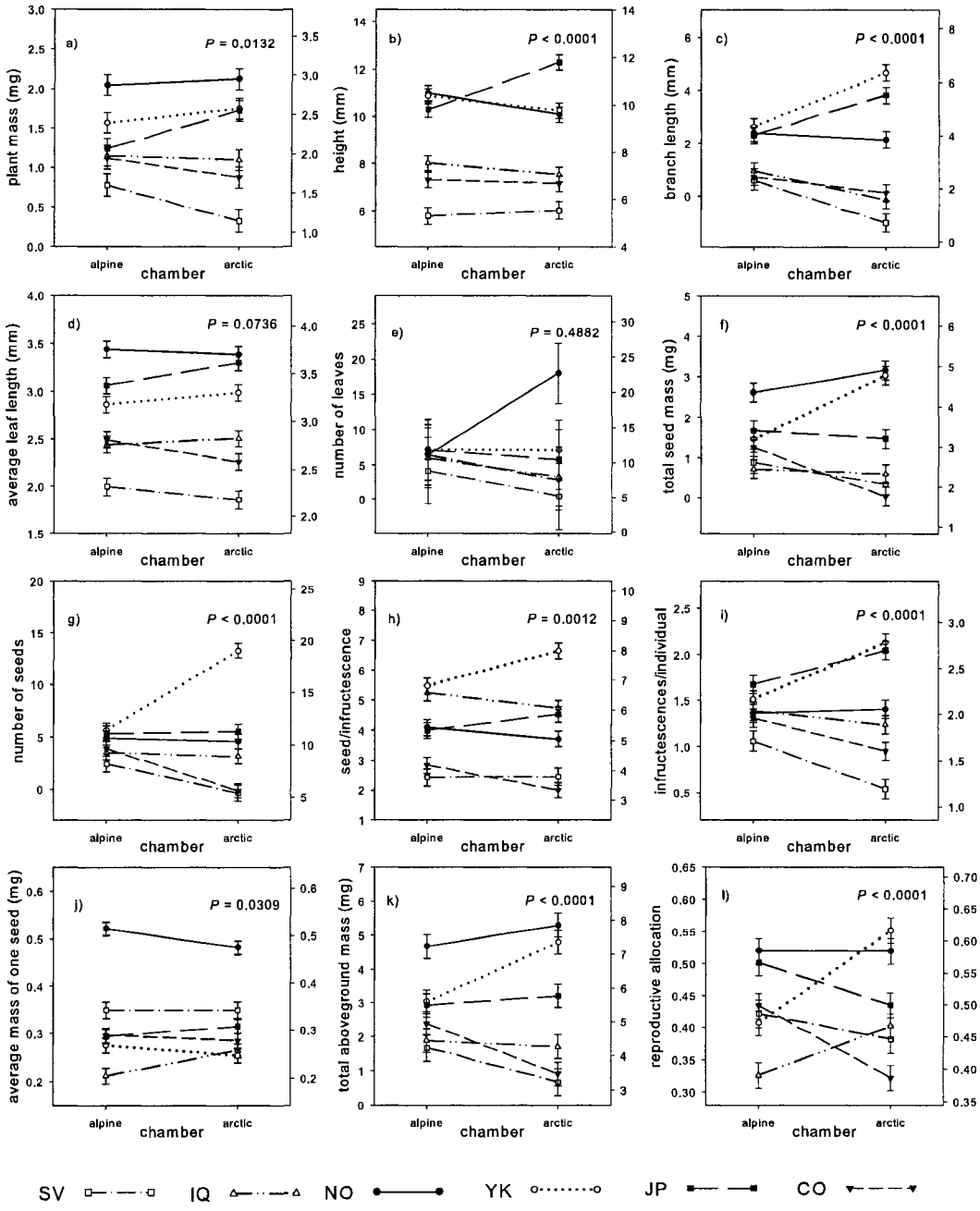

Figure 6.2. Vegetative (a–e), reproductive (f–j) and life-history traits (k–l) of six populations of *Koenigia islandica* in reciprocal simulated alpine and arctic conditions. Trial 2. SV (dash-dotted line)—Svalbard; IQ (dash-dot-dotted line)—Iqaluit; NO (solid line)—Norway; YK (dotted line)—Yukon; JP (long dashed line)—Jasper; CO (short dashed line)—Colorado. To represent Chamber*Population interactions rather than simple differences in trait means across environments, means for each trait are shifted to coincide along the two y-axes (see Methods). Left y-axis is for alpine chamber values; right y-axis for arctic chamber values. P-values are for Chamber*Population interaction. No false rejections (type-I errors) at α=0.05 were detected after controlling for false discovery rate. Error bars indicate ±1SD.

Table 6.5. Multivariate analysis of variance for repeated measures for vegetative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions. Stages 0 and 6 (Table 6.1) were not included in the analysis.

Source	Value	Exact F	*df*	DenDF	Prob>F
Between Subjects:					
Chamber	F=0.07	2.81	1	42	0.10
Population	F=0.36	5.04	3	42	0.004
Chamber*Population	F=0.03	0.45	3	42	0.72
Within Subjects:					
Stage	F=155.24	679.18	8	35	<.0001
Stage*Chamber	F=0.69	3.04	8	35	0.01
Stage*Population	Wilks' λ=0.40	1.56	24	102.11	0.065
Stage*Chamber*Population	Wilks' λ=0.50	1.15	24	102.11	0.31

Table 6.6. Multivariate analysis of variance for repeated measures for generative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions. Stages 0, 2 and 5 (Table 6.1) were not included in the analysis.

