Longevity of arctic and alpine clonal plants

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"One of the great biological principles is that the development, adaptation, and survival of living organisms are the results of closing down options. The genome represents the impractical unedited totality of what the organism is capable of. Successful organisms do more than possess, express, and pass on the right genes - they refrain from expressing inappropriate potential. Music provides an analogy. Western music uses the 12 notes of the chromatic scale. Imagine sitting at the keyboard of an organ. Simultaneously hold down the 12 keys corresponding to the chromatic scale. Cease playing 3 minutes later. Within that cacophonous 3 minute block of sound are all possible 3-minute musical works. But "Tea for two" (Tatum 1933) is 3 minutes of musical genius, and why? Overwhelmingly, because of the notes that were not played. So it is with living organisms - the genome is the chromatic scale, the surviving organism is the harmonised musical line. The selectivity that orchestrates expression of genomic potential comprises cellular processes that repress and destroy. Might it not be that ageing is the long-term revelation of these negative, but nonetheless essential, forces that animate the machinery of living matter?"

Howard Thomas (2003) Do green plants age, and if so, how?

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Contents

Chapter 1

General Introduction

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Introduction

Arctic and alpine regions, characterized by cold climates, are expected to be vulnerable to climate warming and land use change. Therefore, arctic-alpine habitats are the focus of numerous research projects trying to assess past vegetation patterns and to predict future vegetation changes and ecosystem responses to environmental change (e.g. Arft et al. 1999, Walther et al. 2002, Grabherr & Nagy 2003, Thuiller et al. 2005, Pearman et al. 2008, Thuiller et al. 2008, Randin et al. 2009, Scherrer & Körner 2010). In these habitats, vegetative reproduction and slow growth enable most plants to survive long periods of time, in which harsh environmental conditions hamper or prohibit sexual reproduction (Schröter 1926, Billings & Mooney 1968, Bliss 1971, Callaghan 1988, Sonesson & Callaghan 1991, Körner 2003). Consequently, arctic and alpine plants tend to be perennial and long-lived (Hartmann 1957, Klimeş et al. 1997). Based on the discovery of extremely old plants and the presence of 'remnant populations' that were found to persist despite negative growth rates, extended longevity of plants is believed to enlarge persistence of populations and to have a positive relationship with ecosystem functioning (Eriksson 1996, Weiher et al. 1999, Eriksson 2000). If the longevity of plants can positively affect community stability and ecosystem resilience, it has the potential to prevent major vegetation changes to present and future global change (Steinger et al. 1996, Grabherr & Nagy 2003, Guisan & Thuiller 2005, García et al. 2008, Morris et al. 2008). Community stability is meant here as the persistence of a species assemblage, in which the relative abundance of the species may fluctuate but species do not become extinct (Begon et al. 1990, Grimm & Wissel 1997). Ecosystem resilience comprises resistance of a community towards adverse influences and particularly the ability to regenerate rapidly from disturbances (Harrison 1979).

Longevity of plants is of high biological interest, because it is a key trait for understanding life history, population dynamics and evolutionary fitness (Harper 1977, Silvertown 1991, Silvertown & Lovett Doust 1993). However, lifespan is one of the least accessible traits in plants, especially when they reproduce mainly vegetatively. Moreover, the lifespan of a genet, which comprises the entire vegetative offspring of a sexually produced zygote, often is independent from the lifespan of its ramets, the vegetatively produced plant parts. Therefore, direct age estimates like annual growth ring analysis can only be used to estimate the age of ramets, which may only form part of the genet in many naturally fragmenting clonal plants such as *Picea mariana* (Legère & Payette 1981, Laberge et al. 2000).

A few years ago, indirect age estimates based on genet size and annual horizontal growth became feasible due to the development of highly polymorphic molecular fingerprint markers that can properly distinguish genets within a population, if error probabilities during genotype assignment are critically evaluated (Bonin et al. 2007). Using molecular fingerprint studies, genet size structure can be detected within dense homogeneous populations of clonal plants that dominate arctic-alpine vegetation (Pornon et al. 2000). The annual horizontal growth rates used to calculate genet age, however, usually receive little attention and sometimes they are simply obtained from literature. Therefore, the variability of genet size increments within populations and differences among regions and years are yet unknown for most long-lived arctic-alpine clonal species.

In plants in which size is not related to age, indirect methods using genet size are not suitable and alternative estimators for genet lifespan, for example somatic mutations (Heinze & Fussi 2008) have to be considered. Another promising approach to estimate lifespan or population age distribution is the use of stage- or size-classified matrix population models (Cochran & Ellner 1992, Barot et al. 2002). The transition probabilities in such matrix models can additionally be used to estimate demographic properties of long-lived plants that are also important for analyses of population persistence and evolutionary fitness (Callaghan

1976, Molau 1997, Erschbamer & Winkler 2005, Diemer 2002, Nicolè et al. 2005, Weppler et al. 2006).

It may be assumed that populations of long-lived clonal plants are able to persist locally despite past, present and future climate changes, but we actually know very little about the longevity of clonal plants. Clearly, there is a need to investigate the lifespan of clonal plants and the persistence of their populations. Moreover, methods to estimate lifespan in clonal plants require improvements, especially for those plants, which are strongly fragmented or in which genet age by far surpasses ramet age. If plants and populations are found to have survived past climate changes that are of the same magnitude as expected climate warming, we may better predict their future fate. Finally, genet lifespan, population age structure and dynamics among species and geographic regions need to be compared. This will allow to properly assess the ability of long-lived clonal plants to positively influence ecosystem functioning, and to predict the resilience of arctic-alpine vegetation to future climate change.

The aim of this thesis

This thesis aimed to study the longevity of arctic and alpine clonal plants with an extended focus on population persistence, community stability and ecosystem resilience under future climate change. This work was part of the European Commission's FP6 ECOCHANGE project "Challenges in assessing and forecasting biodiversity and ecosystem changes in Europe", which tries to improve predictions of species distribution patterns and ecosystem responses to climate warming. The main research questions of this thesis were:

(i) Why is longevity of clonal plants important and how can we measure it? In particular, what is the quality of the methods used to measure age in clonal plants, which loose their main root or become fragmented? (Chapter 2)

(ii) Will arctic-alpine clonal plant populations persist despite future climate change? In particular, how long-lived are clonal plants, which dominate late-successional vegetation in arctic-alpine regions? And what is their resilience to future climate change? (Chapter 3)

(iii) How is annual horizontal growth, in this study used to estimate the lifespan of several clonal plants, influenced by successional stage and climatic variability through time and space? (Chapter 4)

(iv) What is the longevity and population age structure of a clonal pioneer species that lives on glacier forelands and exhibits an expansive growth strategy? (Chapter 5)

Experimental approach

To measure longevity in arctic-alpine plant species dominating large parts of late-successional vegetation, indirect lifespan estimation based on genet size and annual horizontal growth was applied (Chapter 3). The species *Carex curvula*, *Dryas octopetala*, *Salix herbacea*, *Vaccinium uliginosum* and *Empetrum nigrum* were chosen according to the following criteria: (i) important and dominant species in arctic and alpine ecosystems; (ii) existence of large, homogeneous populations; (iii) phalanx strategy with horizontal and centrifugal growth and thus suitable for indirect age estimates based on genet size and size increment data. In order to compare size and age structure of clonal plant populations in geographically distant regions, a standardized sampling design by selecting four populations for each species in two different regions was applied: two populations in the Alps and two in the Romanian Carpathians for *Carex curvula* and *Dryas octopetala*; two populations in the Alps and two in Fennoscandia (northern Norway and northern Sweden) for *Salix herbacea*, *Vaccinium uliginosum* and *Empetrum nigrum*.

Genets and their size were identified using the molecular fingerprinting technique "Amplified Fragment Length Polymorphism" (AFLP), which is a highly sensitive method for detecting polymorphisms in DNA. This method enabled to assign every collected sample to a genet and then to calculate its size based on the fixed sampling distance between them. The AFLP method yielded not enough fingerprint markers for the genotype assignment in the species *Empetrum nigrum*, probably due to the secondary metabolites produced in the leaf tissue. Therefore, genet size and age structure could not be

investigated in this species. Based on genet size and the in situ annual horizontal growth measurements, a minimum and maximum genet age was then calculated.

The annual horizontal growth data was statistically analyzed in order to investigate differences among species, between pioneer and climax sites, between the two subsequent years, as well as between the distant geographical regions (chapter 4). Additionally, these differences were compared to the soil temperature and season length measured in climax populations.

In the clonal pioneer species *Geum reptans* living on glacier forelands, genet lifespan was estimated using a projection matrix model based on demographic field data of ramets collected at two sites in three subsequent years (Weppler et al. 2006, chapter 5). Genet age structure at different population ages was calculated by multiple simulations, which included a maximum carrying capacity and density dependent mortality. Additionally, the age of the two field populations was calculated by comparing results from simulations with the population structure observed in the field.

Outline

Chapters 2, 3, 4 and 5 are written for publication in peer-reviewed scientific journals. Below, a short outline of each chapter is given. Co-authorship and the reference are indicated therein.

Chapter 2:
Longevity of clonal plants: why it matters and how to measure it
L.C. de Witte & J. Stöcklin
Annals of Botany 106: 859–870, 2010

This chapter critically reviews present knowledge on the longevity of clonal plants and on the various methods used to measure plant lifespan. The background for this chapter is that species' life history and population dynamics are strongly shaped by the lifespan of genets. However, genet lifespan remains relatively poorly known, because it is one of the least accessible demographic traits, particularly in clonal plants, which can lose their main stem or root and can get strongly fragmented. Therefore, especially indirect methods using genet size, demographic approaches and somatic mutations are addressed. Finally, the functional significance of plant longevity for population persistence and community stability under changing climates is discussed.

Chapter 3:
AFLP markers reveal high clonal diversity and extreme longevity in four arctic-alpine key species
L.C. de Witte, G.F.J. Armbruster, L. Gielly, P. Taberlet & J. Stöcklin
Molecular Ecology 21: 1081–1097, 2012

This chapter contains the investigation of genet diversity, genet size structure and longevity of late-successional arctic-alpine plants in order to evaluate their persistence under past climate oscillations and their resilience to future climate change. A standardized sampling design was applied for a comparative study of four homogeneous climax populations of the four key species *Carex curvula*, *Dryas octopetala*, *Salix herbacea* and *Vaccinium uliginosum* that dominate arctic-alpine vegetation in the Alps, the Carpathians and Fennoscandia. Genet age was indirectly estimated by dividing genet size, identified by molecular fingerprint markers (AFLP), by a mean annual size increment from in situ growth measurements in all four populations. Results are used to discuss the effect of clonality and longevity of arctic-alpine plants on population persistence and ecosystem resilience. Furthermore, the implications for models of future species distributions and vegetation patterns are considered.

Chapter 4:
Horizontal growth in arctic-alpine clonal plants is not affected by climatic variability among regions
L.C. de Witte & J. Stöcklin
Plant Ecology & Diversity 4(4): 329–340, 2011

This chapter presents the results from the in situ measurements of annual horizontal growth in five arctic-alpine species. Such data are essential to investigate life-history and population parameters in long-lived clonal species, but are

still scarce. Field measurements of annual horizontal growth in the long-lived clonal plant species *Carex curvula*, *Dryas octopetala*, *Salix herbacea*, *Vaccinium uliginosum* and *Empetrum nigrum* growing in three arctic and alpine regions of Europe were compared and statistically analysed to study the differences between species, successional stages, years and between distant geographical regions. The differences in growth were also compared to the climatic variability. The results indicate that horizontal growth in arctic-alpine clonal plants may not be strongly affected by differences in climate and thus by a warmer climate in the future.

Chapter 5:
Genet longevity and population age structure of the clonal pioneer species *Geum reptans* based on demographic field data and projection matrix modelling
L.C. de Witte, D. Scherrer & J. Stöcklin
Preslia 83: 371–386, 2011

In this manuscript the focus is again on the estimation of genet longevity and population age structure, this time of a clonal alpine pioneer species, using a projection matrix approach. Its inter-ramet connections are short-lived and its ramets move independently in space by an expansive growth strategy. For the lifespan estimation, the projection matrix model was based on demographic field data of ramets collected at two sites in three subsequent years. Population age structure was then calculated at different population ages by multiple simulations, including a maximum carrying capacity and density dependent mortality. Additionally, the age of the two field populations was estimated by comparing results from simulations with the population structure observed in the field.

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Chapter 2

Longevity of clonal plants: why it matters and how to measure it

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REVIEW

Longevity of clonal plants: why it matters and how to measure it

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• *Background* Species' life-history and population dynamics are strongly shaped by the longevity of individuals, but life span is one of the least accessible demographic traits, particularly in clonal plants. Continuous vegetative reproduction of genets enables persistence despite low or no sexual reproduction, affecting genet turnover rates and population stability. Therefore, the longevity of clonal plants is of considerable biological interest, but remains relatively poorly known.
• *Scope* Here, we critically review the present knowledge on the longevity of clonal plants and discuss its importance for population persistence. Direct life-span measurements such as growth-ring analysis in woody plants are relatively easy to take, although, for many clonal plants, these methods are not adequate due to the variable growth pattern of ramets and difficult genet identification. Recently, indirect methods have been introduced in which genet size and annual shoot increments are used to estimate genet age. These methods, often based on molecular techniques, allow the investigation of genet size and age structure of whole populations, a crucial issue for understanding their viability and persistence. However, indirect estimates of clonal longevity are impeded because the process of ageing in clonal plants is still poorly understood and because their size and age are not always well correlated. Alternative estimators for genet life span such as somatic mutations have recently been suggested.
• *Conclusions* Empirical knowledge on the longevity of clonal species has increased considerably in the last few years. Maximum age estimates are an indicator of population persistence, but are not sufficient to evaluate turnover rates and the ability of long-lived clonal plants to enhance community stability and ecosystem resilience. In order to understand the dynamics of populations it will be necessary to measure genet size and age structure, not only life spans of single individuals, and to use such data for modelling of genet dynamics.

Key words: Age, community stability, genet size, global change, life history, population dynamics, somatic mutation, vegetative reproduction.

INTRODUCTION

The life span of plants, as in any other organism, is a key demographic trait for understanding life history (Weiher *et al.*, 1999), population dynamics (Harper, 1977; Silvertown and Lovett Doust, 1993) and evolutionary fitness (Silvertown, 1991). Extended longevity of plants is believed to enlarge persistence of populations and thus affects community stability and vegetation responses to present and future climate change (Steinger *et al.*, 1996; Eriksson, 2000; Körner, 2003; García *et al.*, 2008; Morris *et al.*, 2008). Unfortunately, there are few reliable data on genet longevity and genet turnover rates in plants, because these are difficult to measure (Dietz and Schweingruber, 2002). Known maximum longevity ranges from a few weeks in annuals (e.g. Bliss, 1971; Sharitz and McCormick, 1973) to thousands of years in some clonal herbs and trees (Table 1; e.g. Wherry, 1972; Lynch *et al.*, 1998; Brundu *et al.*, 2008). This wide variation seems to be due to trade-offs between life span and other fitness traits and because the modular construction of plants and their indeterminate growth counteract intrinsic senescence. The broad range in longevity also implies that there are considerable differences in the timescale of population dynamics and in the selective forces acting on individual plants.

In clonal plants, temporal gaps between years with successful sexual recruitment were found to be highly variable in length, from zero to thousands of years (Eriksson, 1989). For example, in high alpine meadows, sexual reproduction can be hampered due to a lack of pollinators or from low temperatures that inhibit seed germination. In such habitats, clonality can enhance genet longevity considerably, it can compensate for the partial loss of genets due to disturbance, and thereby it can secure population persistence for long periods of time. In general, clonal reproduction allows plants to benefit from a potential two-fold fitness, persistence of the product of a single zygote plus repeated economical offspring production (Aarssen, 2008).

Persistent clonal reproduction of an individual not only enhances longevity, but it can also lead to genets inhabiting large areas, because clonal plants have a pronounced capacity to spread horizontally (Stöcklin, 1992; Herben and Hara, 1997; Hutchings and Wijesinghe, 1997). Therefore, many studies use size and annual size increments of a genet to measure its age (e.g. Vasek, 1980; Steinger *et al.*, 1996; Reusch *et al.*, 1998; Wesche *et al.*, 2005), although size and age are not always linearly correlated. It is important to note that longevity of a genet is independent of ramet life span, and thus the spatial structure of all ramets belonging to the same genet is only an incomplete mirror of the life history of the entire genet. When genets become fragmented and when annual growth increments indicate high interannual variability, the relationship between size and age becomes particularly weak.

TABLE 1. *Size (usually diameter) and longevity (in years) of clonal plants from the literature, separated into trees, shrubs, herbs, grasses, other species, and with an indication of the method used for size or age determination*

(a) Clonal trees

	Method to estimate the size of the clone	Size of clone [diameter (m, or as indicated)]	Estimated age of oldest genet (years)	Estimated age of youngest genet (years)	Reference(s)
Olea europaea subsp. *laperrinei*	Molecular markers	80 m^2 +	1000 +	–	Baali-Cherif and Besnard (2005)
Picea abies	Radiocarbon dating	–	10 000 –12 000	–	Kullman (2008)
Picea mariana	Morphological and growth ring analysis, statistical analysis	14	300	–	Legère and Payette (1981)
	Molecular markers and dendrochronological analysis	691·3 m^2	1800 +	100	Laberge *et al.* (2000)
Pinus longaeva	Growth ring analysis	–	4900	–	Schulman (1958), Johnson and Johnson (1978), Brown (1996)
Populus alba	Molecular markers	–	>12 000	–	Brundu *et al.* (2008)
Populus tremuloides	Morphological analysis, aerial photographs	510	10 000 +	–	Kemperman and Barnes (1976)
	Microsatellite divergence based on mutation accumulation	–	12 000	14	Ally *et al.* (2008)
Populus tremula	Molecular markers	16	152	2	Suvanto and Latva-Karjanmaa (2005)
Ulmus procera	Molecular markers and microsatellite divergence based on mutation accumulation	–	2000	–	Gil *et al.* (2004)

(b) Clonal shrubs

	Method to estimate the size of the clone	Size of genet [diameter (m, or as indicated)]	Annual growth rate (cm $year^{-1}$)	Estimated age of oldest genet (years)	Estimated age of youngest genet (years)	Reference(s)
Arctostaphylos alpina	Growth ring analysis	–	–	93	–	Schweingruber and Poschlod (2005)
Calluna vulgaris	Growth ring analysis	–	–	58	–	Mork (1946)
Dryas octopetala	Growth ring analysis	–	–	108	–	Kihlman (1890)
Empetrum nigrum ssp. *nigrum*	Growth ring analysis	–	–	140	–	Bell and Tallis (1973)
Erica carnea	Growth ring analysis	–	–	82	–	Schweingruber and Poschlod (2005)
Juniperus sabina	Growth ring analysis	–	–	67–70	–	Molisch (1929)
	Molecular markers	795 m^2	1·8–6·8	770–2940	–	Wesche *et al.* (2005)
Larrea tridentata	Molecular markers, growth rings, radiocarbon dating	16·6	–	11 700	–	Vasek (1980)
	Growth rings, radiocarbon dating	11	–	9170	–	Vasek (1980)
Loiseleuria procumbens	Growth ring analysis	–	–	110	–	Schweingruber and Poschlod (2005)
Lomatia tasmanica	Molecular markers, chromosome counts and radiocarbon dating	1200	–	43 600	–	Lynch *et al.* (1998)
Rhododendron ferrugineum	Growth ring analysis	–	–	202	–	Schweingruber and Poschlod (2005)
	Molecular markers	20 m^2	2·6	300	–	Escaravage *et al.* (1998)
	Molecular markers	25 m^2	115	283 +	28	Pornon *et al.* (2000)
Salix arctica	Growth ring analysis	–	–	150	–	Kraus (1873)
Vaccinium vitis-idaea	Growth ring analysis	–	–	109	–	Callaghan (1973)

(c) Clonal herbs (except grasses and sedges)

	Method to estimate the size of the genet	Size of genet [diameter (m, or as indicated)]	Annual growth rate (cm year^{-1})	Estimated age of oldest genet (years)	Reference
Acantholimon diapensoides	?	–	–	400	Agakhanyantz and Lopatin (1978)
Anemone nemorosa	Growth ring analysis	–	–	>5	Shirreffs (1985)
	Molecular markers	12	1·9–3·1	190–320	Stehlik and Holderegger (2000)
Calamagrostis epigejos	Comparative analysis of site history and genet size	50	–	400	Oinonen (1969)
Convallaria majalis	Comparative analysis of site history and genet size	850	–	670 +	Oinonen (1969)
Cypripedium calceolus	Molecular markers	39 ramets	1–1·5	370	Brzosko *et al.* (2002)
Gaylusaccia brachycerium	Morphological analysis	1980	–	13 000 +	Wherry (1972)
Silene acaulis	Growth ring analysis	–	–	252	McCarthy (1992)
	Modelling: size-based population projection matrices	>0·2	–	300 +	Morris and Doak (1998)
Teucrium scorodonia	Morphological analysis	Several square metres	–	50–100	Hutchinson (1968)
Trifolium alpinum	Growth ring analysis	–	–	50	Schweingruber and Poschlod (2005)

(d) Clonal grasses and sedges

	Method to estimate the size of the genet	Size of genet [diameter (m, or as indicated)]	Annual growth rate (cm year^{-1})	Estimated age of oldest genet (years)	Reference
Calamagrostis epigejos	Comparative analysis of site history and genet size	50	–	400 +	Oinonen (1969)
Carex curvula	Molecular markers	1·6	0·04	2000	Steinger *et al.* (1996)
Carex ensifolia ssp. *arctisibirica*	Molecular markers	40	–	3800 +	Jónsdóttir *et al.* (2000)
Carex stans	Molecular markers	7·4	–	Approx. 150	Jónsdóttir *et al.* (2000)
Festuca ovina	Morphological analysis, cross-pollination tests	8·25	0·3	1000 +	Harberd (1962)
Festuca rubra	Morphological analysis, cross-pollination tests	220	22·9	1000 +	Harberd (1961)
Holcus mollis	Morphological and phenological analysis, chromosome analysis	880	–	1000 +	Harberd (1967)
Sasa senanensis	Molecular markers	300	Approx. 100	Several decades	Suyama *et al.* (2000)
Stipa pennata	Calendar age determination (Gatsuk *et al.*, 1980)	–	–	75	Vorontzova and Zaugolnova (1985)

(e) Clonal pteridophytes and marine species

	Method to estimate the size of the genet	Size of genet [diameter (m, or as indicated)]	Annual growth rate (cm year^{-1})	Estimated age of oldest genet (years)	Reference
Lycopodium annotinum	Comparative analysis of site history and genet size	Up to 250	–	250	Oinonen (1967)
	Morphological analysis	–	–	21	Callaghan (1980)
	Molecular markers	36	20	90 +	Wittig *et al.* (2007)
Lycopodium comoplanatum	Comparative analysis of site history and genet size	250	–	850	Oinonen (1969)
Pteridium aquilinum	Comparative analysis of site history and genet size	489	–	1400	Oinonen (1967)
	Molecular markers	1015	43	1180	Parks and Werth (1993)
Zostera marina	Molecular markers	33	13	134	Reusch *et al.* (1998)

Methods include growth ring analysis, morphological analysis, radiocarbon dating, comparative analysis of site history, molecular markers and microsatellite divergence (see text for more explanation).

With this in mind, genet age seems to be difficult to measure, even when the spatial extension of a genet is known. Nevertheless, there have been many attempts to measure maximum longevity in clonal plants, either for curiosity or because it can serve as an indicator of population persistence.

Currently, there is considerable effort to find alternative methods to estimate longevity that are not based on genet size. For example, molecular divergence based on somatic mutations and cell-growth estimates (Ally *et al.*, 2008) or the proportion of ramets to genets (variation due to somatic mutation vs. recombination; Mock *et al.*, 2008) are being used. Also stage-based population or transition-matrix models can be useful tools to investigate life history, dynamics and individual longevity (Ehrlén and Lehtilä, 2002).

Here we critically review the present knowledge on genet longevity in clonal plants, which ranges from a few months up to several thousand years. We summarize and discuss the methods that have been used to estimate genet age and we examine their suitability. A comprehensive overview of published life-span data for clonal trees, shrubs, herbs and grasses is presented in Table 1. Next to the discussion on the recent progress in genet life-span determination and its importance, we examine the literature on the topic of somatic mutations and the role of genet longevity for population dynamics and community stability.

MAXIMUM LONGEVITY OF CLONAL TREES, SHRUBS, HERBS AND GRASSES

Genet life span, a fundamental aspect for understanding life history, is one of the highly attractive but least accessible traits in plants (Dietz and Schweingruber, 2002). Measurements of life span in plants that goes beyond the simple classification into annuals, biennials and perennials is available primarily for trees, in which counting the annual growth rings is a convenient and direct way to determine age (Ehrlén and Lehtilä, 2002). With dendrochronology the 'oldest living tree' was found in Nevada, USA, a bristlecone pine (*Pinus longaeva)* about 4800 years old (Schulman, 1958; Brown, 1996; Lanner and Connor, 2001). For trees that are able to reproduce clonally, genet longevity was found to exceed the maximum age of single tree stems considerably. With dendrochronological analysis, an age of about 300 years was determined for a *Picea mariana* tree in 1981 (Legère and Payette, 1981; Table 1c). Twenty years later, using molecular markers, a genet of the same species consisting of several stems was estimated to be more than 1800 years old (Laberge *et al.*, 2000). Genets of *Populus tremuloides* were found to form large forest patches up to 80 ha based on morphological analyses and analyses of aerial photographs. From this, an estimated longevity of 10 000 years has been suggested by Kemperman and Barnes (1976). Analysis of microsatellite divergence based on mutation accumulation about 30 years later revealed an age of 12 000 years for this species (Ally *et al.*, 2008). Radiocarbon dating applied to fossil wood resulted in extreme life-span estimates for several clonal species (e.g. *Picea abies*, Kullman, 2008; *Lomatia tasmanica*, Lynch *et al.*, 1998).

Genet age of non-trees has long been ignored in the literature, for example in biological floras (but see Poschlod *et al.*, 1996). Only in the second half of the 20th century did researchers start to determine the life span of shrubs, herbs and grasses. Direct measurements of morphological structures, such as via herbchronology, usually account for maximum ages of only a few decades, for example 50 years for the clonal herb *Trifolium alpinum* (Schweingruber and Poschlod, 2005; Table 1c). With more recent methods, which will be described below, longer genet life spans have been reported in shrubs, herbs and grasses (e.g. Escaravage *et al.*, 1998; Stehlik and Holderegger, 2000; Wesche *et al.*, 2005), indicating that these life forms can reach maximum ages of one to several hundreds of years and, in some cases, thousand years (Table 1b–e). Hence, there is no indication from the available literature that genets of shrubs, herbs or grasses have potentially lower life spans than trees, but plant life forms of shrubs, herbs and grasses that can be safely attributed to a single genet are usually much younger than the massive outliving stems of trees.

Maximum age estimates may be in part a product of curiosity. Scientifically, they are an indication of the slowest possible genet turnover rate in a population. Moreover, they tell us more about adult survival relevant for an understanding of the life history and demography of a species (Silvertown *et al.*, 1993; Franco and Silvertown, 1996). However, the maximum longevity ever recorded for a species depends on the sampling effort taken and of the methods used, making it difficult to compare the data.

METHODS TO MEASURE LIFE SPAN IN PLANTS

Direct methods

The following direct methods have been used to determine the life span of clonal plants. (1) Analysis of annual growth rings, a widely used method usually applied to stems of trees, can also be applied to herbs and shrubs that have primary root systems or woody stems with visible growth rings (herbchronology; Zoller, 1949; Dietz and Ullmann, 1997; Schweingruber and Dietz, 2001; Dietz and Fattorini, 2002). Schweingruber and Poschlod (2005) determined the life span of many species with this method and included a critical evaluation of the method. Growth ring analysis is relatively quick, and makes comparisons among successional stages or ecosystems easily possible (Dietz and Ullmann, 1998; Kuen and Erschbamer, 2002; Erschbamer and Retter, 2004; Jónsson, 2004; Von Arx and Dietz, 2005; Perkins and Parks, 2006; Kuss *et al.*, 2008). With this method, for example, it was found that *Vaccinium myrtillus* ramets were significantly younger on ski pistes in the Swiss Alps than in control plots (Rixen *et al.*, 2004). (2) Radiocarbon (C^{14}) dating is usually applied to organic remains of archaeological sites (e.g. Vasek, 1980; Kullman, 2008), but is relatively expensive. These first two methods are only reliable for clonal plants when the oldest parts of the genet are still in place and can be identified. Another disadvantage is that these two methods normally result in single age estimates not useful for population demographic analysis. (3) Growth-form or phenological analysis based on annual morphological markers (e.g. Troll, 1937; Harberd, 1967; Kemperman and Barnes, 1976; Kull and Kull, 1991; García and Antor, 1995;

Jäger *et al.*, 1997) is used to study growth strategies, age-related patterns, size and age distribution or survivorship curves. By counting annual growth increments, Callaghan (1980) estimated an age of 21 years for a genet of the clonal plant *Lycopodium annotinum* (Table 1e). (4) Permanent plot research involves long-lasting research efforts, but yields highly reliable age determinations (e.g. Bärlocher *et al.*, 2000; Erschbamer and Winkler, 2005). This method is especially appropriate for use in geophytes, such as orchids, which may disappear from above ground for years (Tamm, 1948, 1956; Inghe and Tamm, 1985). (5) Age determination by colour band analysis in grasstrees allows for the reconstruction of fire history (Ward *et al.*, 2001; Colangelo *et al.*, 2002). Less known and seldomly applied methods include (6) comparative analysis of site history (Oinonen, 1967), (7) age state determination (Rabotnov, 1950; Gatsuk *et al.*, 1980; Kawano, 1985; Vorontzova and Zaugolnova, 1985) and (8) chromosome analysis (Harberd, 1967).

Only rarely has a life span longer than 200 years been found with the above listed direct methods (Table 1). The main drawback is that only surviving and connected plant structures can be measured and attributed, with certainty, to a particular genet. Therefore, direct measurements systematically underestimate the longevity of clonal plants.

Indirect estimates of age

The size or diameter of a genet can be divided by a measure of mean annual size increment (Suvanto and Latva-Karjanmaa, 2005), yielding an indirect estimate of its age. Several maximum age estimates are based on this method (e.g. Steinger *et al.*, 1996; Reusch *et al.*, 1998). Clonal plants covering large areas can intermingle with other genets and the longer they survive, the more likely they are to become fragmented or to partially die. This hampers easy recognition of entire genets by eye. To overcome these difficulties, some scientists have used genet-specific morphological markers or self-incompatibility tests to detect the size of genets and to determine their age. Harberd (1962, 1967; Table 1d), for example, reported extremely large sizes and old ages for *Festuca rubra* (diameter 220 m) and *Holcus mollis* (880 m) based on self-incompatibility tests. Barsoum *et al.* (2004) identified genets by excavation of root connections, but this method is strongly invasive and causes biases when roots graft naturally or connections are lost over time. Today, the use of DNA fingerprinting techniques, discussed further below, facilitates precise genet identification.

The accuracy of such indirect age estimates largely depends on the reliability of the annual size increment measurement. Size increments can be highly variable among individuals depending on ontogenetic development, successional stage, competitive and nutritional conditions, and environmental factors. The larger and older a genet is, the more critical it is to estimate its expansion rate over the entire life span. Age estimates are therefore generally less accurate than estimates of genet size, and also because the relationship between size and age is not always linear in clonal plants. Therefore, age estimates of genets should include such putative variation, but this is rarely the case (but see Vasek, 1980).

The use of DNA fingerprinting

Although the methods used to measure the size of clonal plants, discussed above, may be doubtful or might not recognize the total size of large genets, modern molecular analysis of leaf samples now allows for a better identification of entire genets. In recent decades, genet identity has been revealed by genetic markers such as allozymes (e.g. Stehlik and Holderegger, 2000) and DNA fingerprinting techniques such as microsatellites (e.g. Suvanto and Latva-Karjanmaa, 2005), random amplification of polymorphic DNAs (e.g. Laberge *et al.*, 2000) or amplified fragment length polymorphisms (e.g. Escaravage *et al.*, 1998). With molecular markers, individuals can be distinguished, allowing spatially explicit sampled plant material to be assigned to genets. Based on the use of a defined sampling distance, genet size can be determined and then divided by a measure of annual growth increment to obtain age information. Using DNA fingerprinting, the oldest genet occurring in a population of the alpine clonal dwarf shrub *Rhododendron ferrugineum* was estimated to be 300 years (Escaravage *et al.*, 1998; Table 1b) and a genet of the alpine grassland species *Carex curvula* was found to be an estimated 2000 years old (Steinger *et al.*, 1996; Table 1d).

The use of DNA fingerprinting techniques has the advantage that a large number of markers can be developed easily and at low cost (Jones *et al.*, 1997; Mueller and Wolfenbarger, 1999). Further advantages include the possibility to sample over large spatial scales and that it causes minimal impact on populations. Unfortunately, there is still some ambiguity associated with two types of molecular assignment errors: misidentification of genetically similar ramets as one genet and misidentification of dissimilar fingerprints as genetically distinct individuals (Widen *et al.*, 1994). Repeated samples coming from the same genet but from different ramets do not always have identical fingerprints. This may result from somatic mutations, from contamination in the laboratory, or from scoring errors that may happen during data analysis (Arens *et al.*, 1998; van der Hulst *et al.*, 2000; Douhovnikoff and Dodd, 2003). Bias introduced by scoring errors has been underestimated until recently (Pompanon *et al.*, 2005; Arnaud-Haond *et al.*, 2007; Bonin *et al.*, 2007), but it is now accepted how crucial it is to apply repeatability tests and statistical tools to critically evaluate error probability in molecular fingerprinting studies (Lasso, 2008).

In crop science, DNA fingerprinting has achieved importance because this technique is used to identify genetic relationships between cultivars and establishes pedigree reconstructions. Thereby, the life span of several cultivars was revealed, for example grapes (*Vitis vinifera*). For the clonally propagated and economically important grapevine cultivar ‘Albarino’, from north-western Spain, which is being used in a recent breeding programme, was given an estimated age of 200–300 years (Alonso *et al.*, 2007). ‘Rouge du Pays’, presently cultivated in the Valais (Switzerland), was already mentioned in a manuscript from the year 1313 (Vouillamoz *et al.*, 2003), suggesting an even longer life expectancy for grapevine cultivars.

The crop plant vanilla (*Vanilla planifolia*) is propagated only vegetatively in many areas due to a lack of pollinators. On islands in the Indian Ocean, where the plants have been

cultivated since the early 1800s, almost all accessions were found to constitute a single and probably very old genet (Bory *et al.*, 2008; Lubinsky *et al.*, 2008).