Source	Value	Exact F	*df*	DenDF	Prob>F
Between Subjects:					
Chamber	F=0.02	0.54	1	30	0.466
Population	F=0.41	6.09	2	30	0.006
Chamber*Population	F=0.05	0.79	2	30	0.46
Within Subjects:					
Stage	F=200.40	687.09	7	24	<.0001
Stage*Chamber	F=0.71	2.45	7	24	0.048
Stage*Population	Wilks' λ=0.09	7.86	14	48	<.0001
Stage*Chamber*Population	Wilks' λ=0.36	2.30	14	48	0.016

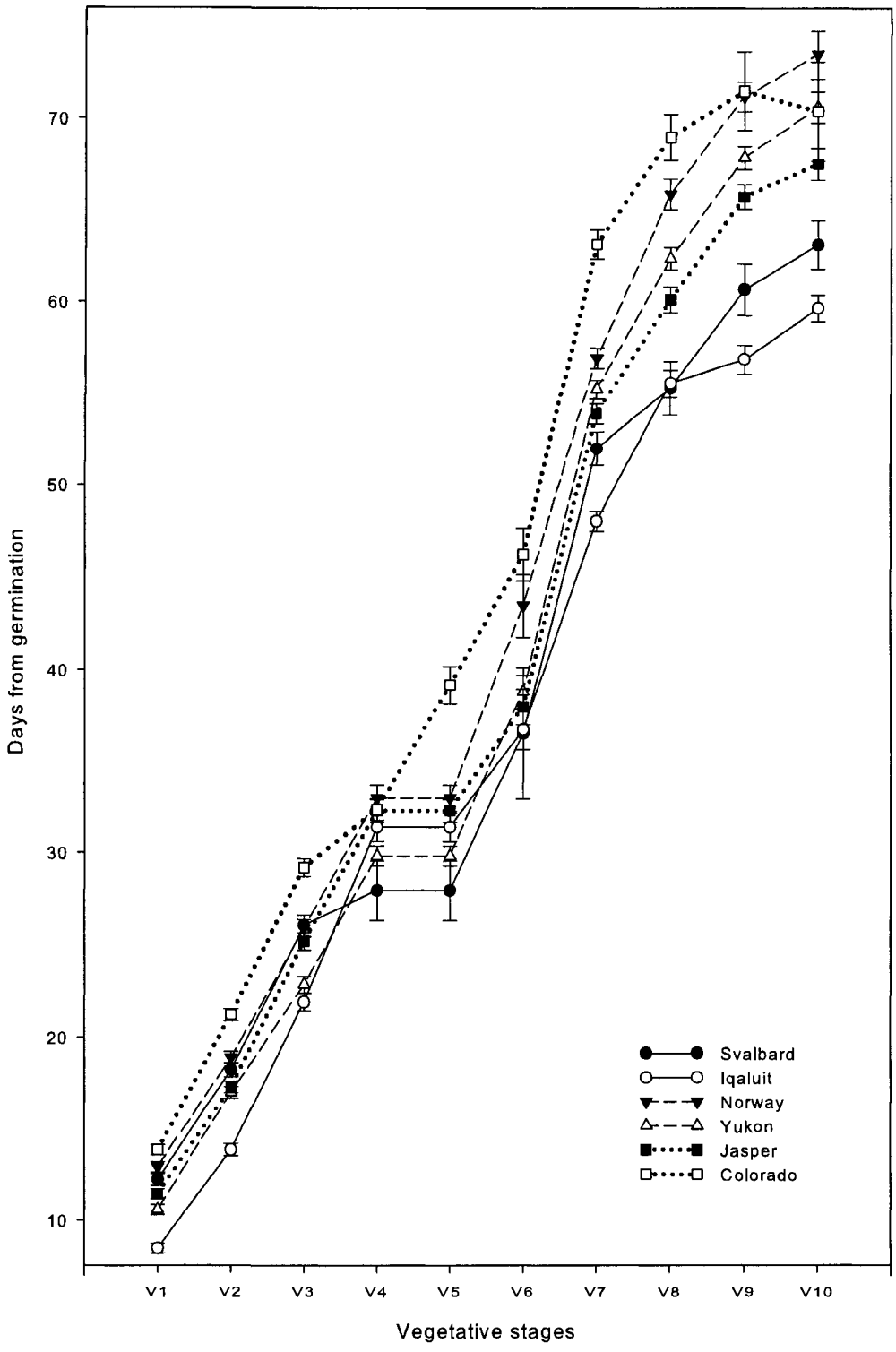

Figure 6.3. Vegetative phenology of six populations of *Koenigia islandica*. Mean timing of vegetative phenological stages (Table 6.1) of plants grown in simulated alpine and arctic conditions are represented.