Overall, molecular size determination in clonal plants has led to better insights into population size and age structure owing to the extensive and qualitative genet detection. However, its accuracy can be impaired by genetic assignment errors due to somatic mutations and scoring mistakes. Therefore, efforts to improve the molecular assignement are necessary for reliable results.

Demographic approaches to longevity

Increasingly, studies are using size- or stage-structured matrix models to estimate demographic properties of long-lived plants (Callaghan, 1976; Erschbamer, 1994; Erschbamer and Winkler, 1995; Molau, 1997; Erschbamer *et al.*, 1998; Diemer, 2002; Nicolè *et al.*, 2005; Weppler *et al.*, 2006). Such models are usually based on ramet dynamics but are nevertheless helpful because they allow us to overcome the difficulties of the long observation periods necessary to understand population processes in clonal plants (Watkinson and Powell, 1993). Demographic data of long-lived clonal plants at the genet level are still scarce (Menges, 2000), and very few studies have used matrix models and population viability analysis techniques to investigate genet longevity and population persistence. A notable exception is the work of Colling and Matthies (2006) on *Scorzonera humilis*, which revealed low mortality of adult genets and a life expectancy of several decades.

In a few cases, the transition probabilities between plant size stages or age stages in matrix models were used to estimate life span or population age distribution (Cochran and Ellner, 1992; Barot *et al.*, 2002). For *Silene acaulis*, for example, a size-based projection-matrix model revealed a life expectancy of more than 300 years for genets (Morris and Doak, 1998). Ehrlén and Lehtilä (2002) reviewed population matrix models for 71 herbaceous perennials and calculated species life spans ranging from 4 to approx. 1000 years, whereby more than half of the studied species had a life expectancy over 35 years. Their results agree reasonably well with previously published age estimates for long-lived plant species. However, most matrix models for clonal plants used in their study (86 %) were based on ramet data. It is important to recognize that understanding the life history of clonal plants should involve investigations at the genet level, too (Harper, 1977; Cook, 1985; de Steven, 1989; Eriksson, 1993; Fair *et al.*, 1999; Tanner, 2001; Araki *et al.*, 2009), although ramet dynamics may be used as an indirect measure of genet fitness, popualation growth and persistence (Caswell, 1985; Eriksson and Jerling, 1990; Weppler *et al.*, 2006). For example, Eriksson (1994) predicted that clonal populations of *Potentilla anserina*, *Rubus saxatilis* and *Linnaea borealis* consisting of more than 250 ramets are able to persist much longer then 50 years despite a negative population growth rate.

A challenge will be to employ demographic techniques on genet data obtained by molecular genotyping studies to make more accurate predictions at the genet level. For example, in a combined demographic–molecular approach to study growth patterns, reproduction and spatial expansion at the ramet level, it was possible to reveal spatio-temporal patterns at the genet level, and thus the characteristics particularly relevant to clonal life histories and population viability (Araki *et al.*, 2009; see also de Steven, 1989; Torimaru and Tomaru, 2005).

Somatic mutations and life span measurements

The use of genetic divergence generated by somatic mutations is a novel approach to measure genet size and to estimate life span (Heinze and Fussi, 2008). It is based on the fact that constant division of mitotic cells in clonal plants leads to the accumulation of somatic mutations over time ('somatic mutation theory of clonality', Klekowski, 1997). With this method, Gil *et al.* (2004; Table 1a) were able to date the origin of an *Ulmus procera* genet back to the time of the Romans, with some of its ramets growing as far apart as in Spain and Britain. The effective vegetative propagation and the deliberate plantation of this elm variety by humans explain the large distance between its ramets. In *Populus tremuloides*, molecular divergence detected by microsatellites was related to clone age with the help of demographic models of ramet and genet dynamics (Table 1a; Ally *et al.*, 2008). The resulting age estimates were up to 12 000 years, indicating that genet size of *Populus tremuloides* actually is not related to their life span. The formation of extra petals due to somatic mutations in buttercup (*Ranunculus repens*) was the key to establish a quick method to estimate the age of meadows by Warren (2009). Based on the frequency of this phenotypic change in buttercup of pastures of known age, he established a relationship between phenotypic change and meadow age. There is a similar link between increased frequency of pollen abortion and genet age for several clonal species (Harberd, 1967; Brighton *et al.*, 1983). However, thus far, somatic mutation rates have rarely been used for life span estimates, because somatic mutations cannot yet be detected efficiently (Gil *et al.*, 2004), and because somatic mutations are difficult to distinguish from allelic variation (Heinze and Fussi, 2008). Moreover, molecular divergence, due to somatic mutations, may differ between species (Klekowski and Godfrey, 1989), among populations (Gill *et al.*, 1995) and among genets (Schaal and Leverich, 1996), probably because somatic mutations may occur in response to environmental stress. In genets of *Pinus longaeva* ranging in age from 23 to 4713 years, no age-related accumulation of somatic mutations was detected at all (Lanner and Connor, 2001), while molecular divergence was found in distinct ramets of wild olive trees about 1000 years old (Baali-Cherif and Besnard, 2005). The incertitude concerning measured rates of somatic mutations remains a main concern for the precision of indirect life span estimates.

SENESCENCE AND AGEING IN CLONAL PLANTS

Clonal plants are considered to be immortal and several extreme life spans reported seem to confirm this. Senescence, defined here as the apparent weathering or a highly regulated deteriorative process (Leopold, 1975; see also Munné-Bosch, 2008), has indeed never been observed

in several plant species (e.g. *Rhododendron ferrugineum*, Escaravage *et al.*, 1998). Thus, genets do not reach their maximum age and eventually dying parts or ramets of clonal plants are constantly replaced by new ones (Watkinson and White, 1986). Senescence is not a necessary consequence of ageing with time in plants and there are many examples of death without senescence and of senescence without death (Thomas, 2002, 2003). Ecologically interpreted, a long life span is a compensation for erratic and hazardous seed production that is common in monocarpic plants (Molisch, 1938; Grime, 2001). Clearly, in clonal plants, fitness does not only rely on sexual but also on vegetative reproduction and is further enlarged by a long life span (Eriksson, 1988; Schmid, 1990; Fagerström, 1992). Clonal fitness is best defined as the 'rate of increase of a genet' (Fagerström *et al.*, 1998) and is maximized by the combination of three possible options of a meristem: (1) to propagate vegetatively, (2) to propagate sexually or (3) to remain dormant (Fagerström, 1992). Indeed, Tanner (2001) found a positive correlation between the expected remaining life span and genet size in clonal plants. Additionally, selection can act on eventual genetic variability among the modules of a genet resulting from somatic mutations (Antolin and Strobeck, 1985; Gill *et al.*, 1995; Fagerström *et al.*, 1998; Lushai and Loxdale, 2002). At least in theory, somatic mutations could give plants the ability to adapt to changing conditions throughout their lifetime (Salomonson, 1996; Pineda-Krch and Fagerström, 1999) and could thereby positively affect longevity of clonal plants (Breese *et al.*, 1965; Breese and Hayward, 1972; Klekowski, 1997).

On the other hand, genetic deterioration is sometimes assumed to cause senescence in long-lived plants (Thomas *et al.*, 2000). In his 'somatic mutation theory of clonality', Klekowski (1997, 2003) proposed that sexual reproductive success is inversely proportional to longevity, because the increasing age of a genet will make the accumulation of deleterious somatic mutations more likely. The accumulation rate of genetic load by somatic mutations in genets is not known, but infertility caused by mutations at one or only a few loci has been found, for example, in *Decodon verticillatus* (Eckert *et al.*, 1999). Another genetic mechanism leading to such a 'sexual extinction' is a change in polyploidy (Stebbins, 1971), as seed production can covary strongly with ploidy level (see, for example, *Butomus umbellatus*, Eckert, 2002). Despite these examples, an inherent molecular process leading to the death of a genet has, so far, not yet been identified in long-lived clonal plants. The long time persistence of genets in natural clonal populations will largely depend on meristem demography (e.g. Watson and Casper, 1984).

LONGEVITY AND POPULATION PERSISTENCE OF CLONAL PLANTS

Among the many traits enhancing population persistence, longevity of genets is, by far, the most important (Weiher *et al.*, 1999). Even populations that have a negative population growth rate are able to persist for long time periods due to the slow turnover rates of genets. The low extinction probability of genets results in a high persistence of well-established populations, which is typical for most clonal plant species (Helm *et al.*, 2006). Eriksson (1996, 2000) assessed the causal relationship between long-lived remnant populations and their function within their ecosystem. He suggests that remnant populations increase community and ecosystem stability as well as ecosystem resilience. First, this is due to vegetative recruitment that can directly buffer environmental variation experienced by a clonal population. Second, community resilience is increased by the continuous maintenance of similar habitat conditions created by the populations themselves, by balanced nutrient cycling and by enhanced (re-)colonization after disturbances. This phenomenon of positive species interactions stabilizing communities is also known as facilitation, an important process in community organization (Bertness and Leonard, 1997; Bruno *et al.*, 2003).

Arctic and alpine permanent vegetation types, such as grasslands and dwarf-shrub heaths, have been found to be very stable communities that have not been affected by past and recent climate changes (Grabherr and Nagy, 2003). The main reason for this appears to be the longevity of the mainly vegetatively reproducing members of such communities, reinforcing the hypothesis that long-lived clonal plants can enhance community and ecosystem resilience, thereby slowing vegetational change as a consequence of global warming (Guisan and Thuiller, 2005). On the other hand, analysis of available observational data has also revealed range expansions for several clonal species towards higher altitudes or latitudes (Pauli *et al.*, 1996; Walther *et al.*, 2002). Plant responses to artificially applied climate change included increased flowering, increased senescence of old modules and altered internal resource ratios (Carlsson and Callaghan, 1994; Callaghan *et al.*, 1997; Grabherr *et al.*, 2000). The indirect consequences were an increased rate of genet turnover and an increase of youger age-classes, indicating changes in population dynamics and structure. But more empirical data on current changes and the potential buffering of environmental variation by clonal plants will be necessary to make safe predictions about the future fate of old clonal populations when climate change is accelerating. Moreover, there is a need for studies that investigate population demography and viability. There are many age estimates for single genets, but there is only limited information on the variability of genet size and age at the population level that can form a basis for studies on the dynamics and persistence of clonal plant populations (Pornon *et al.*, 2000; van Kleunen *et al.*, 2001; Erschbamer and Winkler, 2005; Scheepens *et al.*, 2007). Depending on competitors and the success of seedling recruitment in dense populations, genets within a clonal population can differ considerably in size or age.

The level of genetic diversity is another important issue for the population viability of clonal plants. High genetic diversity can enable adaptation to changing climates, which in turn increases the persistence of populations. Asymmetric competition among differently sized genets can result in self-thinning, diversity loss and, in extreme cases, a monoclonal stand (Harberd, 1962; 1967; Oinonen, 1967; Williams, 1975). However, because clonal plants grow horizontally rather than in height, competition among genets is often found to be symmetric and genet diversity is maintained (Soane and Watkinson, 1979; Hartnett and Bazzaz, 1985; Cain, 1990; de Kroon *et al.*,

1992; Hara, 1994; van Kleunen *et al.*, 2001). Low sexual recruitment has long been reported to be a common feature of clonal plant populations (Eriksson, 1989; Schmid, 1990), but their size and age structure seem to be strongly shaped by sexual recruitment patterns (Kudoh *et al.*, 1999; Weppler *et al.*, 2006; Stöcklin *et al.*, 2009). Molecular studies of clonal plants found on average similar high levels of genetic diversity in clonal populations as in other plant species, indicating that seed recruitment does at least occasionally occur (Nybom, 2004) and that low levels of seedling recruitment in clonal plants are compensated for by the longevity of genets. In several populations of *Rhododendron ferrugineum*, next to very large and old individuals of about 260–300 years, many small and probably young genets were found (see Table 1; Escaravage *et al.*, 1998; Pornon *et al.*, 2000). Repeated seedling recruitment was also detected in populations of *Ranunculus repens* (Soane and Watkinson, 1979), *Calystegia collina* (Wolf *et al.*, 2000), in the rare orchid species *Cypripedium calceolus* (Brzosko *et al.*, 2002; Table 1c) and in populations of *Uvularia perfoliata* (Kudoh *et al.*, 1999). Studies that combine the estimation of maximum age with an investigation of genet size and age structure and a demographic analysis of ramet growth and seedling recruitment will help us to better understand population persistence, and will allow inferences to be made about their fate.

CONCLUSIONS

Our knowledge of plant longevity remains limited, particularly for clonal species. Methods to measure clonal plant age are either not appropriate, laborious or have inherent uncertainties. However, life span estimates of genets achieved by indirect methods, demographic approaches and the use of somatic mutations have increased our empirical knowledge considerably and thereby understanding of the structural and demographic properties of clonal plant populations. Maximum age estimates range from a few to several thousands of years and are an indicator for the slowest possible genet turnover rate and for population persistence. New molecular tools, used to estimate age indirectly, allow the investigation of size and age structure of whole populations instead of single genets and also on larger scales. Plant size estimates based on molecular fingerprinting can be critically evaluated with statistical methods, improving their accuracy. Because this is less the case for estimates of annual growth increments over hundreds or even thousands of years, age estimates are generally less accurate than estimates of genet size. Nevertheless, together with information on demography at the ramet and genet level, molecular data on whole populations provide a better tool to evaluate species life history and population viability. Next to maximum longevity, genet size and age structure, demography and genet diversity will be important for predicting population persistence in clonal plants and their ability to enhance community stability and ecosystem resilience under global change.

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Chapter 3

AFLP markers reveal high clonal diversity and extreme longevity in four arctic-alpine key species

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AFLP markers reveal high clonal diversity and extreme longevity in four key arctic-alpine species

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Abstract

We investigated clonal diversity, genet size structure and genet longevity in populations of four arctic-alpine plants (*Carex curvula*, *Dryas octopetala*, *Salix herbacea* and *Vaccinium uliginosum*) to evaluate their persistence under past climatic oscillations and their potential resistance to future climate change. The size and number of genets were determined by an analysis of amplified fragment length polymorphisms and a standardized sampling design in several European arctic-alpine populations, where these species are dominant in the vegetation. Genet age was estimated by dividing the size by the annual horizontal size increment from *in situ* growth measurements. Clonal diversity was generally high but differed among species, and the frequency distribution of genet size was strongly left-skewed. The largest *C. curvula* genet had an estimated minimum age of *c.* 4100 years and a maximum age of *c.* 5000 years, although 84.8% of the genets in this species were <200 years old. The oldest genets of *D. octopetala*, *S. herbacea* and *V. uliginosum* were found to be at least 500, 450 and 1400 years old, respectively. These results indicate that individuals in the studied populations have survived pronounced climatic oscillations, including the Little Ice Age and the postindustrial warming. The presence of genets in all size classes and the dominance of presumably young individuals suggest repeated recruitment over time, a precondition for adaptation to changing environmental conditions. Together, persistence and continuous genet turnover may ensure maximum ecosystem resilience. Thus, our results indicate that long-lived clonal plants in arctic-alpine ecosystems can persist, despite considerable climatic change.

Keywords: climate change, genet size, maximum age, offspring recruitment, population persistence, spatial structure

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Introduction

Perennial plants that reproduce clonally become more abundant with increasing altitude and increasing latitude (Klimeš *et al.* 1997; Peck *et al.* 1998) and can cover large areas of late-successional vegetation, such as arctic tussock tundra, dwarf shrub heath or alpine grassland. In these vegetation types, a short growing season, cold temperatures, desiccation and low availability of mineral nutrients reduce growth and increase individual longevity (Schröter 1926; Klötzli 1991; Sonesson & Callaghan 1991; Körner 2003). For some arctic-alpine plant species, particularly long lifespans have been reported: 300 years for the alpine dwarf shrub *Rhododendron ferrugineum* (Pornon *et al.* 2000), 400 years for *Acantholimon diapensoides* in the Pamir Mountains (Agakhanyantz & Lopatin 1978), 2000 years for the high alpine sedge *Carex curvula* (Steinger *et al.* 1996), 3800 years for *Carex ensifolia* in northern Siberia (Jónsdóttir *et al.* 2000) and 2940 years for an individual of the woody shrub *Juniperus sabina* (Wesche *et al.* 2005). These age estimates suggest that individuals and populations have persisted locally, despite considerable climatic oscillations.

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Traits that allow clonal plants to survive harsh climates and changing environmental conditions include resource storage, tight regulation of resource acquisition, and cycling and maintenance of dormant buds, which enable branching following death of apical meristems (de Kroon & van Groenendael 1997). In addition, single genets may survive variable selective pressures and tolerate considerable environmental variation and disturbances (Harper 1977). As a consequence of individual longevity, populations of many clonal plants are expected to be highly persistent over time. The longevity and persistence of clonal plant populations are believed to enhance community stability and ecosystem resilience to climate change (e.g. Steinger *et al.* 1996; Grabherr & Nagy 2003; Guisan & Thuiller 2005). Here, community stability refers to the persistence of a species assemblage without substantial changes in composition. Ecosystem resilience alludes to the resistance of a community to adverse influences, particularly the ability to regenerate from disturbances induced by climate or land-use change.

Stability and resilience are thought to be high in arctic and alpine ecosystems, where more than 50% of the plant species are able to reproduce clonally (Körner 2003). Except for some age estimates of single plants, we know very little about the longevity of species that dominate arctic-alpine vegetation, and therefore how persistent their populations are. Information on the size and age structure of populations and their dynamics will allow for a better understanding of the functional role of long-lived clonal plants in cold habitats and under climate change. Longevity may depend on life-history parameters and consequently be very different among clonal plant species. Moreover, clonal diversity, genet size and age structure should be compared among different populations and geographical regions. To date, there are only a few studies comparing size, age structure and clonal diversity among populations; for example, one study on two populations of *R. ferrugineum* in the Pyrenees revealed different patterns of genet diversity and density, as well as population dynamics (Pornon *et al.* 2000), whereas another study on *Dryas octopetala* in Scandinavia showed decreasing clonal diversity but an unchanged level of clonality at increasing latitudes (Vik *et al.* 2010).

In many clonal plants, the age of individual genets is not easy to measure. For clonal species forming continuous patches, the best approach is to estimate age by dividing genet size, determined by molecular fingerprint markers, by a measure of annual size increment (de Witte & Stöcklin 2010). For instance, in a population of the subalpine shrub *R. ferrugineum*, the largest genet was found to be more than 7.6 m in diameter. This size divided by an annual horizontal growth rate of 2.7 cm resulted in an age estimate of 280 years (Pornon *et al.* 2000). Several other studies have estimated genet age based on the same calculation (e.g. Reusch *et al.* 1999; Stehlik & Holderegger 2000; Ruggiero *et al.* 2002; Wesche *et al.* 2005; van der Merwe *et al.* 2010; Takahashi *et al.* 2011; see also de Witte & Stöcklin 2010). For a long time, genetic assignments had to be taken with caution, because they were based on low numbers of polymorphic loci, leading to underestimated clonal diversity and overestimates of genet size (Bonin *et al.* 2004; Arnaud-Haond *et al.* 2005). Fortunately, genotype assignment studies have improved over the last few years, because of the critical evaluation of error probabilities in molecular marker scoring and the more sophisticated sampling designs (Arnaud-Haond *et al.* 2007; Bonin *et al.* 2007). Annual growth increment measures, however, often get much less attention and are sometimes simply replaced by literature data. Therefore, they may not account for variability over the plant's lifetime, between individuals or between different geographical locations.

We chose to investigate diversity, structure and age in arctic-alpine clonal plant populations to better understand their ecology, as well as their persistence through past climatic oscillations and their potential resistance to future climate change. To achieve this, we utilized improved molecular methods and sampling designs to compare clonal diversity, structure and age within different populations and species. Our objectives in this study were (i) to improve the methodological approach for determining clonal age estimates by systematically analysing populations of four clonal species in arctic and alpine key ecosystems, where these species are dominant; (ii) to investigate and compare the size and age of a large number of individuals within several populations, as well as their clonal diversity; and (iii) to draw conclusions about the persistence of these individuals and populations under historical cooling and warming events. Finally, we discuss the effect of clonality on ecosystem resilience and the implications for models of future species distributions and vegetation patterns.

Methods

Study species

We selected four clonal species, *C. curvula* All. ssp. *curvula* (Cyperaceae), *D. octopetala* L. (Rosaceae), *Salix herbacea* L. (Salicaceae) and *Vaccinium uliginosum* L. s. lat. (Ericaceae), according to the following criteria: (i) dominant species in arctic and alpine ecosystems; (ii) existence of large and dense populations; (iii) genets grow horizontally in all directions from the starting

point, forming round patches, and are, based on their morphology, not expected to fragment by clonal dispersal (Klimešová & Klimeš 2011; personal observation). *Carex curvula* is a dominant sedge in late-successional grassland vegetation on siliceous substrate in the Central and Eastern Alps, the Southeastern Carpathians, the Eastern Pyrenees and in some of the Balkan Mountains between 2200 and 2700 m above sea level (Schröter 1926; Grabherr 1987; Puşcaş *et al.* 2008). The perennial dwarf shrub *D. octopetala* has a circumpolar distribution in Arctic, sub-Arctic and Alpine regions of Europe, Asia and North America. It is restricted to calcareous rock, but often occurs in nearly pure stands (Hultén 1959; Elkington 1971; McGraw & Antonovics 1983). The deciduous dwarf shrub *S. herbacea* has an amphi-Atlantic but mainly Arctic range, occurring in Northern Europe, western Siberia and North America, in addition to the mountainous regions of Central Europe (Myklestad & Birks 1993; Beerling 1998). In the Alps, this species occurs between 2200 and 2800 m, mainly on snowbeds, and in the high Arctic, it is found on wet tundra soils, wind-exposed fell-fields and screes (Ellenberg 1988). The woody, deciduous dwarf shrub *V. uliginosum* occurs throughout the circum-boreal and circum-Arctic regions, as well as in many alpine areas (Alsos *et al.* 2005). This is an abundant, sometimes dominant species in boreal mires, shrub lands, forests, dry upland, arctic and alpine tundra, heaths and ridges throughout all successional stages (Schröter 1926).

Sampling strategy

To compare the size and age structure of clonal populations, sampling was carried out on a large spatial scale in the Swiss Alps, the Romanian Carpathians and Fennoscandia (northern Norway and northern Sweden). For each species, we selected four populations in two geographically distant regions, the populations within a region being separated by at least 50 km. A description of each population is given in Table 1. Sampling of plant material for DNA fingerprinting was carried out in large and dense populations on flat relief with no intervening larger patches of other species or bare ground during the summer of 2008. To identify genets and determine their diameters, plant tissue was collected on 100 sampling points within each population along two point transects crossed at right angles (Fig. 1, also see Supporting information). Based on the specific morphology of each species, we took samples at fixed distances of 30 cm for *C. curvula* and 60 cm for the other three species. For each sample, at least three leaves from a single shoot were collected, so as to have sufficient material for the blind replicates necessary for repeatability tests. These tissues were stored in tubes containing silica gel until DNA extraction. *C. curvula* leaves become senescent very early, and leaf tips often turn brown because of infection by the fungus *Clathrospora elynae* and other endophytes. Therefore, only young green leaves without any visible infection were collected. For the other species, to avoid contamination with foreign DNA, only young and healthy-looking leaves were collected. Within each population, three control samples were taken from connected ramets of three different genets at the same fixed distances that were applied on the transect (30 cm for *C. curvula* and 60 cm for the other three species). These intra-genet-variability control samples were used to determine the genetic variability between ramets of a single genet

Table 1 Location of populations studied. *Dryas octopetala* and *Carex curvula* populations are from the Swiss Alps and the Romanian Carpathians. *Vaccinium uliginosum* and *Salix herbacea* populations are from the Swiss Alps and Fennoscandia (Sweden and Norway)

Species	Region	Location	Coordinates	Elevation (m a.s.l.)	Slope
C. curvula	Alps	Biedmer (Furkapass)	46°33′N 8°23′E	2489	30°, North
	Alps	Valetta Schlattain (St. Moritz)	46°30′N 9°48′E	2622	20°, Southeast
	Carpathians	Vf. Paltinului (Lacul Bâlea)	45°35′N 24°36′E	2265	30°, Northwest
	Carpathians	Vf. Pietrosu (Borşa)	47°35′N 24°38′E	2232	45°, Southwest
D. octopetala	Alps	Il Jalet (Pass dal Fuorn)	46°38′N 10°17′E	2022	45°, North
	Alps	Calanda (Chur)	46°53′N 9°28′E	2100	45°, East
	Alps	Bonistock (Melchsee-Frutt)	46°46′N 8°17′E	2130	30°, Northwest
	Carpathians	Baba Mare (Buşteni)	45°24′N 25°28′E	2260	30°, North
S. herbacea	Alps	Blauberg (Furkapass)	46°34′N 8°25′E	2460	45°, Northeast
	Alps	Flüelapass (Davos)	46°44′N 9°56′E	2400	30°, Northeast
	Fennoscandia	Lulip (Abisko)	68°21′N 18°38′E	1060	30°, Southeast
	Fennoscandia	Kåfjorddalen (Birtavarre)	69°22′N 21°05′E	670	30°, Southeast
V. uliginosum	Alps	Gotthard (Andermatt)	46°32′N 8°34′E	2085	20°, Northwest
	Alps	Stillberg (Davos)	46°46′N 9°51′E	2220	20°, East
	Fennoscandia	Lulip (Abisko)	68°21′N 18°38′E	1000	10°, South
	Fennoscandia	Steindalen (Elvenes)	69°23′N 19°58′E	460	30°, Southeast

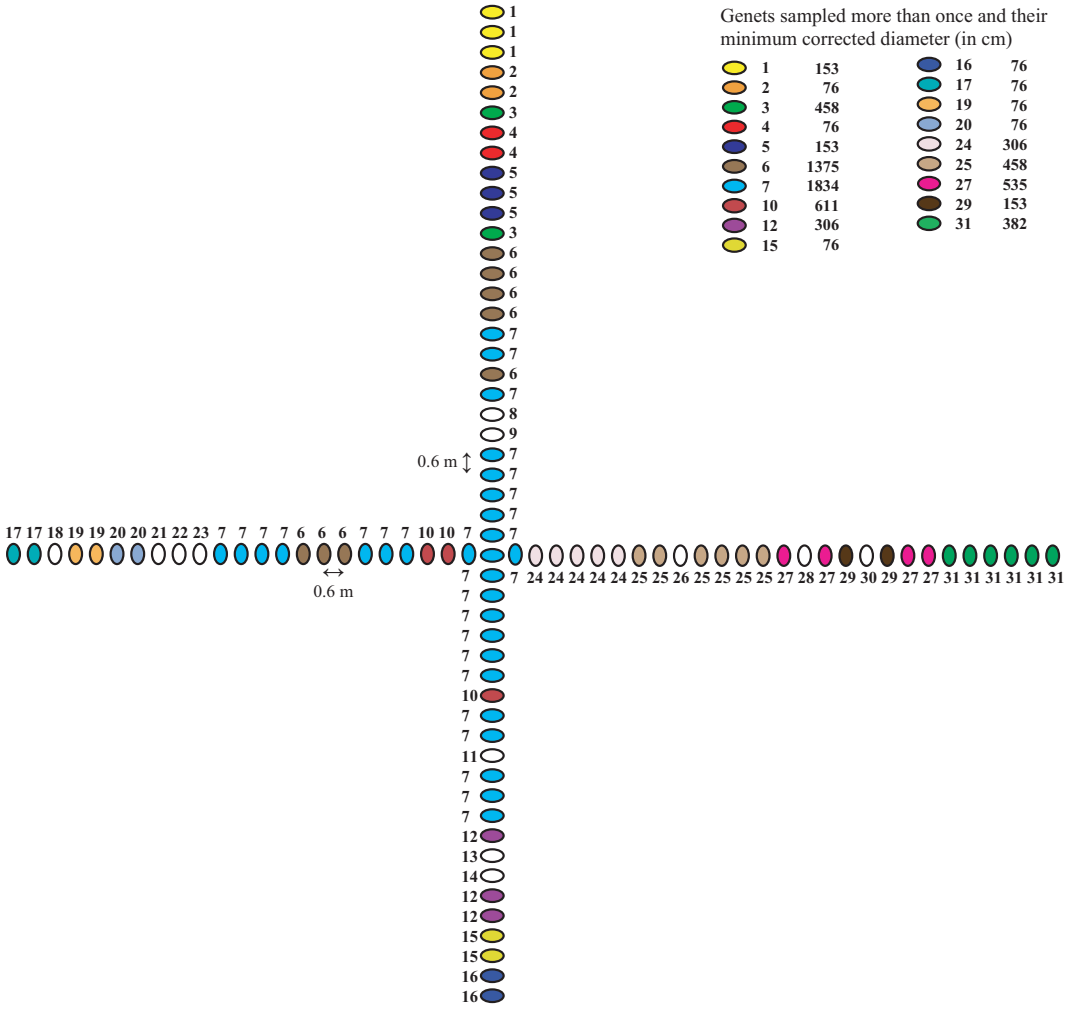

Fig. 1 Map showing the spatial distribution of *Vaccinium uliginosum* genets in the population 'Gotthard' as an example of one of our sampling transects. Sampling distance was 0.6 m and transect length was 30 m. Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

caused by endophytes, scoring errors or somatic mutations. Extreme care was taken to ensure that these control samples were taken within the same genet, and thus were connected by rhizomes or branched shoots.

DNA extraction, AFLP procedure and marker scoring

The amplified fragment length polymorphism (AFLP) procedure was performed according to Vos *et al.* (1995) with minor modifications (Puşcaş *et al.* 2008). DNA was extracted from 10 to 15 mg of dried leaf material using the DNeasy™ Plant 96 Kit (Qiagen, Hilden, Germany), according to the manufacturer's handbook. The three primer pairs (E-NNN for *Eco*RI FAM-fluorescent primers with selective nucleotides; M-NNN for *Mse*I) used in the selective amplification were as follows: (i) *Carex curvula*: E-ATC/M-CAC, E-ATC/M-CAT and E-ATC/M-CTG (Puşcaş *et al.* 2008); (ii) *Dryas octopetala:* E-ATG/M-CTG, E-AAC/M-CTG and E-ACT/M-CTG (Gugerli *et al.* 2008); (iii) *Salix herbacea*: E-ACT/M-CTA, E-ACA/M-CTA and E-AGC/M-CTC (Alsos *et al.* 2007); and (iv) *Vaccinium uliginosum*: E-ATC/M-CAG, E-ACT/M-CTA and E-ATG/M-CAG (Eidesen *et al.* 2007), respectively. Starting from the leaf sample, we included 10% blind samples for each species according to Bonin *et al.* (2004). Throughout the laboratory procedure, we adopted a strict sample arrangement on 96-well plates. To ensure repeatability, 'within-plate' and 'between-plate' replicates (5% each) and negative controls (2%) were also included. For fragment sizing and marker scoring after electrophoresis on an automated sequencer, GeneMapper 3.7 (Applied Biosystems) was used. Samples were excluded if they failed in the DNA extraction or the AFLP procedure. The scoring process

was carried out for each population separately, yielding more AFLP markers per population.

We used the blind samples, replicates and negative controls to reliably ensure the reproducibility of AFLP banding patterns and to achieve consistency of AFLP scoring, following Bonin *et al.* (2007). For stringent and consistent marker selection and locus presence–absence scoring, the marker selection algorithm implemented in scanAFLPv1-1 (Herrmann *et al.* 2010) was used. This software allows an automated selection procedure that reduces mismatch error rates and labour-intensive visual scoring of AFLP markers. The program converts the markers into presence–absence phenotype tables according to quality and repeatability criteria and then calculates the repeatability rate at the marker level. Further data analysis was only carried out when the analysis of blind samples and replicates yielded a probability of ≥0.94 of finding the same band in a replicate. To assess scoring differences and their effects on the biological conclusions, another scientist scored one population per species a second time, as recommended by Bonin *et al.* (2004).

Genetic and clonal data analysis

The samples from each population, including the intra-genet-variability controls, were assigned to genets using GenoDive 2.0b17 (Meirmans & Van Tienderen 2004). To ascertain that each multilocus genotype (MLG) belonged to a distinct genet, the frequency distribution of genetic distances among MLGs was investigated to determine whether it followed a strict unimodal distribution or showed high peaks at low distances. A bimodal distribution indicated the existence of somatic mutations or genotyping errors in the data set that evoked low distances among slightly distinct MLGs actually deriving from a single reproductive event (Woods *et al.* 1999; Peatkau 2004). Genetic distances were calculated using an infinite allele model that assumes single mutation steps to get from a certain allelic state to another (Kimura & Crow 1964). The largest pairwise genetic distance within the intra-genet-variability controls, together with the bimodal frequency distribution of genetic distances, was used to set an appropriate threshold for the genet or multilocus lineage (MLL) assignment (Halkett *et al.* 2005; Arnaud-Haond *et al.* 2007). Consequently, any genetic distance below this threshold was defined as variability occurring within MLLs. This approach is shown in Fig. 2, in which the vertical lines depict the applied assignment threshold (threshold values are given in Table 2). Pairs of MLGs that were on the left side of this threshold, and thus had a genetic distance below the threshold value, were assigned to the same MLL. Because the number of small genetic distances

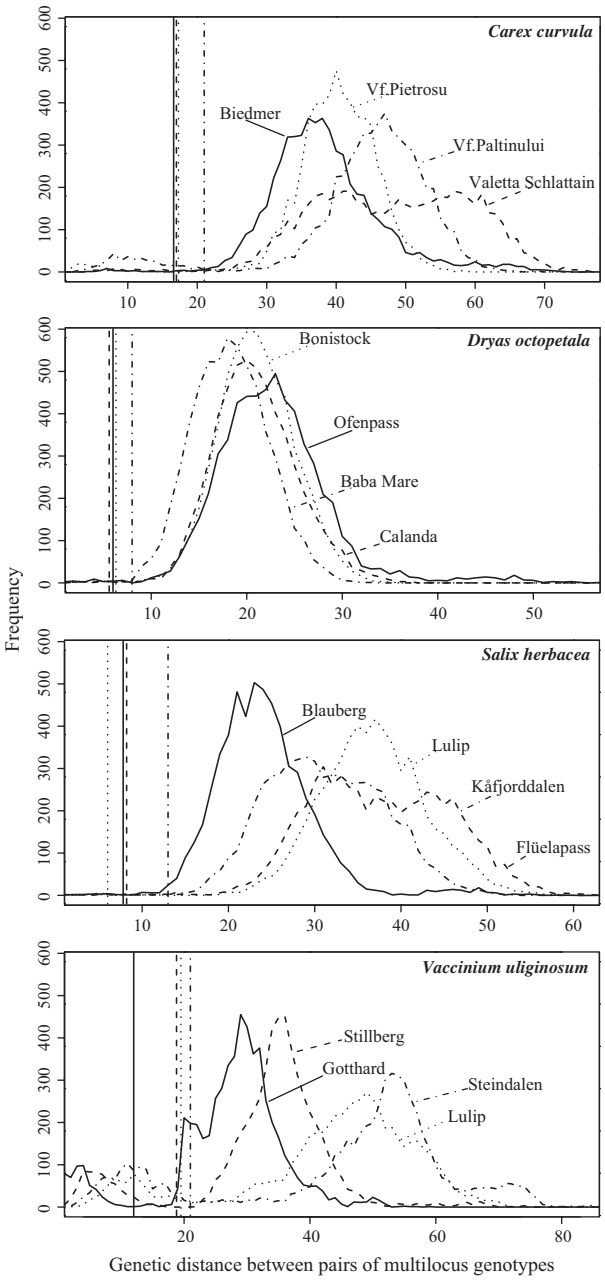

Fig. 2 Frequency distribution of genetic distances between pairs of genotypes in all four populations of each species and their applied assignment thresholds (vertical lines). Genetic distances refer to the number of amplified fragment length polymorphism markers in which genotype pairs differ and were calculated using an infinite allele model. Genetic distances below the assignment threshold represent scoring errors and somatic mutations, with variable proportions observed among species. Pairs of genotypes on the left side of the threshold were assigned to the same multilocus lineage.