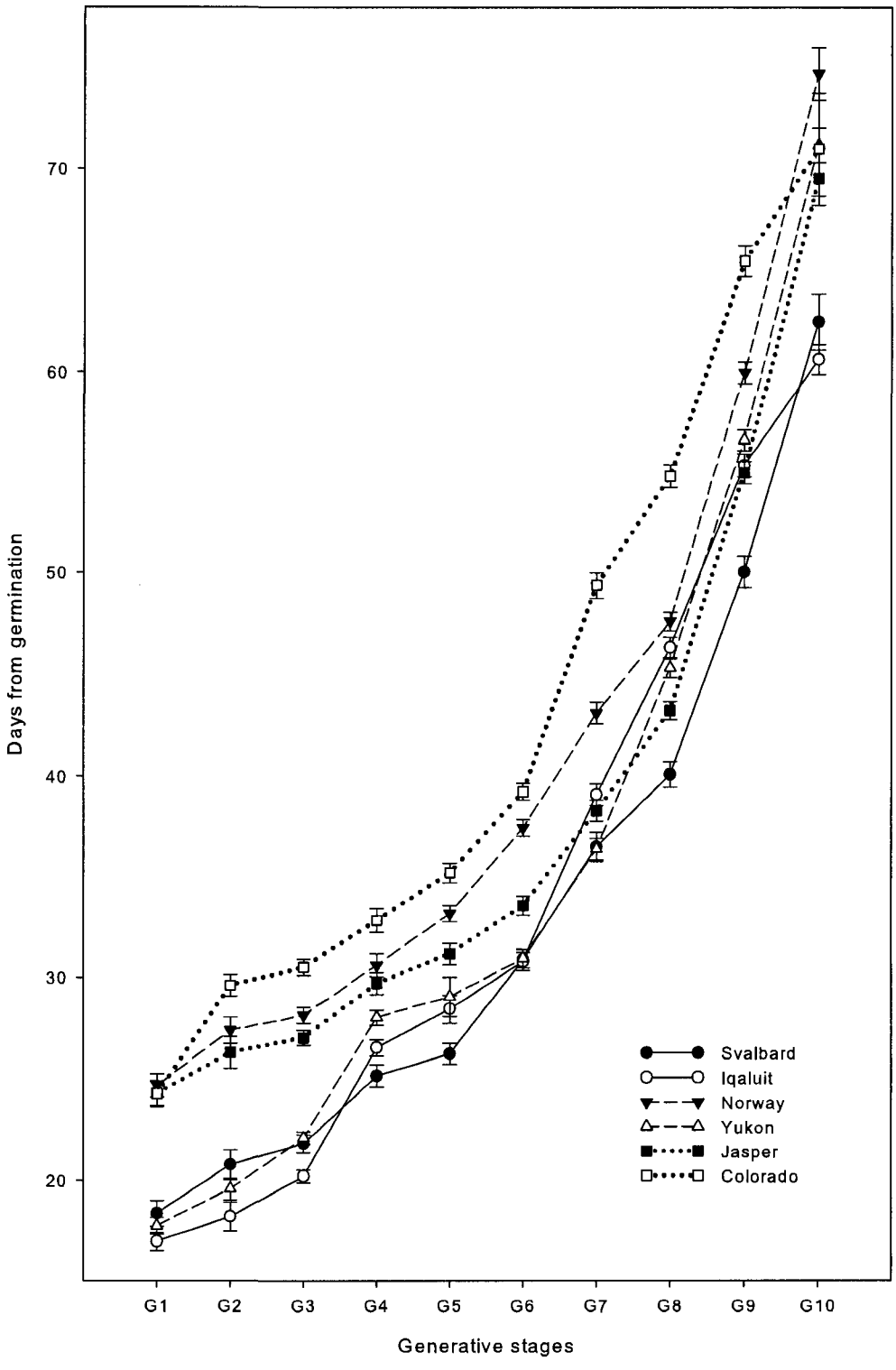

Figure 6.4. Generative phenology of six populations of *Koenigia islandica*. Mean timing of generative phenological stages (Table 6.1) of plants grown in simulated alpine and arctic conditions are represented.

Table 6.7. Multivariate analysis of variance for repeated measures for vegetative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions. Stages 0 and 6 (Table 6.1) were not included in the analysis.

Source	Value	Exact F	*df*	DenDF	Prob>F
Between Subjects:					
Chamber	F=0.03	1.37	1	44	0.25
Population Category	F=0.32	7.08	2	44	0.002
Chamber*Population Category	F=0.01	0.28	2	44	0.76
Within Subjects:					
Stage	F=141.76	655.63	8	37	<.0001
Stage*Chamber	F=0.50	2.32	8	37	0.04
Stage*Population Category	Wilks' λ=0.51	1.88	16	74	0.04
Stage*Chamber*Population Category	Wilks' λ=0.63	1.18	16	74	0.30

Table 6.8. Multivariate analysis of variance for repeated measures for generative phenological stages of six populations of *Koenigia islandica* in reciprocal simulated arctic and alpine conditions). Stages 0, 2 and 5 (Table 6.1) were not included in the analysis.

Source	Value	Exact F	*df*	DenDF	Prob>F
Between Subjects:					
Chamber	F=0.02	0.54	1	30	0.47
Population Category	F=0.02	0.54	1	30	0.47
Chamber*Population Category	F=0.05	0.79	2	30	0.46
Within Subjects:					
Stage	F=200.40	687.09	7	24	<.0001
Stage*Chamber	F=0.71	2.45	7	24	0.05
Stage*Population Category	Wilks' λ=0.09	7.86	14	48	<.0001
Stage*Chamber*Population Category	Wilks' λ=0.36	2.30	14	48	0.02