deriving from somatic mutations or genotyping errors was low in *Salix* and *Dryas* MLGs, the bimodality of the distribution curve is almost indiscernible in these

Table 2 Descriptive statistics of genetic diversity parameters in the investigated populations of all four species

Population	*N*	No. of markers	Polymorphic markers (%)	RR	*T*	MLL	P_{sex}	$P_{(ID)sib}$	Clonal fraction	$MLL_s = 1\times$	$MLL_s > 1\times$	D_{pop}	eve
Carex curvula													
Biedmer	100	203	93.5	0.89	15	77	<0.001	0.001	0.23	62	15	0.18	0.59
Valetta Schlattain	100	193	95.9	0.96	16	68	<0.001	<0.001	0.32	45	23	0.24	0.63
Vf. Paltinului	99	178	91.1	0.96	20	37	<0.001	<0.001	0.62	15	22	0.23	0.38
Vf. Pietrosu	100	169	84.0	0.96	16	62	<0.001	<0.001	0.38	39	23	0.22	0.50
Mean		186	91.1	0.94	17	61	<0.001	<0.001	0.39	41	21	0.22	0.52
Dryas octopetala													
Il Jalet	100	91	78.3	0.98	8	78	<0.001	<0.001	0.22	62	16	0.13	0.80
Calanda	96	187	94.7	0.94	7	91	<0.001	0.004	0.05	82	9	0.13	0.34
Bonistock	100	149	77.2	0.98	7	75	<0.001	<0.001	0.25	58	17	0.13	0.89
Baba mare	99	126	65.9	0.96	6	76	<0.001	0.010	0.23	66	10	0.14	0.24
Mean		138	79.0	0.97	7	80	<0.001	0.004	0.19	67	13	0.13	0.57
Salix herbacea													
Blauberg	96	110	90.0	0.91	9	79	0.006	0.004	0.18	68	11	0.21	0.60
Flüelapass	93	150	97.3	0.95	16	76	<0.001	0.010	0.18	62	14	0.24	0.82
Lulip	99	110	95.5	0.95	18	92	<0.001	<0.001	0.07	85	7	0.20	0.64
Kåfjorddalen	100	119	98.3	0.96	13	91	<0.001	0.004	0.09	85	6	0.27	0.61
Mean		122	95.3	0.94	14	85	<0.001	0.005	0.13	75	10	0.23	0.66
Vaccinium uliginosum													
Gotthard	100	170	92.9	0.96	11	31	<0.001	<0.001	0.69	12	19	0.23	0.29
Stillberg	100	179	76.5	0.96	18	17	<0.001	<0.001	0.83	7	10	0.17	0.54
Lulip	100	237	75.1	0.94	18	20	<0.001	<0.001	0.80	8	12	0.17	0.45
Steindalen	100	192	76.0	0.96	20	12	<0.001	<0.001	0.88	2	10	0.20	0.61
Mean		195	80.1	0.96	17	20	<0.001	<0.001	0.80	7	13	0.19	0.47

N, number of ramets sampled; RR, repeatability rate; *T*, threshold for the multilocus genotype assignment; MLL, number of detected multilocus lineages (MLLs); P_{sex}, probability that the repeated genotypes of a MLL originate from distinct sexual reproductive events; $P_{(ID)sib}$, probability of two related individuals displaying the same multilocus genotype by chance; clonal fraction: (*N*-MLL)/*N*; $MLL_s = 1\times$, genets sampled only once; $MLL_s > 1\times$, genotypes sampled more than once; D_{pop}, Simpson's complement; eve, evenness according to Simpson's index.

species. Additionally, discrepancies in pairwise distances were evaluated using AFLPDAT (Ehrich 2006) to split or merge genotypes assigned by the threshold methodology. To assess whether any of the *n* repeated genotypes of a given MLL was the result of an independent sexual reproduction event, the probability that the repeated genotypes actually originated from distinct sexual reproductive events was calculated using the binomial probability function P_{sex} (Arnaud-Haond *et al.* 2007; modified from Parks & Werth 1993):

$$P_{sex} = \sum_{x=n}^{N} \frac{N!}{x!(N-x)!} (P_{dgen})^x (1 - P_{dgen})^{N-x} \quad (1)$$

where

$$P_{dgen} = \prod_{i=l}^{L} p_i \quad (2)$$

and p_i = the estimated frequency of band presence or absence at locus *i* among MLLs in the population. *L* = the number of loci used for a given population.

When using genetic fingerprinting methods, another potential error risk lies in assigning genetically similar sibs to the same MLL, especially when mating opportunities are confined to relatives within the neighbourhood, and thus are not random (Douhovnikoff & Dodd 2003). Although *D. octopetala* and *S. herbacea* are self-incompatible species, we could not fully exclude the occurrence of inbreeding in any of the four species studied. However, with dominant fingerprint markers, deviations from Hardy–Weinberg equilibrium cannot be calculated. To avoid incorrect assignments and maximize the sensitivity with which the analysis distinguished sibs and clones, we used only polymorphic AFLP markers and calculated the probability of identity [$P_{(ID)}$] among sibs for dominant markers (Paetkau & Strobeck 1994; Waits *et al.* 2001). While the more frequently calculated P_{sex} statistic represents the probability of two unrelated individuals displaying the same multilocus genotype by chance, $P_{(ID)}$ between sibs serves as a conservative measure for the probability of observing identical genotypes among relatives (Taberlet *et al.* 1999), with

$$P_{(\mathrm{ID})\mathrm{sib}} = 1 - \left\{(3/2p)\left(q^2\right)\right\} \quad (3)$$

where p is the frequency of a 'present' band (allelic state 1), and q is the absence of a band (allelic state 2). Values of $P_{(\mathrm{ID})}$ below 0.01 are recommended for studies of population structure (Waits *et al.* 2001).

Subsequently, clonal diversity and heterogeneity were calculated according to Arnaud-Haond *et al.* (2007) using the software GenoDive and the following indices: (i) the number of genets or multilocus lineages (MLLs) relative to the number of samples analysed (N) (Ellstrand & Roose 1987); (ii) Simpson's complement, $D_{\mathrm{pop}} = (1 - \sum_{i=1}^{k} p_i^2[n/(n-1)]$, where n is the sample size, k is the number of markers and p is the frequency of the MLL; and (iii) evenness (eve), according to Simpson's index. Clonal diversity was compared among populations of each species with respect to altitude, slope, season length, mean season temperature and sum of degree–days above 0 °C and 5 °C calculated from *in situ* hourly soil temperature data.

To determine whether within-population genetic distances between MLLs were associated with the spatial distance between MLLs, we performed a Mantel test with 10^4 permutations using GenAlEx 6 (Peakall & Smouse 2006). For this test, which estimates the association between two independent matrices (Sokal & Rohlf 1995), we constructed a spatial distance matrix in centimetres using the distance between the centres of each MLL.

Analysis of genet size and age structure

Genet radius was calculated as half of the maximum distance (diameter) between samples assigned to the same MLL. The calculated genet sizes are minimum values, because it is possible that detected genets cover more area outside the sampling transect. Because transects were laid out at random, and therefore crossed genets randomly, the estimated size of each genet was multiplied by the correction factor $4/\pi$, calculated from the probability density corresponding to the linear distance between the centres of a chord and a circle, when a random chord in a circle is chosen (Jaynes 1973). To test whether genet expansion was independent of cardinal direction, we compared the genet size distribution on the north–south and east–west axes of the sampling transect using a Kolmogorov–Smirnov test. The frequency distributions of genet size within species were compared between regions and among populations within regions with the same statistical test.

Average annual horizontal growth was estimated based on year-to-year measurements in several plants of the same four populations selected for the measurement of genet size. To take into account the expected variability in annual horizontal growth among individuals of unknown age, in each population, a minimum of 20 shoots or tillers that were at least one metre apart were marked on a single day in summer 2008. Horizontal shoot length or tiller diameter and position were precisely measured, and the specific morphology was noted. Annual horizontal growth was then measured in millimetres to the nearest decimal for each shoot or tiller exactly one and two years later in 2009 and 2010. The data were analysed using JMP'8 statistical software (SAS Institute Inc., Cary, NC, USA). Because the annual horizontal growth of shoots may differ owing to seasonal variation, microclimate or the position of a shoot in a genet, we accounted for the variability of annual growth by calculating the mean and the upper 95% confidence interval as measures for the annual size increase in genets. As we cannot exclude the possibility that faster growing shoots may be present along the genet margin, and thereby increase the diameter of genets even when other shoots grow more slowly, we used the upper 95% confidence interval as a measure of maximum size increase. The mean of all measurements within a population was used, because all growing shoots contribute equally to genet size increases. We then calculated the minimum and maximum age of each genet by dividing the genet radius derived from the molecular analysis by the upper 95% confidence interval and the mean genet size increase, respectively. It is of course difficult to reconstruct the annual size increase for genets that are hundreds or even thousands of years old. Thus, the longer the genets live, the less precise the age estimates probably are. By including minimum and maximum age estimates, we account for the inherent uncertainty of age estimates for slow-growing clonal plants based on actual growth measurements.

Results

DNA extraction, AFLP procedure and marker scoring

For all four species, a total of 1636 samples were analysed with the AFLP fingerprinting method. Only 1.1% of the samples displayed an irregular AFLP pattern. Using three pairs of primers per species and applying a restrictive scoring process based on blind samples and replicates, the following average numbers of AFLP markers per population, ranging from 50 to 500 bp, were identified: 186 for *C. curvula*, 138 for *D. octopetala*, 122 for *S. herbacea* and 195 for *V. uliginosum*. The resulting average repeatability rate, based on the stringent marker selection algorithm, was 99.7%. The percentage of polymorphic loci within populations varied between 65.9 and 98.3%, with a mean of 80.5% across all species

(Table 2). The repeatability of the AFLP scoring results was high, as the probability of finding the same band in a replicate ranged between 0.89 and 0.98 among populations, with a mean of 0.96 (Table 2).

Genetic and clonal data analysis

The frequency distribution of genetic distances between pairs of multilocus genotypes was found to be bimodal in all populations of each species, revealing the existence of somatic mutations and/or genotyping errors (Fig. 2). The threshold defining clonal lineages (MLLs) was set to 6 to 20 pairwise distances, according to the bimodal distribution of distances and the largest pairwise distance between the intra-genet-variability controls of the respective populations. Scoring by a second scientist resulted in a marker set that matched only 39% with that of the first scientist, but subsequent genotypic identification was very similar, with only three mismatches in the MLL assignment. Therefore, genotyping errors did not affect the biological conclusions that were made from our AFLP data set. Overall, genet assignment ambiguities represented a very small proportion of the total number of genotypes identified. From the 400 samples collected for each species, 244 unique MLLs were detected for *C. curvula,* 320 for *D. octopetala*, 338 for *S. herbacea* and 80 for *V. uliginosum* (see the total number per population in Table 2). The samples belonging to each MLL (genet) were usually spatially grouped. In only a few cases, samples belonging to the same MLL were separated by other MLLs. We assigned these to the same genet, assuming that intermingling at the genet margins occasionally occurs. Maps showing the spatial distribution of the samples assigned to genets in all 16 populations are shown in Fig. 1 (one population) and the Supporting information (15 other populations). The probability that the sampled units determined to be from the same clonal lineage, which actually originate from distinct sexual reproductive events (P_{sex}), was unlikely (0.006) for the *S. herbacea* population 'Blauberg' and very unlikely (<0.001) for all other populations (Table 2). The estimated probability of identity between sibs was never higher than the recommended maximum value ($P_{(ID)sib} < 0.01$; Waits *et al.* 2001) (Table 2).

Clonal diversity was highest in the *S. herbacea* populations (mean $D_{Pop} = 0.23$) and lowest in the *D. octopetala* populations (mean $D_{Pop} = 0.13$). Clonal evenness was intermediate for all four species, indicating that MLLs had neither equal nor unequal abundances. The mean clonal fraction, calculated as the ratio of MLLs to samples, was much higher in *V. uliginosum* (0.80) compared with the other species (0.4 in *C. curvula,* 0.19 in *D. octopetala* and 0.13 in *S. herbacea*). Clonal diversity was not correlated with any of the population characters or climatic parameters measured. Results of the Mantel tests indicated that genetic distance between MLLs was not correlated with spatial distance for any population of the four species (data not shown).

Analysis of genet size and age structure

Genet diameter varied strongly within and among the four species, from a few centimetres in *C. curvula* to approximately 18 m in *V. uliginosum* (Table 3). For each species, we found genets represented by a single sample, which were therefore smaller than twice the sampling distance. The largest genets were 4.97 m in diameter for *C. curvula* (Făgăraş mtns, RO), 6.11 m in *D. octopetala* (Baba Mare, RO), 3.82 m in *S. herbacea* (Blauberg, CH) and 18.34 m in *V. uliginosum* (Gotthard, CH, Fig. 1). We found no significant differences in genet size between the north–south and the east–west axes of the sampling cross (Kolmogorov–Smirnov test, $P > 0.1$ for all populations and species). For all four species, the genet size distributions were strongly left-skewed in each population, indicating the dominance of small genets and the presence of a few very large genets (Fig. 3). In all four species, the population size distribution did not differ among the distant geographic regions (Kolmogorov–Smirnov test, $P > 0.1$). However, there were more large genets present in populations of *C. curvula* and *D. octopetala* in the Carpathians compared with populations in the Alps (see Fig. 3a,b). In all four populations of *V. uliginosum*, genet sizes were more evenly distributed, with fewer small genets compared with the other species, although the distribution was also left-skewed (Fig. 3d).

The mean annual horizontal growth was significantly different between species (d.f. = 4/25, $F = 4.9$, $P < 0.01$), but not among regions or between years within the four species. The minimum and maximum annual horizontal growth rates used for the age estimates were 0.4 and 0.6 mm in *C. curvula*, 5.4 and 5.9 mm in *D. octopetala*, 3.7 and 4.3 mm in *S. herbacea*, and 4.8 and 6.6 mm in *V. uliginosum*. For the largest genets of each species, the minimum and maximum age estimates were 4140 and 4970 years in *C. curvula*, 520 and 570 years in *D. octopetala*, 450 and 520 years in *S. herbacea*, and 1390 and 1880 years in *V. uliginosum*. Age estimates for each measured genet are shown in Table 3. Many *C. curvula* genets (84.8%) were smaller than 0.2 m and were, thus, younger than 215 years (Fig. 3). In *D. octopetala*, 94.1% of all genets found in the four populations had a diameter smaller than 0.8 m and were, thus, not older than 80 years. In *S. herbacea*, even more genets (96.5%) were smaller than 0.8 m, and thus younger than 100 years. In

Vaccinium, however, only about half (53.8%) of the genets were smaller than 0.8 m, and thus were no more than 80 years old. The population size and age structure in *D. octopetala* and *S. herbacea* populations clearly indicated that only a small number of 'young' genets survive for long periods of time (i.e. more than 100 years) (5.9% and 3.5%, respectively). In contrast, many genets of *V. uliginosum* survived for more than 100 years (>46.2) and many genets of *C. curvula* were more than 300 years old (15.2%). Several genets of *C. curvula* (5.7%) and *V. uliginosum* (5.0%) had even reached a minimum age of at least 1000 years.

Discussion

Several previous studies found that clonality in plants has the potential to ensure persistence of populations over a long time period and that some clonal plant

Table 3 Measured size (in metres) and age estimates (in years) of all genets detected in the populations of all four species. Bold characters indicate genets with largest size and largest age estimate per species

Species	Population	Number of genets	Corrected diameter	Minimum age	Maximum age
Carex curvula	Biedmer	61	<0.38	<320	<380
		10	0.38	320	380
		2	0.76	640	760
		1	1.15	960	1150
		1	1.53	1270	1550
		1	1.91	1590	1910
		1	3.06	2550	3060
	Valetta Schlattain	45	<0.38	<320	<380
		14	0.38	320	380
		3	0.76	640	760
		4	1.15	960	1150
		1	1.53	1270	1550
		1	2.29	1910	2290
	Vf. Paltinului	15	<0.38	<320	<380
		6	0.38	320	380
		6	0.76	640	760
		2	1.15	960	1150
		2	1.53	1270	1550
		1	2.29	1910	2290
		1	2.67	2230	2670
		2	3.06	2550	3060
		1	3.44	2870	3440
		1	**4.97**	**4140**	**4970**
	Vf. Pietrosu	40	<0.38	<320	<380
		16	0.38	320	380
		3	0.76	640	760
		2	1.15	960	1150
		1	4.20	3500	4200
Dryas octopetala	Il Jalet	62	<0.76	<65	<75
		10	0.76	65	75
		4	1.53	130	145
		2	2.29	190	215
	Calanda	82	<0.76	<65	<75
		9	0.76	65	75
	Bonistock	58	<0.76	<65	<75
		12	0.76	65	75
		3	1.53	130	145
		2	2.29	190	215
	Baba Mare	66	<0.76	<65	<75
		2	0.76	65	75
		3	1.53	130	145
		1	2.29	190	215
		2	3.06	260	285
		1	4.58	390	425
		1	**6.11**	**520**	**570**

Table 3 *Continued*

Species	Population	Number of genets	Corrected diameter	Minimum age	Maximum age
Salix herbacea	Blauberg	68	<0.76	<90	<100
		7	0.76	90	100
		2	1.53	180	210
		1	2.29	270	310
		1	3.06	360	410
	Flüelapass	62	<0.76	<90	<100
		9	0.76	90	100
		2	1.53	180	210
		3	2.29	270	310
	Lulip	85	<0.76	<90	<100
		6	0.76	90	100
		1	1.53	180	210
	Kåfjorddalen	85	<0.76	<90	<100
		4	0.76	90	100
		1	2.29	270	310
		1	**3.82**	**450**	**520**
Vaccinium uliginosum	Gotthard	12	<0.76	<60	<80
		7	0.76	60	80
		3	1.53	120	160
		2	3.06	230	310
		1	3.82	290	390
		1	4.58	350	470
		2	5.35	410	550
		1	6.11	465	630
		1	13.75	1045	1410
		1	**18.34**	**1390**	**1880**
	Stillberg	7	<0.76	<60	<80
		1	0.76	60	80
		1	1.53	120	160
		1	2.29	170	235
		2	5.35	410	550
		1	6.88	520	700
		1	7.64	580	780
		1	10.70	810	1095
		2	12.99	990	1330
	Lulip	9	<0.76	<60	<80
		3	0.76	60	80
		1	1.53	120	160
		1	2.29	170	235
		1	3.06	230	310
		1	4.58	350	470
		2	7.64	580	780
		1	11.46	870	1170
		1	12.99	990	1330
		1	13.75	1045	1410
	Steindalen	2	<0.76	<60	<80
		2	0.76	60	80
		2	2.29	170	235
		1	6.11	465	630
		1	6.88	520	700
		2	8.40	640	860
		1	17.57	1340	1800

individuals can grow very old (Steinger *et al.* 1996; Escaravage *et al.* 1998; Eriksson 2000; Helm *et al.* 2006; reviewed in de Witte & Stöcklin 2010). The present study confirms that individual arctic-alpine clonal plants often become very old and that their populations in late-successional communities have persisted through

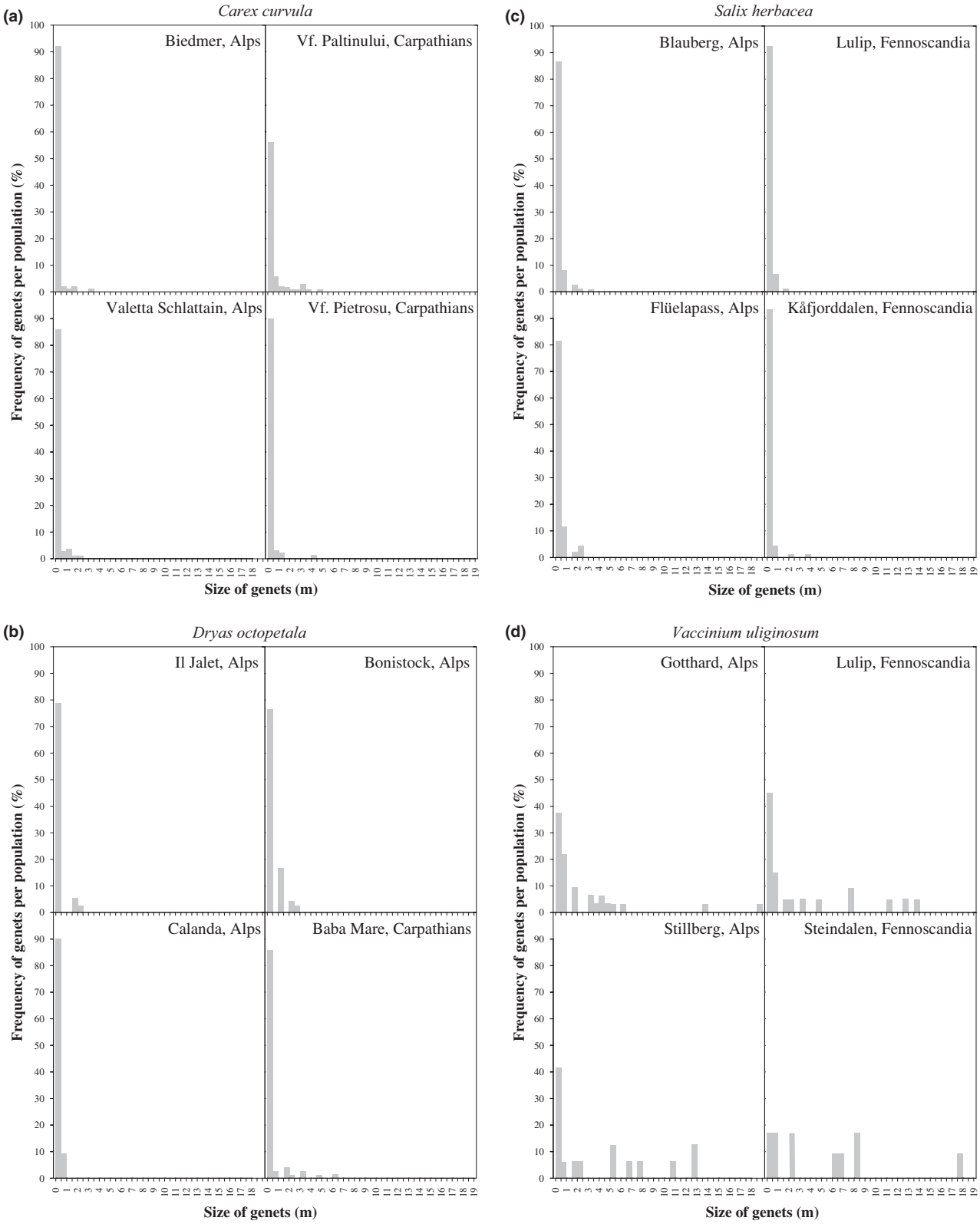

Fig. 3 Frequency distribution of genet size for the four populations of each species.

past climatic oscillations. Our results enable us to make the following conclusions: (i) AFLP fingerprinting proved capable of safely assigning all samples to clonal lineages; (ii) clonal diversity is generally high in all sampled populations of the four species studied; (iii) the frequency distribution of genet size is left-skewed in all populations and species studied herein, with a dominance of small genets and a low number of large genets; (iv) there are almost no differences in size structure among the four populations of each species; (v) many of the oldest genets are several hundred or sometimes even more than one thousand years old; (vi) the observed frequency distribution of genet size and age indicate regular seedling recruitment and a high genet turnover in all four species; and (vii) populations of arctic-alpine clonal plants in the Alps and the Arctic regions of Europe should be able to cope with considerable climatic changes in the future, at least as long as the shifts are of similar magnitude and time scale as in the past. Our conclusions are discussed in further detail later.

Clonal assignment

Our study is methodologically important, as the AFLP technique has proved to be an efficient tool for detecting and measuring the sizes of large numbers of genets in several populations using an appropriate sampling strategy. A critical prerequisite was the optimization of the AFLP scoring procedure by automated and stringent marker selection to yield highly reproducible markers. In this study, the robustness of the inferred biological message to scoring differences (i.e. deviating marker sets of two scientists) was derived from the redundancy of the information contained in the large number of AFLP markers, which enabled us to assign our samples to genotypes and to genets (Peatkau 2004).

Clonal diversity

Within-population clonal diversity was found to differ among species, but the values were still rather high for clonal species. Despite the important role of clonal growth, most clonal plants usually maintain sexual reproduction, and their populations can be as diverse as those of nonclonal plant species (Till-Bottraud & Gaudeul 2002; Stöcklin *et al.* 2009; Thiel-Egenter *et al.* 2009). Similar levels of clonal diversity were previously found in populations of *V. uliginosum* in Svalbard and *D. octopetala* throughout Scandinavia (Alsos *et al.* 2002; Vik *et al.* 2010). Moreover, our findings are in accordance with studies on the same species (Steinger *et al.* 1996; Albert *et al.* 2005; Skrede *et al.* 2006; Stamati *et al.* 2007), other arctic and alpine species (Persson & Gustavsson 2001; Stöcklin *et al.* 2009) and the average clonal diversity found in a wide range of alpine and lowland species (Till-Bottraud & Gaudeul 2002).

Genet size structure

The frequency distribution of genet size and age is left-skewed, as is typical for polycarpic perennials (Stearns 1992). Frequency distributions were not noticeably different between populations (Fig. 3), indicating that population dynamics are very similar among the populations of a given species and largely independent of local conditions. This conclusion is consistent with the findings in several populations of the clonal perennial *Ranunculus repens* (Sarukhán & Harper 1973; Lovett-Doust 1981).

The majority of multilocus genotypes detected in the *C. curvula*, *D. octopetala* and *S. herbacea* populations were sampled only once. This suggests that many small and presumably relatively young genets are present within late-successional populations, indicating that repeated seedling recruitment is common in populations of these species and that there is a considerable turnover rate. Repeated recruitment is an important mechanism to maintain population persistence, as it also creates genetic diversity, a precondition for microevolution and adaptation to changing environmental conditions. On the other hand, few genets of *C. curvula*, *D. octopetala* and *S. herbacea* seem to survive long enough to grow to a large size. In contrast, we found a relatively high fraction of medium-sized and very large genets in the populations of *V. uliginosum*, indicating higher survival of recruits. Furthermore, occasional intermingling of genets was found to have occurred in *V. uliginosum*, e.g. in the genets with code numbers 6, 7, 10, 12 and 27 in the population 'Gotthard' (Fig. 1). While making shoot increment measurements for this species in the field, we observed that large portions of the shoots died or were destroyed by trampling, indicating higher sensitivity to disturbance. These slightly different demographic patterns might be related to the morphology of this species, which is deciduous, has a higher degree of woodiness and exhibits layering shoots.

The observed genet size structures in our study species show concordance as well as discrepancies with some previous findings. For example, the average genet size in our four transects of *S. herbacea* populations in the Swiss Alps and Fennoscandia (1.21 m) is similar to the average genet size of 0.84 m found for the same species in a 3×3 m plot in the Austrian Alps (Reisch *et al.* 2007). However, none of our *S. herbacea* genets was as large as the largest genet found in a population in Britain (7 m, Stamati *et al.* 2007). The strongest concordance with our results for the *C. curvula* population

on Biedmer (Furkapass) came from Steinger *et al.* (1996), who found several small genets next to one very large genet of at least 1.6 m in diameter, using random amplified polymorphic DNA markers and a sampling grid of only 2.0×0.4 m. Based on a similar mean annual rhizome growth of 0.4 mm, they estimated a minimum age of about 2000 years for this large genet. This concordance between studies involving different approaches and methodologies strengthens our confidence in our results. Our systematic investigation of genet size, however, proved to be more efficient at detecting large numbers of genets and allowed us to analyse a much larger fraction of the studied populations, as well as to compare data between regions, populations and species.

We emphasize that all investigated genets may have larger diameters than we were able to detect with our sampling strategy, especially the outermost genets on the transect ends. There are several reasons for this. First, most sampled genets were probably not bisected by the transect, so their measured diameters are actually only secants. We accounted for this bias by applying a correction factor, but this only yields an approximation of the real genet diameter. Second, our estimation of genet age assumes strict symmetrical and centrifugal growth, whereas in reality, horizontal growth may be more irregular, and sections of unknown size may have died and consequently changed the shape of the genet, departing from the ideal circle. Even genotypes sampled only once in our sampling design could be the relicts of much larger (and older) genets, and the largest genets might have been much larger in the past.

Population age structure and dynamics

Previous studies have suggested that individuals of several arctic-alpine clonal grass and sedge species are probably much older than one thousand years (de Witte & Stöcklin 2010). This phenomenon has once more been confirmed by our findings. For *C. curvula,* we found genets with estimated ages of at least one thousand years in all four studied populations. For this species, even the conservative minimum age is clearly more than 1000 years. Meanwhile, very few shrubs have previously been found to be very long lived. The few examples include *Juniperus sabina*, which received an age estimate of 770–2940 years (Wesche *et al.* 2005), and an individual *Rhododendron ferrugineum*, which was estimated to be at least 280 years old (Pornon *et al.* 2000). We suspect that this trend should be attributed to the methodological constraints or ignorance about the life history and longevity of these species rather than to a real biological tendency. Here, we present maximum lifespan estimates that exceed any existing age estimates for dwarf shrubs, the maximum age estimate of almost 1900 years for a *V. uliginosum* genet in the central Swiss Alps being the most extreme.

Based on the genet size structures, we assume that there is a large range of age classes in the four species studied. From this, we deduce that seedling recruitment has taken place repeatedly. However, we cannot resolve the question of whether genets of the same age have different sizes, as was found, for example, in the tree species *Pinus sylvestris* (Ågren & Zackrisson 1990). In addition, in other plant populations, only one or two age classes are usually found, because of partial or complete demise of the younger plants or the absence of repeated recruitment (Crawley & Ross 1990; Silvertown & Lovett-Doust 1993). There is also evidence for local cyclical dynamics in some clonal heathland shrubs like *Calluna vulgaris* (Watt 1964). Unfortunately, there are only a few long-term demographic studies on herbaceous perennials that can shed light on the variability in annual growth, population dynamics and the relationship between genet size and age. One exception is a study on terrestrial orchids that revealed unequal age structures with strong age classes and only periodic recruitment (Wells 1981; Inghe & Tamm 1985; Kull & Hutchings 2006). In addition, slow turnover and negligible seedling recruitment were found in the species *Potentilla anserina* (Eriksson 1986). In contrast, for the arctic-alpine plants studied here, we found a large range of size classes, high genet diversity and a large amount of small genets. These results suggest relatively high genet turnover, repeated seedling recruitment and the presence of many different age classes.

Persistence under past climatic oscillations

During the last 2000 years, Arctic and Alpine regions have experienced several climatic oscillations. In the European Alps, for example, temperature fluctuations with an amplitude of 3.1 °C over the past 1250 years were estimated based on tree-ring analysis (Büntgen *et al.* 2006). This study revealed that summer temperatures in the European Medieval Warm Period between the 9th and 13th century A.D. were similar to the measurements of the last ten years (+1.7 °C). During the Little Ice Age between 1350 and 1820, temperatures in the Alps dropped considerably (−4.5 °C), and glaciers reached their maximal advance of the last two thousand years. Since the beginning of the 17th century, a consistent temperature increase has taken place in Europe, with record-breaking heat in the year 2003. Considerable climate changes have also occurred in Arctic regions, where average annual temperature increased at almost twice the global rate during the past 150 years

(ACIA 2004). In Swedish Lapland, where several of our study populations are located, mean winter temperatures increased by approximately 5 °C (Callaghan *et al.* 2009). The results of our study clearly indicate that a considerable number of individuals of the four arctic-alpine species have survived these past climate changes. This conclusion still holds true for all four species when we consider only the very conservative minimum age estimates.

Resistance to future climate change

In recent years, vegetation changes owing to global warming have been reported for many ecosystems, including arctic-alpine regions (Carlsson & Callaghan 1994; Pauli *et al.* 1996; Callaghan & Carlsson 1997; Callaghan *et al.* 1997; Grabherr *et al.* 2000; Walther *et al.* 2002). In general, however, communities dominated by long-lived clonal plant species seem to be very stable (Grabherr & Nagy 2003). The persistence of slow-growing clonal species is believed to have a positive effect on the resilience of whole ecosystems, because they may enhance the community's resistance to adverse environmental fluctuations and the ability to recover from disturbances. The species studied here are widespread and reproduce sexually as well as clonally, which theoretically enables them to recolonize and migrate by dispersal. From our data, we conclude that late-successional, long-lived clonal species exhibit high genetic variability and turnover, and may indeed show a previously underestimated resilience to changing climatic conditions. Our findings suggest that moderate climate change with an average temperature increase of 1.8 °C over the next hundred years [as predicted by the B1 scenario of the IPCC report (IPCC 2007)] and a moderate frequency of extreme climatic events will not lead to local extinctions of long-lived clonal plant populations. Unless future climatic changes occur substantially faster, with temperature increases of more than 3 °C, an increased amount of precipitation and more extreme weather events (as predicted by the A2 and A1FI scenario of the IPCC report), it is likely that these species are able to resist or adapt to the environmental changes.