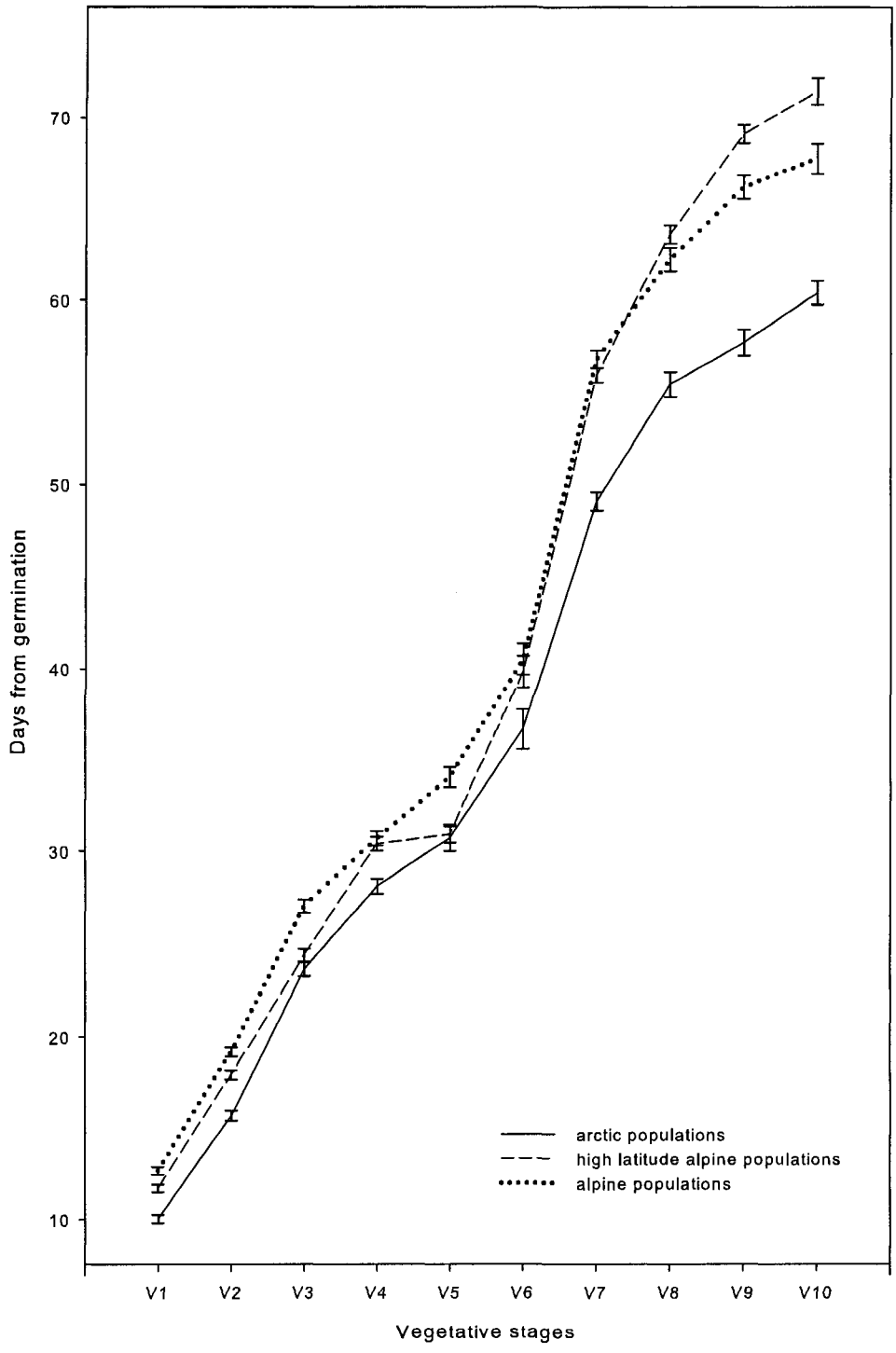

Figure 6.5. Vegetative phenology of six populations of *Koenigia islandica* grouped into one of three population categories: arctic, high latitude alpine and alpine. Mean timing of vegetative phenological stages (Table 6.1) of plants grown in simulated alpine and arctic conditions are represented.

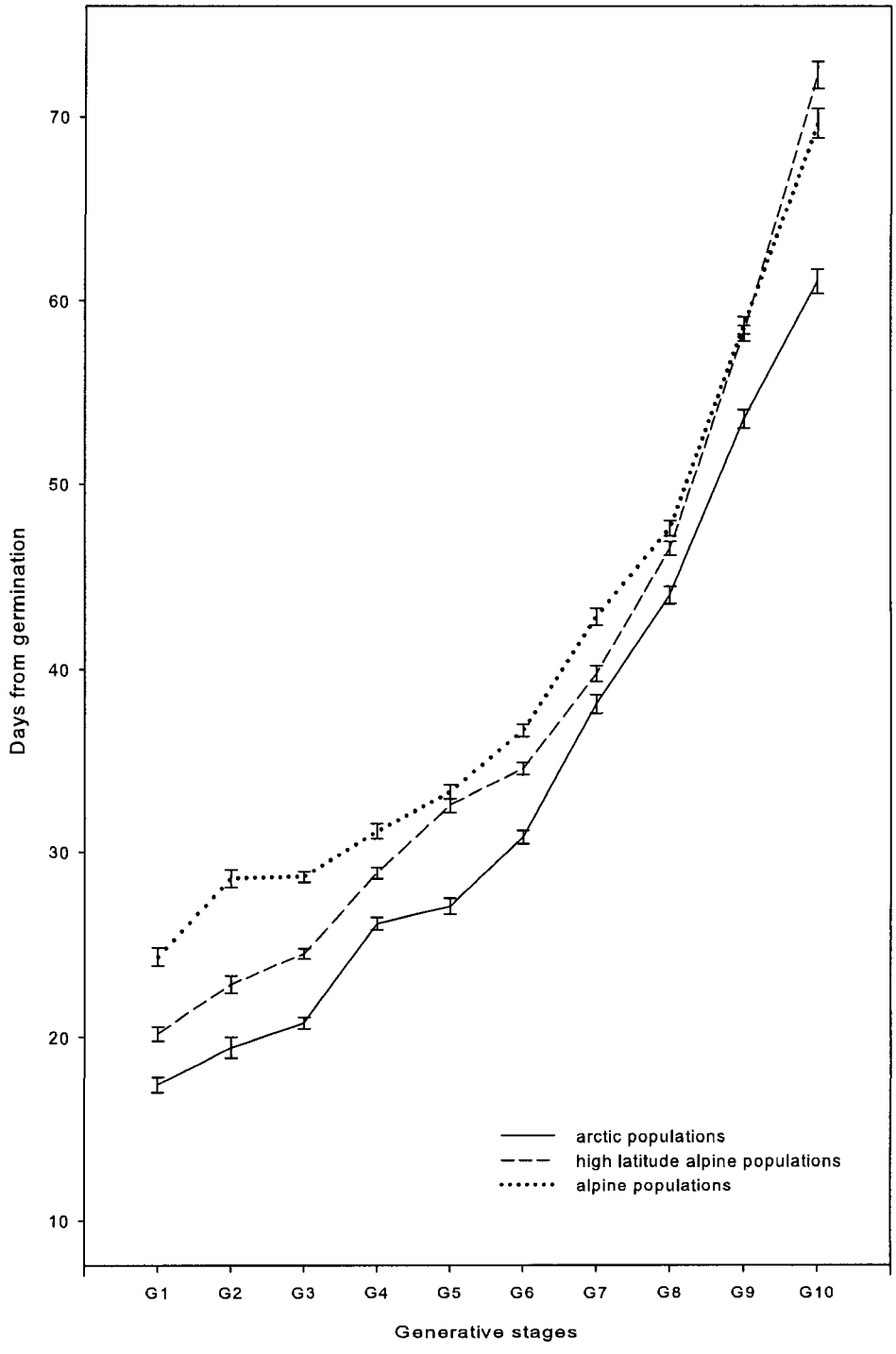

Figure 6.6. Generative phenology of six populations of *Koenigia islandica* grouped into one of three population categories: arctic, high latitude alpine and alpine. Mean timing of generative phenological stages (Table 6.1) of plants grown in simulated alpine and arctic conditions are represented.

6.4. Discussion

Annual plant species in arctic and alpine environments are subjected to particularly strong selective pressures from abiotic factors (Billings, 1974), and are therefore expected to differentiate into marked ecological races. In this set of experiments we test for genetically based differentiation in morphology, phenology and life-history traits in the six arctic and alpine populations of *Koenigia islandica*, originating from sites spread over a wide range of latitudes and elevations, with a correspondingly great variety of climatic conditions (Table 2.2).

The results of the experiments performed in the simulated arctic and alpine environments suggest significant differentiation among populations. The use of material obtained in the laboratory, in which population positions were randomized during development, suggests that the differences are ecotypic rather than due to phenotypic plasticity generated by maternal or other environmental effects. The ecotypic nature of these dissimilarities is reinforced also by the comparatively minor differences observed among subpopulations of the Colorado population (Table 6.3).

Important differences were observed among populations, regardless of the experimental environment during development. For example, the larger average mass per seed of the Norway population appears to be a population-specific trait (Figures 6.1, 6.2), and smaller overall sizes and lower seed production seem to be characteristics of the plants from Svalbard, Iqaluit and Colorado (Figure 6.1). The populations from Svalbard and Iqaluit are arctic, the northernmost among those studied, and their small sizes confirm the general finding that ecotypes originating from higher latitudes or from harsher environments show smaller stature and decreased fecundity relative

to more southern populations, or those from milder climates (Mooney and Billings, 1961; Hickman, 1975; Potvin, 1986; Gurevitch, 1992a; Winn and Gross, 1993; Li et al., 1998; Heide, 2005). This is apparently contradicted by the growth exhibited by the Colorado population. However, while this population is the southernmost in this study and among the most southern populations in the global distribution of *Koenigia islandica*, it also originates from the environment with one of the highest elevations and harshest climate, with some of the lowest growing season temperatures (Table 2.2).

It is the relative performance of populations growing in the contrasting chamber environments, given by the Chamber*Population interaction, that provides information on local adaptation. In both trials, three populations from northern latitudes—Iqaluit, Norway and Yukon—grew better in the environment more closely simulating their environment of origin, i.e. the arctic chamber, while the performance of the low latitude alpine population from Colorado was enhanced relative to other populations when grown under alpine conditions (Figures 6.1, 6.2). This result is consistent with local adaptation and the general observation that populations in reciprocal transplants do better in conditions closer to their environment of origin (Waser and Price, 1985; Potvin, 1986; Galen et al., 1991; Van Tienderen and Van der Toorn, 1991a; Joshi et al., 2001; Macel et al., 2007). However, important exceptions to expected trends are the Svalbard and the Jasper populations (Figure 6.2). The negative slopes of reaction norms across alpine to arctic chambers for both vegetative mass and total seed mass of plants from Svalbard (Figure 6.2) indicate that, relative to other populations, they did not thrive under the continuous photoperiod and cooler temperatures of the simulated arctic environment; a most surprising outcome considering the high arctic origin of this population. These growth patterns, as well as most morphological

traits of the Svalbard plants seem to be very similar to those of the Colorado population. Intriguing similarities between these two geographically remote populations were observed also in seed germination patterns (see Chapter 4): both required high germination temperatures and germinated slowly and incompletely, regardless of stratification treatment.

Also contrary to predictions is the finding that growth of the plants of the alpine population from Jasper was promoted, relative to other populations, in the arctic chamber (Figure 6.2). This may be partially explained by the higher latitude of the Jasper population relative to that of Colorado. Still, individuals from Jasper accumulated a significantly higher biomass under the continuous photoperiod of this environment, a photoperiodic effect normally encountered in high latitude plants (Heide, 1982) and confirmed here in the more northern Iqaluit, Norway and Yukon populations.

The cases in which observed growth patterns do not fit predictions require further exploration. The similar growth of the Svalbard and Colorado plants in both chambers lend support to the finding that ecotypes from harsh environments show relatively small stature due to lower growth rates, regardless of growth environment (Chapin and Chapin, 1981; Potvin, 1986; Winn and Gross, 1993; Li et al., 1998). Also, the simulated arctic and alpine environments used in our experiments are only crude approximations of the complex climates experienced by *Koenigia islandica* in each location, which makes the classification in arctic and alpine sites imprecise and difficult. Moreover, the similarities in growth patterns between some populations seem to be more related to their geographic proximity, and therefore probable phylogeographic relationships, than to the particular climate they experience. This may be the case for the Yukon and Jasper populations (Figure 6.2), a

hypothesis that is consistent with observations that their seed germination patterns are also similar (see Chapters 4 and 5).

Reproductive allocation in plant species is usually constant within a population (Hickman, 1977), with the few examples of environmentally-cued reproductive strategies found as adaptive responses to the short-term unpredictability of the environments in which the populations grow (Hickman, 1975). In the case of *Koenigia islandica*, reproductive allocation is variable among populations (Tables 6.2, 6.4), and seems to be plastic within some populations; the plants from Norway in the first trial, and the plants from Iqaluit and Yukon in the second trial invested more in reproduction relative to other populations in the simulated arctic environment. This strategy, however, seems to be inconsistent between the trials. The populations with the least growth—Svalbard, Iqaluit and Colorado—also had the smallest reproductive allocation (Figures 6.1, 6.2). These observations contradict the findings that populations from harsher, more northern environments have greater reproductive allocations (Potvin, 1986), or in the case of plastic reproductive strategies, that populations invest more in reproduction when growing in harsher environment (Hickman, 1975). However, in these experiments, mean growth was lower in the alpine chamber rather than in the arctic chamber, a situation that may not be reflected in natural environments.