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This study is part of the PhD thesis of L.C.d.W., which was supervised by J.S.. L.C.d.W. is interested in the population ecology and longevity of arctic-alpine clonal plants and the speciation of orchids. J.S. is group leader of the Population Ecology and Genetics lab at the Botanical Institute, University

of Basel and conducts research on biodiversity, reproductive ecology, evolutionary biology and conservation of Alpine plants. G.F.J.A., supported L.C.d.W. as a research engineer, and is interested in the molecular ecology of plants, bird migration and the biology of land snails. P.T., project coordinator of the EcoChange project and scientific collaborator of the Laboratoire d'Ecologie Alpine/CNRS, focuses on the conservation genetics and molecular ecology of different plant and animal species. L.G., research engineer at the Laboratoire d'Ecologie Alpine, taught L.C.d.W. molecular methods and was responsible for the AFLP protocols.

Data accessibility

AFLP binary data: DRYAD entry doi: 10.5061/dryad.05b10.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Map showing the spatial distribution of the 77 *Carex curvula* genets in the population 'Biedmer'. Genets sampled only once are shown without colour filling, while samples belonging to the same genets are shown in the same colour and have the same number.

Fig. S2 Map showing the spatial distribution of the 68 *Carex curvula* genets in the population 'Valetta Schlattain'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S3 Map showing the spatial distribution of the 37 *Carex curvula* genets in the population 'Vf. Paltinului'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S4 Map showing the spatial distribution of the 62 *Carex curvula* genets in the population 'Vf. Pietrosu'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S5 Map showing the spatial distribution of the 78 *Dryas octopetala* genets in the population 'Il Jalet'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S6 Map showing the spatial distribution of the 91 *Dryas octopetala* genets in the population 'Calanda'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S7 Map showing the spatial distribution of the 75 *Dryas octopetala* genets in the population 'Bonistock'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S8 Map showing the spatial distribution of the 76 *Dryas octopetala* genets in the population 'Baba Mare'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S9 Map showing the spatial distribution of the 79 *Salix herbacea* genets in the population 'Blauberg'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S10 Map showing the spatial distribution of the 76 *Salix herbacea* genets in the population 'Flüelapass'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S11 Map showing the spatial distribution of the 92 *Salix herbacea* genets in the population 'Lulip'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S12 Map showing the spatial distribution of the 91 *Salix herbacea* genets in the population 'Kåfjorddalen'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S13 Map showing the spatial distribution of the 17 *Vaccinium uliginosum* genets in the population 'Stillberg'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S14 Map showing the spatial distribution of the 20 *Vaccinium uliginosum* genets in the population 'Lulip'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

Fig. S15 Map showing the spatial distribution of the 12 *Vaccinium uliginosum* genets in the population 'Steindalen'. Genets sampled only once are shown without colour filling, while samples belonging to the same genet are shown in the same colour and have the same number.

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Chapter 4

Horizontal growth in arctic-alpine clonal plants is not affected by climatic variability among regions

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Horizontal growth in arctic-alpine clonal plants is not affected by climatic variability among regions

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Background: Many arctic and alpine plant species from cold environments reproduce mainly vegetatively and can be extremely long-lived. To understand the life history and population dynamics of such species, careful in-situ measurements of growth are essential, but reports of such measurements are still scarce.
Aims: Our objectives were to compare annual horizontal growth in populations of five clonal arctic-alpine species in different geographic regions, successional stages and years, and to test how much their mean annual growth is affected by season length.
Methods: We performed replicated measurements of annual size increments in 36 populations of *Carex curvula*, *Dryas octopetala*, *Salix herbacea*, *Vaccinium uliginosum* and *Empetrum nigrum* in three arctic-alpine regions of Europe for 2 years (2008–2010).
Results: The mean annual horizontal growth was different among the species and between early and late successional stages in both years. In late successional populations, the mean growth over both years was between 0.46 mm (*Carex curvula*) and 13.2 mm (*Empetrum nigrum*), and in early successional populations, the growth was between 0.85 mm and 19.0 mm, respectively. Across geographical regions, growth rates were not different, despite a difference of as much as 50 days among season lengths.
Conclusions: Our results indicate that horizontal growth in arctic-alpine clonal plants may not be strongly affected by a warmer climate in the future. As a consequence, changes in arctic-alpine late successional vegetation dominated by the clonal species studied here might be slower in the face of global warming than changes in other vegetation types.

Keywords: climate change; community stability; ecosystem resilience; season length; shoot increment; soil temperature; successional stage

Introduction

Clonal growth and longevity are among the most notable adaptations of plants in alpine and arctic habitats, which are characterised by severe climatic conditions, steep environmental gradients, sharp boundaries and high disturbances (Hartmann 1957; Billings and Mooney 1968; Bliss 1971; Callaghan 1988; Klimeš et al. 1997). Dominant clonal plants can cover large areas in late successional vegetation, such as alpine grassland, arctic tussock tundra or dwarf shrub heath. In such vegetation types, a short growing season, low temperatures, desiccation and a low availability of mineral nutrients generally reduce growth and biomass production and contribute to long individual lifespan (Schröter 1926; Bliss 1962; Chapin and Shaver 1985; Eriksson and Jerling 1990; Klötzli 1991; Sonesson and Callaghan 1991; Jónsdóttir et al. 2000; Körner 2003; de Witte and Stöcklin 2010). Moreover, the longevity of plants is believed to support the persistence of populations and thus to positively affect community stability (Steinger et al. 1996; Eriksson 2000; Körner 2003; Morris et al. 2008).

To understand the growth dynamics and life history of long-lived clonal plants, long-term studies and field experiments are necessary. Furthermore, because ramet and genet dynamics can differ greatly, data collection should be carried out at both the ramet and genet level. However, there is a general lack of information about the life history and population dynamics of arctic-alpine clonal plants. This gap in our basic knowledge hampers our understanding of the functional role of clonal plants in community stability and ecosystem resilience in cold habitats and under climate change. To circumvent labour-intensive long-term efforts, indirect methods and modelling approaches have been applied (e.g. Callaghan 1976; Watkinson and Powell 1993; Steinger et al. 1996; Molau 1997; Morris and Doak 1998; Diemer 2002; Wesche et al. 2005; Erschbamer and Winkler 2005; Weppler et al. 2006). In clonal plants that can lose their main root or main stem and become fragmented, lifespan can only be indirectly estimated by dividing the genet size, determined by using molecular fingerprint markers, by a measure of the annual size increment (de Witte and Stöcklin 2010). This estimation has been made, for instance, for genets of the subalpine shrub *Rhododendron ferrugineum* L, of which the largest genet was found to exceed 7.6 m in diameter (Pornon et al. 2000). This genet size divided by an annual shoot increment of 2.7 cm resulted in an age estimate of ca. 280 years. The two critical components of the method are (i) the assignment of plant samples to genetic individuals and (ii) the

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accuracy of the annual horizontal growth measurements, which must be valid for the sometimes extremely long lifespan of clonal individuals. Genotype assignment has improved recently by using improved sampling designs and by the application of highly polymorphic fingerprint markers and statistical tools that critically evaluate the error probability in molecular marker scoring. Annual horizontal growth measurements, however, are often given much less attention, and sometimes values obtained from the literature are used. Such published values may not include the full variation expected among individuals, different locations, different successional stages or over time.

Numerous climate manipulation experiments have shown that arctic-alpine plant species have different growth responses (usually measured in terms of biomass production) to changes in growth conditions, such as light, temperature or nutrients (e.g. Chapin and Shaver 1985; Chapin et al. 1995; Shaver et al. 1996; Graglia et al. 1997; Arft et al. 1999). However, to date, only few studies have compared horizontal growth patterns in the field and under different climatic conditions (but see Bliss 1956). Variability in horizontal growth was found to be affected by season length (Myneni et al. 1997), temperature (Kojima et al. 1997; Welker et al. 1997; Wada et al. 2002), nutrient availability (Sammul et al. 2003; Shevtsova et al. 2005), competition (Shevtsova et al. 1995; 1997; Erschbamer et al. 1998), grazing (Elkington 1971), pollution (Kopponen et al. 2001; Zverev et al. 2008) and anthropogenic disturbances (Rixen et al. 2004). Furthermore, biomass production and horizontal growth is expected to differ depending on successional stage, because in pioneer vegetation competition is absent and nutrient availability differs compared with vegetation of late successional stages (Suzuki and Hutchings 1997; van Kleunen et al. 2001).

Our work addressed this hiatus. Our objectives were to estimate and compare annual horizontal growth in populations of five clonal arctic-alpine key species in different geographic regions, successional stages and years, and to test how much their mean annual growth was affected by season length. We hypothesised that the annual horizontal growth in the five arctic-alpine species was larger in early successional populations compared with that in late successional populations and that, depending on the season length, the annual horizontal growth varied between regions and years.

Materials and methods

Study species

We selected the five clonal species *Carex curvula* All., *Dryas octopetala* L., *Salix herbacea* L., *Vaccinium uliginosum* L. and *Empetrum nigrum* L., because they (i) are widespread and dominant in arctic and alpine ecosystems; (ii) form large and dense populations; and (iii) have a phalanx strategy with horizontal and centrifugal growth, and are thus suitable for indirect age estimates based on genet size and size increase data (de Witte and Stöcklin 2010).

The sedge *Carex curvula* (Cyperaceae) is the dominant species in the most important grassland vegetation (Caricetum curvulae) on siliceous substrate in the Central and Eastern Alps between 2300 m and 3000 m a.s.l. (Schröter 1926; Oberdorfer 1959; Grabherr 1987). It develops relatively homogeneous tussocks with many erect and densely packed tillers produced by clonal proliferation (Reisigl and Keller 1987) and with an extensive underground rhizome system (Grabherr et al. 1978). Genets of *C. curvula* usually form a 'fairy ring' because they mainly grow at the outside of the tussock, and the ramets inside eventually die. The genets have also been found to become fragmented and intermingle with other genets (Grabherr 1989). Growth is very slow, and only approximately every sixth tiller was found to produce a new tiller (Grabherr et al. 1978), which is developed within the leaf sheath of an adult tiller (intravaginal tillering sensu Erschbamer and Winkler 2005). The tillers live for at least 6–7 years and up to 15–20 years, while the leaves remain alive for 2–3 years. *C. curvula* genets can be extremely long-lived; the age of a genet in the Central Alps was estimated to be several thousand years (Steinger et al. 1996).

The dwarf shrub *Dryas octopetala* (Rosaceae) has a broad, circumpolar distribution in arctic, sub-arctic and alpine regions of Europe, Asia and North America. It is found on calcareous ridges or fell-fields that have thin snow cover in winter, and it often occurs in nearly pure stands (Elkington 1971; Hultén 1959; McGraw and Antonovics 1983). Each genet starts with a deep-growing tap-like root, eventually dying off. The vegetative growth of *D. octopetala* occurs primarily by the sympodial extension and branching of individual prostrate ramets, with each ramet having between two and five leaves (McGraw and Antonovics 1983; Welker et al. 1993). The genets can grow up to several metres across and commonly live for more than 100 years (Kihlman 1890; Elkington 1971; Crawford 1989).

The deciduous dwarf shrub *Salix herbacea* (Salicaceae) has an amphi-Atlantic but mainly arctic range, being distributed in the northern regions of Europe, western Siberia and North America in addition to the mountainous regions of central Europe (Myklestad and Birks 1993; Beerling 1998). It occurs in the Alps between 2200 m and 2800 m a.s.l. and in the high Arctic in late-melting snow-beds, wind-exposed fell-fields and screes, and as a pioneer on glacier foreland. *Salix herbacea* is a dioecious, prostrate dwarf shrub with an extensive ramifying system of branching underground rhizomes, forming loose, flat mats. Its horizontal growth usually takes place below ground by rhizomes and runners (Hartmann 1957), while the aboveground shoots elongate sympodially and are usually shorter than 5 cm (Resvoll 1917; Wijk 1986). The genets of *S. herbacea* become very large and old; diameters of more than 1 m and life spans of several hundred years have been found (Reisch et al. 2007; Stamati et al. 2007).

The deciduous dwarf shrub *Vaccinium uliginosum* (Ericaceae) occurs throughout both the circum-boreal and the circum-arctic regions, as well as in many southern

mountain areas (Alsos et al. 2005). It is an abundant, sometimes dominant species in boreal mires, shrub lands, forests, dry upland, arctic and alpine tundra, heaths and ridges throughout all successional stages (Schröter 1926). The highly branched *V. uliginosum* exhibits sympodial horizontal growth (Shevtsova et al. 1995) and reproduces clonally by layering or sprouting from rhizomes (Calmes and Zasada 1982; Jacquemart 1996). Ramet life spans of up to 93 years have been recorded (Shevtsova et al. 1995), and the genets can be very long-lived; genets that were 1000 years old or more have been found in the Swiss Alps and in Fennoscandia (de Witte et al. 2012).

The evergreen dwarf shrub *Empetrum nigrum* (Ericaceae) dominates heathland ecosystems on acidic and nutrient-poor soils in cool climates (Tybirk et al. 2000). This drought-sensitive species forms dense mats in nearly monospecific vegetation stands. The young plants have a strong primary root, but as the plant ages a shallow root system with many lateral roots develops (Bell and Tallis 1973). The main form of clonal growth of *E. nigrum* occurs via sprouting from underground or basal meristems. In addition, adventitious roots form where shoots come into contact with the ground. The shoots elongate more or less horizontally and monopodially from a terminal bud formed in the previous season (Hagerup 1946; Shevtsova et al. 1995). *E. nigrum* individuals, if not disturbed by fire, trampling or mowing, can grow very large; a genetic analysis of samples located 40 m apart indicated the same genotype (Szmidt et al. 2002).

Experimental design and measurements

We compared the annual horizontal growth of the species in the Alps, the Carpathians and in northern Norway and northern Sweden (Fennoscandia) in 2008–2010. For each of the five species, we selected four late successional populations in two different geographic regions and separated by at least 50 km within these regions. Where available within the same geographic regions, we additionally selected early successional populations on glacier foreland free from ice for 30–100 years. The location of the 36 studied populations is given in Table 1.

The average annual size increment was estimated for each population, based on year-to-year measurements of approximately 20 randomly selected shoots. These shoots were permanently marked in 2008 with plastic cable ties or cotton twines and, depending on the species, either the terminal shoot or all of the ramifications were measured. Each population was revisited on exactly the same date in 2009 and 2010, to measure the annual horizontal growth of the shoots to the nearest tenth of a millimetre. Growth was measured as shoot elongation in the horizontal direction and orthogonal to the growing front.

Horizontal growth patterns depend on life- or growth-form, and thus can strongly differ among species. We adjusted measurement methods for each species as follows: *C. curvula* has very short internodes between the tillers, and horizontal growth is only visible if a new tiller is developed at the growing front. Therefore, the diameter of new tillers at the growing front was measured at the soil surface. In *D. octopetala* and *V. uliginosum*, the length of the five outermost internodes of the marked shoots was recorded in the first year. In the following 2 years, the changes in internode length and the length of new internodes were measured and summed. Whenever offshoots were produced on the marked shoots that elongated farther than the main shoot, this growth was also recorded as shoot elongation. In *E. nigrum,* the shoot tip position of the chosen shoots was permanently marked by yellow acrylic paint on the undersides of the outermost leaves. In the following years, shoot elongation was measured as the distance from the base of the painted leaves to the shoot tip. The same method was applied to the short above-ground shoots and eventual below-ground runners of *S. herbacea*. In early successional populations, in addition to shoot growth, we also measured the size of each genet by taking the average of the smallest and largest genet diameter because the genet margins were clearly visible.

To compare the local temperature at the late successional sites, we measured soil temperature in almost all of the populations by using small waterproof temperature loggers buried at 5 cm of depth (iButtons; Maxim Integrated Products, Sunnyvale, CA, USA). The total annual precipitation in each of the 2 years was 1810 mm and 1120 mm, respectively, in the central Swiss Alps (Grimselpass, 46.6° N 8.3° E, 1980 m a.s.l.), 915 mm and 935 mm in the eastern Swiss Alps (Segl Maria, 46.3° N 9.5° E, 1798 m a.s.l.; www.meteosuisse.admin.ch), 740 mm and 780 mm in the southern Carpathians (Vf. Omu, 45.4° N 25.5° E, 2500 m a.s.l.; www.wunderground.com) and 670 mm and 815 mm in Fennoscandia (Bardu, 68.7° N 18.8° E, 314 m a.s.l.; eklima.met.no).

Data analysis

The data were analysed using JMP 8 statistical software (SAS Institute Inc., Cary, NC, USA). For the statistical analysis, a mean annual horizontal growth and the coefficient of variation were calculated for each population and year based on the measurements of the individual shoots. We applied a repeated measures analysis of variance (ANOVA) to the mean annual horizontal growth and the coefficient of variation of the individual measurements from all of the 36 populations to investigate differences among the species, between the two successional stages, and between the two subsequent years. Regional effects were not included. Because the sampling was somewhat unbalanced among the regions and successional stages, we applied separate repeated measures ANOVAs to the population mean values that included all of the species and both of the successional stages, and for each species and successional stage separately, to investigate differences among the geographical regions. For the early successional populations, we also tested the effect of genet size on the annual increment of the individual shoots. For the late successional populations, the following variables were calculated based

Table 1. Location of the populations of five clonal arctic-alpine species studied in different geographic regions across Europe. *Dryas octopetala* and *Carex curvula* populations are from the Swiss Alps and the Romanian Carpathians. *Salix herbacea, Vaccinium uliginosum* and *Empetrum nigrum* populations are from the Swiss Alps and Fennoscandia (Sweden, Norway).