Consistent with previous research (Heide and Gauslaa, 1999), *Koenigia islandica* was found to be completely day-length neutral in flowering requirements; both low and high latitude populations flowered completely and abundantly in both arctic and alpine environments. This is in agreement with the global distribution of this species; its probable origin in low latitude alpine environments of the Himalayas (Hedberg, 1997), from where it spread into the arctic regions (Mesícek and Soják, 1973; P. Hollingsworth, personal

communication) and into the mountain ranges of the rest of the northern hemisphere. Its day-length neutrality also permitted it to cross the short-day regions of equatorial mountains by mountain-hopping (Heide, 2002), and establish itself in southernmost South America.

The significant differences in both vegetative and generative phenologies (Tables 6.5, 6.6) seem to be more closely correlated with the arctic or alpine origin of the populations than are the growth characteristics. The succession of vegetative and generative phenological stages appeared to be clinally delayed from the most northern to the most southern populations. The early development and flowering of the high arctic Svalbard and arctic Iqaluit populations, and late development and flowering of the alpine Colorado population (Fig. 6.3, 6.4) is consistent with the results of other studies, in which more northern populations developed and flowered earlier (Chapin and Chapin, 1981; Potvin, 1986; Winn and Gross, 1993; Li et al., 1998). This tendency is evident also when the days to the occurrence of vegetative and generative phenological stages are plotted for the environmental category of each population (Fig. 6.5, 6.6). The rapid development and early flowering of the arctic populations could be an adaptation to the shortness and increased unpredictability of the growing season (Simons and Johnston, 2003) in these northern environments. By flowering soon after the first leaves are produced, these populations could take advantage and yield a few seeds even in the shortest and most unpredictable growing seasons (Cohen, 1971). Remarkably, the Colorado population, which showed similar growth to the arctic populations from Iqaluit and especially Svalbard, had a markedly dissimilar phenology compared to these arctic populations (Fig. 6.3, 6.4). In fact, this low latitude alpine population had the latest flowering and the longest life cycle of all analysed populations. We speculate that this could be an adaptation to a

longer and more predictable, even if relatively cold, growing season, as well as the relatively short photoperiod typical of these southern latitudes.

The results of this study provide evidence for ecotypic differentiation and some evidence of local adaptation among arctic and alpine populations of *Koenigia islandica* in morphology, phenology and life-history traits. Because of the complex nature of environmental differences among the native sites of the investigated populations, however, it is difficult to unambiguously asses the adaptive significance of these differences. Growth and phenological data from many populations, correlated with molecular evidence on the phylogeographical relations among these populations might further clarify the complex interactions between the observed ecotypes and their environment.

CHAPTER 7

GENERAL DISCUSSION

7.1. The general significance of arctic and alpine habitats

Arctic and alpine tundra environments are among the most demanding terrestrial habitats, especially for vegetal life (see Section 1.2). As such, they provide an opportunity to contribute to the general understanding of evolutionary response to abiotic factors. The winter, with various degrees of severity, lasts here for most of the year, and in the short summer, temperatures remain lower than in any other biome (see Section 2.2). Plants are poikilotherms (Moynihan et al., 1995; Fujiwara, 2003), and therefore have to stay dormant over the long arctic and alpine winter. In order to take advantage of the remaining part of the year, when conditions are more favourable for growth and reproduction, plants in tundra environments have special and unique adaptations (see Section 1.2). It is believed that the main adaptive strategies of plants to arctic and alpine environments are perenniality and clonal reproduction; most arctic and alpine plant species (up to 99%; Billings and Mooney, 1968) are long-lived perennials that reproduce mainly by rhizomes and bulbils and may require several summers for seed maturation and production (see Sections 1.2 and 1.3). In contrast, as much as 62% of the plant species in some warm desert habitats are ephemeral annuals (Venable et al., 1993). I argued (see Section 1.3) that this makes the very few arctic and alpine annual plants, which manage to complete their whole life cycle from germination to seed production in the short, cool and sometimes unpredictable growing season, truly remarkable examples of adaptation to extreme environments.

The work in this thesis exploits the fact that arctic and alpine habitats are not uniform. Their environmental characteristics vary largely (see Section 1.4) and plant species growing in both arctic and alpine regions are expected to be adapted to these environmental differences (see Section 1.5). Because

of the additional constraints they have in tundra environments (see Section 1.3), arctic and alpine annuals are expected to be subjected to stronger selective pressures than perennials and therefore are predicted to be more locally adapted to the environmental differences between arctic and alpine environments. Besides its theoretical importance as an evolutionary example of adaptation to harsh environments, the evidence of population divergence of annuals in dissimilar environments also offers a tool in the study of the effects of environmental change.

7.2. Main contributions to understanding

Studies on intraspecific variability as a response to dissimilar environmental characteristics in arctic-alpine annuals are lacking. Most studies on among-population divergence in perennials (Potvin, 1986; Meyer et al., 1989; Meyer et al., 1990; Winn and Gross, 1993; Schütz and Milberg, 1997; Santamaria et al., 2003; Vandvik and Vange, 2003; Macel et al., 2007; see also citations in Section 1.6), and annuals (Nagy and Rice, 1997), are from temperate regions. Studies on arctic and alpine plants have found that plants inhabiting both environments adapt mostly by evolving ecological races rather than generalist strategies. These studies have been done exclusively on perennials, and on only a few species (Mooney and Billings, 1961; Mooney and Johnson, 1965; Billings et al., 1971; Chapin and Chapin, 1981; Heide, 2005; see also citations in Sections 1.5 and 1.6). The few studies on intraspecific differences in annuals investigated populations along a latitudinal gradient, but not from arctic and alpine environments (Li et al., 1998), or examined populations from high elevation, but not tundra habitats (Hickman, 1975). The few studies on arctic and alpine annuals—which found prominent adaptations of these species to their severe tundra environments (see Section

1.6)—investigated either interspecific differences (Reynolds, 1984a, 1984b), or studied a single population (Löve and Sarkar, 1957b; Heide and Gauslaa, 1999). The present study thus represents an advance in our understanding in that it is the first to investigate ecotypic differentiation among populations of an arctic-alpine species from a wide range of latitudes.

Divergence among the six arctic and alpine populations of *Koenigia islandica* were found in virtually every morphological and life-history trait investigated (see Chapters 4–6). The observed differences were maintained through at least one generation grown in the laboratory, which indicates that these are evolved differences. This general conclusion is further supported by the fact that subpopulations of particular populations differed to a considerably lesser extent than did populations (see Chapters 3 and 6).

The goal here is not only to summarize the interpopulation differences found in the numerous traits, but to provide an interpretation of the differences in suites or combinations of traits among populations.

Seed germination responses to factors such as temperature (Chapter 4), light (Chapter 5) and wet-cold stratification (Chapters 3, 4 and 5) are vastly different among six populations of *K. islandica* (Table 7.1). Seed scarification is an exception in that it promotes germination of every investigated population (see Chapter 3), but in the case of the Colorado population seems to be the only treatment that ensures a high seed germination percentage at suboptimal temperatures, below 27°C (Chapters 3 and 4). This might be an adaptation of the Colorado population to its gravelly, rocky habitat (Figure 1.2); the seeds in the Colorado sites might be subjected to a natural scarification process (Chapter 3).

Table 7.1. General comparison of germination, performance and phenology of six populations of *Koenigia islandica*. The relative strength of positive effects (+), negative effects (-) and no effects (0) of conditions on traits are indicated by the number of symbols used. This is meant as a coarse overview only; for more specific details of differences in components of germination, morphology, life histories and phenology, see Chapters 3 through 6.

Trait	Treatment	Svalbard	Iqaluit	Norway	Yukon	Jasper	Colorado
Germination	Unstratified temperature range (°C)	2 (narrow)	6 (interm.)	21 (very broad)	6 (interm.)	6 (interm.)	2 (narrow)
	Effect of stratification	0	++	-----	+++	++	0
	Effect of light (stratified seeds)	+++	0	+++	0	0	+++
	Effect of light (unstratified seeds)	+++	+	+	+	0	+++
Relative performance	Arctic conditions	0	++	++	++	++	--
	Alpine conditions	0	-	-	-	-	++
Phenology	Arctic & alpine conditions	early	early	interm.	interm.	late	late

The germination requirement of untreated seeds (the laboratory-equivalents of freshly matured seeds shed late in the same growing season in the field) and stratified seeds (corresponding to seeds which spent the winter months under snow cover) are highly divergent (Table 7.1). Considering the germination responses to all of the factors (Table 7.1), distinct germination "syndromes" can be described among the six *K. islandica* populations. A cautious germination strategy of conditionally dormant seeds exists in the Svalbard and Colorado populations, which germinate slowly and in high percentage only in a narrow range of warm temperatures and in light—stratification has effect neither on the germination temperature range, nor on light requirements. The Iqaluit, Yukon and Jasper populations are characterized by a spring germinator strategy typical of summer annuals, with stratification significantly enhancing seed germination and possibly lowering light requirements—although this effect was not apparent at the high temperatures at which light requirements were tested. Finally, a strategy resembling that of autumn germinators is observed in the Norway population. This last syndrome is similar to patterns observed in winter annuals and consists of high light and germination temperature requirements induced by stratification in seeds that would otherwise germinate also at lower temperatures and no light; these are most unexpected germination responses in an arctic-alpine annual.

Surprisingly, the observed germination syndromes are not consistent with the arctic or alpine origin of the investigated *K. islandica* populations. This observation is consistent with the difficulties presented in Section 2.2 in classifying the climates and environments of the sites of origin of the studied populations simply as arctic and alpine, and reflects perhaps the subtle and gradual differences in environmental characteristics along the range of these sites.

The high arctic Svalbard population and the low latitude alpine population from Colorado have similar germination responses, despite being from opposite extremes of tundra environments. The arctic population from Iqaluit, the high latitude alpine population from Yukon and the alpine population from Jasper also show similar patterns, whereas the high latitude alpine population from Norway is highly divergent in germination responses to any other population (Chapters 3–5).

The seed germination patterns seem to be more related to the geographic proximity of the populations (cf. germination responses of Yukon and Jasper populations, and also Montana population in Reynolds, 1984a), as well as to similarities in environmental characteristics of their sites of origin (Table 2.2). Although the exact adaptive nature of this differentiation remains tentative until specifically tested, it appears that the observed germination patterns are adaptive responses to local environmental conditions. The two populations with a cautious germination pattern, Svalbard and Colorado, are from the most severe environments among those studied (see Section 2.2); this is consistent with the finding that seeds from higher elevations and colder climates germinate slowly and at high temperatures (Meyer et al., 1989; Cavieres and Arroyo, 2000; see also Chapters 3 and 4), through a diversification bet-hedging strategy (Simons and Johnston, 2006) as a response to the severity and unpredictability of the climate. Seeds of populations from sites with unpredictable winters, such as the Yukon population—this location records the highest extreme maximum winter temperature among the studied sites (Table 2.2)—are dormant and germinate poorly unless stratified (Table 7.1); this appears to be an adaptation to the high incidence of freeze-thaw events, partially shared also by the Jasper and Iqaluit populations (see Chapters 3 and 4). As for the highly divergent germination patterns of the Norway population—

which germinates readily in autumn conditions, and its light and temperature requirements are increased by stratification (Table 7.1)—these appear to relate to the mild climate in southern Norway, the mildest among the studied sites (Table 2.2). Although previously reported (Milberg and Andersson, 1998), such fundamental differences in germination patterns among populations of the same species are unusual. It seems also unlikely that seedlings of the Norway population, germinated in the autumn, would survive more than 200 days under the snow cover (Table 2.2), and resume their growth and development the following spring. Field investigation might shed light on this issue.

The divergence patterns observed in seed germination among the six populations of *K. islandica* were partially found also in morphological, reproductive and life-history traits (Table 7.1; Chapter 6). Both high arctic Svalbard and low latitude alpine Colorado plants had similar growth patterns and morphological traits, such as smaller overall sizes and lower seed production. This can be attributed again to similarities in environmental characteristics of their sites of origin; ecotypes from harsher climates tend to have smaller statures and decreased fecundity (see citations in Section 6.4). Also, the alpine Jasper population and the high latitude alpine population from Yukon had similar growth and reproductive patterns (Table 7.1); this might be caused by their geographical relative proximity and the similarities in climate.

The arctic or alpine derivation and the latitude of origin of the six analysed *K. islandica* populations is reflected in a greater extent in morphological and life-history traits than in germination patterns (Table 7.1). With some exceptions, the populations grew better in conditions that more closely simulated their environment of origin (Table 7.1; Chapter 6). This indicates local adaptation as well; in reciprocal transplant experiments, populations are consistently found to grow better in conditions closer to their

environment of origin (see citations in Section 6.4).

The significant divergence patterns observed in vegetative and generative phenology among the six populations of *K. islandica* can be closely correlated with their arctic or alpine origin (Table 7.1; Chapter 6). This pattern, following a latitudinal gradient—the vegetative and generative phenological stages were clinally delayed from the northernmost to the southernmost population (Table 7.1)—is in contrast with the complex differentiation patterns observed in germination, growth and life-history characters. This could be explained by the fact that photoperiod is the main environmental factor influencing phenology, and photoperiod depends solely on latitude, being unrelated to elevation or local climate particularities. Remarkably, the Colorado and Svalbard populations, similar in germination, growth, reproductive and life-history patterns, were markedly dissimilar in phenology (Table 7.1). This could be an adaptation not only to the vastly dissimilar photoperiods, but also to differences in length and predictability of the growing season; short and unpredictable in Svalbard, longer and more predictable, even if relatively cold, in the Colorado Front Range.

The results of this research provide evidence for differentiation in ecological races, as well as indications of local adaptation among the six arctic and alpine populations of *Koenigia islandica* in germination, morphology, phenology and life-history traits. Many questions, however, remain unanswered. Many aspects of this species—especially pertaining to its ecophysiology and population dynamics— have not yet been investigated, and many new questions arose during the course of this study. These questions merit attention in future research on this remarkable arctic-alpine annual.

7.3. Future directions

This study's aim was to assess the existence of, and identify the quality of differences among arctic-alpine populations. However, the complex nature of environmental differences among arctic and alpine locations makes the unambiguous assessment of the adaptive significance of observed differences difficult. Taking into study many more populations from a broad range of arctic and alpine environments, and correlating germination, morphology and life-history data with molecular evidence on the phylogeographical relations among the populations would further clarify the complex interactions between ecological races and their environment. This is being attempted in an ongoing study on the molecular phylogeography of several *Koenigia islandica* populations from a wide range of arctic and alpine locations, including the six populations investigated in the present research. Preliminary results on phylogeography indicate, for example, that the Colorado population has, besides common haplotypes shared with other populations, also a unique haplotype, found only in this population (J. Squirrell and P. Hollingsworth, personal communication).

The exact relation between separate environmental components, such as photoperiod and temperature, and growth, phenology and physiology of *K. islandica* is still unclear. Experiments carried out in an environmental chamber which allows compartmentalisation of environmental conditions—a phytotron—would allow subjecting *K. islandica* populations to a range of many different photoperiods and temperatures. This would allow the separation of temperature and daylength effects and their interactions—which is not possible in an experimental set-up where temperature and photoperiod are varied simultaneously, and would allow a more precise pinpointing of the

adaptive nature of the observed differences (Chapter 6).

The small size and the morphology of *K. islandica* precluded the investigation with conventional laboratory equipment of possible differences in rates of photosynthesis and respiration, as well as in adaptations to concentrations of metabolic gases among the studied populations (see Section 1.7). The construction for this purpose of a custom-made system for gas exchange measurements would help in shedding light on these important aspects of adaptation of *K. islandica* populations to their environment.

The analysis of UV-absorbing compounds (flavonoids) of leaf tissues (Rau and Hofmann, 1996; Van de Staaij et al., 1997), and measurements of UV-transmittance of living epidermal strips (Robberecht and Caldwell, 1978; Robberecht et al., 1980), of plants grown in the laboratory with and without addition of UV radiation supplied by UV lamps might reveal differences between arctic and alpine populations in the ability to screen out harmful UV-B radiation. Investigations on flavonoids would require laboratory equipment for biochemical analyses and, for measurements of epidermal UV-transmittance, an integrating sphere, especially designed to accommodate small epidermal tissue samples, would be needed (Robberecht et al., 1980). Although a sophisticated and expensive piece of equipment, results would have a straightforward adaptive interpretation.

Furthermore, field experiments and observations carried out in sites of origin of *K. islandica* populations might help in elucidating many puzzling questions that arose during the present study, such as those related to population dynamics and seed banks of this species. Longer term observations, with more precise instruments on environmental conditions at soil level among *K. islandica* plants—where temperatures and air humidity are expected to differ significantly from conditions measured higher from the soil level (see

Dahl, 1963; and also Chapter 4)—might shed more light on the germination ecology of this species. Observations in the field might clarify also the life-cycle of plants from Norway; the germination requirements would suggest an autumn-germinator strategy for this population. Field investigations on seed banks of the *K. islandica* populations might confirm the predictions arising from some of the observed germination requirements (see Chapters 4 and 5); although previous research found only a small seed bank for an alpine population of *K. islandica* (Reynolds, 1984b), germination patterns recorded in this study would hint on fairly large seed banks for at least some of the investigated populations, such as Svalbard, Colorado and Norway.

Finally, investigations on interpopulation differences could be extended to other arctic and alpine annual species. Examples of other annuals with a wide circumpolar distribution are *Montia fontana* L. (also with southern hemisphere populations) and *Lomatogonium rotatum* (L.) Fries. (which can be also a biennial). This latter species was also initially a focus of the present study. None of the attempts at growing this species in the laboratory, however, were successful, and more research on its requirements would be needed before this species could be successfully studied.

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