Species	Region	Location	Successional stage	Coordinates	Elevation (a.s.l.)	Slope
Carex curvula	Alps	Biedmer, Furkapass	climax	46°33′N 8°23′E	2489	30°, north
	Alps	Valetta Schlattain, St. Moritz	climax	46°30′N 9°48′E	2622	20°, south-east
	Carpathians	Vf. Pietrosu, Borşa	climax	47°35′N 24°38′E	2232	45°, south-west
	Carpathians	Vf. Paltinului, Lacul Bâlea	climax	45°35′N 24°36′E	2265	30°, north-west
	Alps	Muttgletscher	pioneer	46°33′N 8°25′E	2530	20°, north-west
	Alps	Dammagletscher	pioneer	46°64′N 8°45′E	2230	45°, north-east
Dryas octopetala	Alps	Il Jalet, Pas dal Fuorn	climax	46°38′N 10°17′E	2022	45°, north
	Alps	Calanda, Chur	climax	46°53′N 9°28′E	2100	45°, east
	Alps	Bonistock, Melchsee	climax	46°46′N 8°17′E	2130	30°, north-west
	Carpathians	Baba Mare, Buşteni	climax	45°24′N 25°28′E	2260	30°, north
	Alps	Il Jalet, Pas dal Fuorn	pioneer	46°38′N 10°17′E	2022	45°, west
	Alps	Claridenfirn	pioneer	46°85′N 8°86′E	2180	30°, south-east
	Fennoscandia	Kårsajökeln	pioneer	68°22′N 18°22′E	820	30°, north-east
	Fennoscandia	Steindalsbreen	pioneer	69°24′N 19°56′E	480	10°, east
Salix herbacea	Alps	Blauberg, Furkapass	climax	46°34′N 8°25′E	2460	45°, north-east
	Alps	Flüelapass, Davos	climax	46°44′N 9°56′E	2400	30°, north-east
	Fennoscandia	Lulip, Abisko	climax	68°21′N 18°38′E	1060	30°, south-east
	Fennoscandia	Kåfjorddalen, Birtavarre	climax	69°22′N 21°05′E	670	30°, south-east
	Alps	Morteratschgletscher	pioneer	46°26′N 9°56′E	2010	0°
	Alps	Steinlimmigletscher	pioneer	46°43′N 8°25′E	2090	10° north
	Fennoscandia	Kårsajökeln	pioneer	68°22′N 18°22′E	820	30°, north-east
	Fennoscandia	Steindalsbreen	pioneer	69°24′N 19°56′E	480	10°, east
Vaccinium uliginosum	Alps	Gotthard, Andermatt	climax	46°32′N 8°34′E	2085	20°, north-west
	Alps	Stillberg, Davos	climax	46°46′N 9°51′E	2220	20°, east
	Fennoscandia	Lulip, Abisko	climax	68°21′N 18°38′E	1000	10°, south
	Fennoscandia	Steindalen, Elvenes	climax	69°23′N 19°58′E	460	30°, south-east
	Alps	Morteratschgletscher	pioneer	46°26′N 9°56′E	2010	0°
	Fennoscandia	Kårsajökeln	pioneer	68°22′N 18°22′E	820	30°, north-east
	Fennoscandia	Steindalsbreen	pioneer	69°24′N 19°56′E	480	10°, east
Empetrum nigrum	Alps	Gotthard, Andermatt	climax	46°33′N 8°35′E	2085	20°, north-west
	Alps	Piz Calmut, Oberalp	climax	46°39′N 8°41′E	2119	40°, south-west
	Fennoscandia	Torneträsk, Abisko	climax	68°13′N 19°43′E	360	0°
	Fennoscandia	Kåfjorddalen, Birtavarre	climax	69°23′N 21°03′E	670	10°, south-west
	Alps	Morteratschgletscher	pioneer	46°26′N 9°56′E	2010	0°
	Fennoscandia	Kårsajökeln	pioneer	68°22′N 18°22′E	820	30°, north-east
	Fennoscandia	Steindalsbreen	pioneer	69°24′N 19°56′E	480	10°, east

on the cumulative number of days per year with a mean soil temperature above 3.2 °C (Körner 2006): the season length, mean season soil temperature, sum of degree-days above 0 °C (thawing degree-days, TDDs) and the sum of degree-days above 5 °C (growing degree-days, GDDs) representing accumulated 'thermal time'.

Results

Mean annual horizontal growth

The mean annual horizontal growth of the populations was significantly different among the species and between the successional stages (Table 2), with a mean annual growth between 0.46 mm (*C. curvula*) and 13.2 mm (*E. nigrum*) in the late successional populations, and between 0.85 mm (*C. curvula*) and 19.0 mm (*E. nigrum*) in the pioneer populations (Figure 1). The repeated measures ANOVA also indicated that the mean annual horizontal growth differed between the two years (Table 2), with lower annual horizontal growth in 2010, especially in some of the early successional populations. In the pioneer populations of *S. herbacea* and *V. uliginosum*, the shoot increments in 2010 were only about half as large as those in 2009 (Figure 1). Among the three geographical regions, the mean annual horizontal growth was not significantly different for all five of the species, according to the repeated measures ANOVA that included all of the species and both successional stages (Table 3), and based on the comparison within each species separately (Table 4).

Variability within populations

Within the populations, there was a large difference in the annual horizontal growth among the shoots. The coefficient

Table 2. Repeated measures ANOVA of mean annual horizontal growth in 36 populations of five species (2009–2010). Year is the subject of repeated-measures analysis.

Sources of variation	df	*F*	*P*
Between subjects			
Species	4,25	4.90	< 0.01
Successional stage	1,25	6.63	0.02
Within subjects			
Year	1,25	5.70	0.03
Year*species	4,25	1.06	0.40
Year*successional stage	1,25	2.79	0.11

of variation of the mean annual horizontal growth was found to be significantly different among the species (Table 5), with the highest variability in *V. uliginosum* (CV = 0.61) and the lowest in *E. nigrum* (CV = 0.13). However, the variability in the annual horizontal growth was not different among the successional stages, regions and years. In the pioneer populations, a weak positive correlation was found between the annual horizontal growth and genet size in *V. uliginosum* ($r_s = 0.14$, $P < 0.001$, $n = 102$) and *S. herbacea* ($r_s = 0.07$, $P < 0.001$, $n = 169$), but was not found in the other species.

Variability in season length

The shortest mean season length was calculated for sites with *S. herbacea* (108.3 days) and the longest for sites with *D. octopetala* (154.0 days) (Table 6). The lowest mean soil temperature during the growing season was observed in sites with *V. uliginosum* (7.09 °C), and the highest temperature was observed in sites with *D. octopetala* (9.04 °C). The smallest number of TDDs and GDDs was calculated for sites with *S. herbacea* (461.7 / 160.9), and the largest for sites with *E. nigrum* (1107.8 / 464.1). Among the populations of each of the five arctic-alpine species, there was a 61–82% difference in season length between the shortest and longest season across years, and a 68–88% difference in mean soil temperature between the warmest and coldest years.

The season length in 2009 differed among the regions, with the shortest average season length in Fennoscandia (101.5 days), the longest season length in the Carpathians (169 days) and an average season length of 141.3 days in the Alps. The mean soil temperature in 2009 in the Alps (8.27 °C) was similar to that in the Carpathians (8.06 °C), while the temperature was lower in Fennoscandia (6.57 °C). Accordingly, the numbers of TDDs and GDDs were similar in the Alps (886.8/350.46) and the Carpathians (866.8/326.8), but were lower in Fennoscandia (509.36/119.36). Compared with 2009, the season length was on average 20.4 days shorter in 2010 (d.f. = 1,7, $F = 5.64$, $P = 0.05$) in the Alps and Carpathians. During this shorter season, the mean soil temperature was on average 0.91 K higher in most of the sites and, accordingly, the numbers of TDDs and GDDs were higher.

Discussion

Our methods of measurement and standardised sampling design that included several early and late successional populations of five species from different regions across arctic-alpine regions in Europe enabled inferences about environmental factors that could likely affect horizontal growth in clonal plants. The results clearly showed that the annual horizontal growth of arctic-alpine clonal plant species was species specific, even though there was strong stochastic variability among the shoots within every population studied. The hypothesis that populations of the study species exhibited faster dynamics in early successional populations than in late successional populations was supported by the evident differences in horizontal growth found between the two successional stages in all of the regions. Because the sampling was somewhat unbalanced among regions and successional stages, an interpretation of the results concerning the interaction of successional stages and regional climate was not possible. The horizontal growth was smaller in 2010 in most of the populations studied, especially in the pioneer populations of the dwarf shrubs. This finding is in accordance with the differences found in the soil temperatures between the two years. The calculated season length was generally shorter in 2010, but the annual horizontal growth did not differ consistently among the species between the years. For example, the annual horizontal growth was similar in the late successional populations of *C. curvula*, *S. herbacea* and *E. nigrum*, despite the shorter season length in 2010. In contrast, it was unexpected that the population mean values did not differ among regions across Europe, although the differences in season length were larger across regions than the differences between years.

High stochastic variability in annual horizontal growth among individual shoots and considerable variability due to various environmental, ecological or human-induced factors have been reported earlier (Chapin and Shaver 1985; Chapin et al. 1995; Shevtsova et al. 1995; Shaver et al. 1996; Graglia et al. 1997; Kojima et al. 1997; Shevtsova et al. 1997; Welker et al. 1997; Arft et al. 1999; Wada et al. 2002; Sammul et al. 2003; Shevtsova et al. 2005), and demonstrate the need for great caution in any data analysis based on few replicates. In this study, large variability in annual horizontal growth among individual shoots

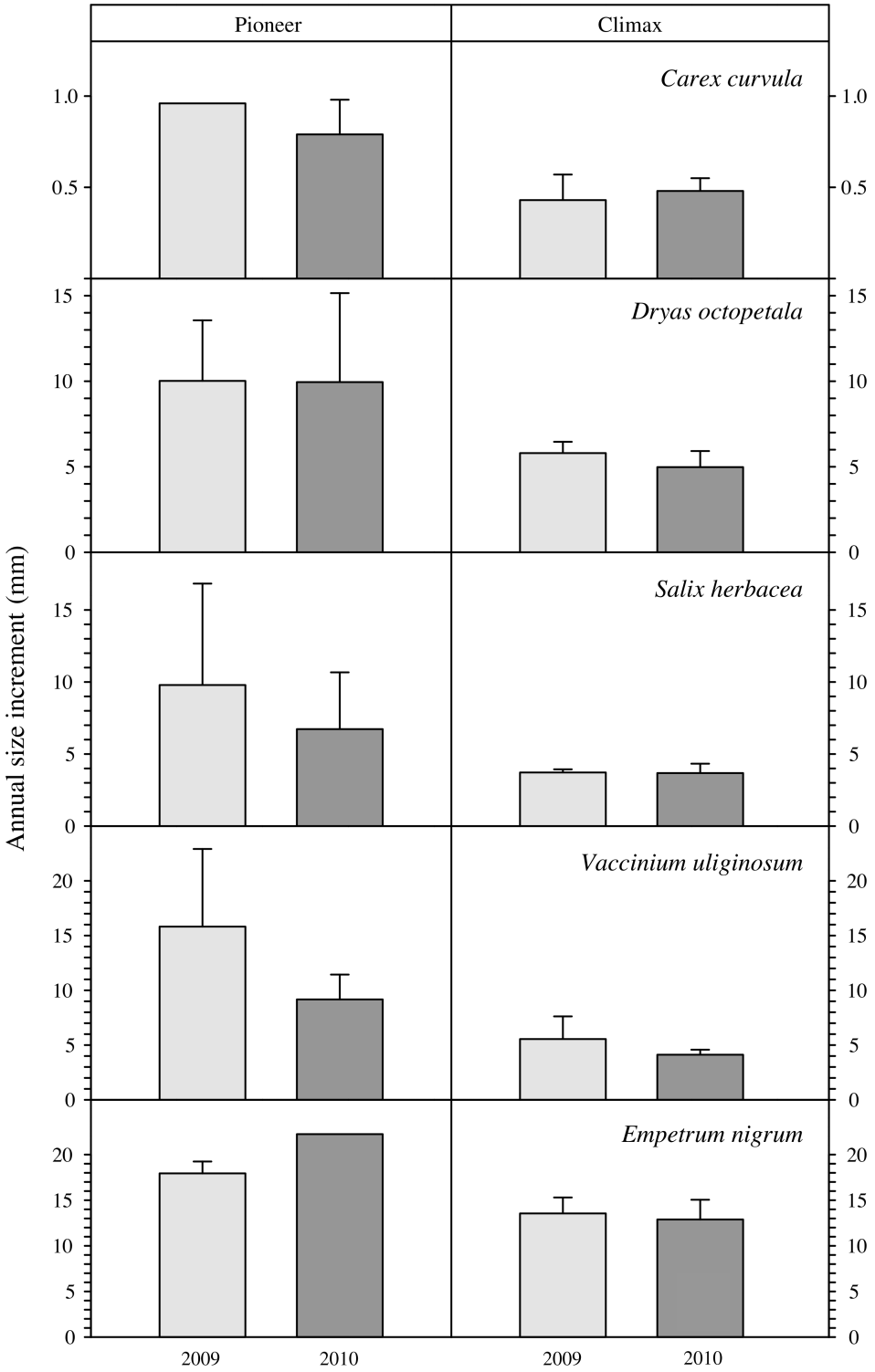

Figure 1. Annual horizontal growth (mean + SE) in climax and pioneer populations of the five clonal species studied, for 2 years separately. Annual horizontal growth was found to be significantly different between species ($P < 0.01$), successional stages ($P = 0.02$) and years ($P < 0.05$; see Table 2).

was observed, and it can be assumed that this result was due to small-scale effects of microhabitat quality influencing the growth dynamics of single shoots. Nevertheless, both the calculated mean annual horizontal growth and the coefficient of variation of each population were found to be species specific, a result that is consistent with the scarce data on horizontal growth in the literature (Bliss 1956, 1971; Shevtsova et al. 1997; Kudo and Suzuki 2003; Wada et al. 2002). Bliss (1956, 1971), for example, found distinct growth patterns for several tundra species in northern

Table 3. Repeated measures ANOVA of mean annual horizontal growth in populations of all five species including both successional stages (2009–2010). Region is the source of variation and year is the subject of repeated-measures analysis.

Sources of variation	df	*F*	*P*
Between subjects			
Region	2,28	1.46	0.25
Within subjects			
Year	1,28	1.58	0.22
Year*region	2,28	0.23	0.80

Table 4. Annual size increment (mean ± SE) in millimeters (mm) in the climax populations of each region for all five species and pooled for both years.

Species	Population	Mean	SE
Carex curvula	Biedmer	0.29	0.12
	Valetta Schlattain	0.38	0.14
	Vf. Paltinului	0.39	0.16
	Vf. Pietrosu	0.76	0.20
Dryas octopetala	Il Jalet	5.55	0.66
	Calanda	6.29	1.32
	Bonistock	5.61	1.54
	Baba Mare	4.12	0.68
Salix herbacea	Flüelapass	3.93	0.96
	Blauberg	4.59	0.69
	Lulip	3.50	0.71
	Kåfjorddalen	2.80	0.36
Vaccinium uliginosum	Gotthard	5.45	2.26
	Stillberg	4.09	3.09
	Lulip	2.08	1.43
	Steindalen	7.73	2.44
Empetrum nigrum	Gotthard	13.80	1.48
	Piz Calmut	15.82	1.10
	Torneträsk	15.50	1.93
	Kåfjorddalen	7.57	1.05

Table 5. Repeated-measures ANOVA of the coefficient of variation of annual horizontal growth in 36 populations of five species (2009–2010). Year is the subject of repeated-measures analysis.

Sources of variation	df	*F*	*P*
Between subjects			
Species	4,25	3.32	0.03
Successional stage	1,25	0.95	0.34
Within subjects			
Year	1,25	0.18	0.68
Year*species	4,25	0.63	0.64
Year*successional stage	1,25	0.48	0.49

Alaska and Wyoming. He also found the horizontal growth of species to vary across microsites and attributed this variation to both genetic tolerances and different environmental conditions. Species-specific growth patterns have also been reported for several dwarf shrubs living in Fennoscandia (Shevtsova et al. 1997) and in alpine regions of Japan (Wada et al. 2002; Kudo and Suzuki 2003).

The sedge species *C. curvula* was found to have the slowest annual mean (± SE) horizontal growth in late successional populations (0.46 ± 0.21 mm), with only 20% of the tillers producing new tillers each year in the Swiss Alps. This result is more or less consistent with previously published data. Grabherr et al. (1978), for example, measured an annual horizontal growth of 0.87 mm and found every sixth tiller of *C. curvula* to produce a new tiller in the Tyrolian Alps. In a field experiment in the Central Alps with elevated CO_2 and mineral fertiliser under seasonally varying summer climates, the annual horizontal growth

Table 6. Temperature variables measured in climax populations. Season length is based on cumulative days with a mean soil temperature above 3.2 °C. The other variables were calculated for the length of the season.

Species	Location	Region	Year	Season length	Mean soil temperature	Sum of degree-days above 0 °C	Sum of degree-days above 5 °C
Carex curvula	Biedmer	Alps	2009	107	9.17	779.11	354.11
			2010	–	–	–	–
	Valetta Schlattain	Alps	2009	155	8.37	1062.63	427.63
			2010	120	10.87	1043.95	563.95
	Vf. Paltinului	Carpathians	2009	148	8.57	797.12	332.12
			2010	–	–	–	–
	Vf. Pietrosu	Carpathians	2009	177	7.14	827.77	247.77
			2010	141	7.14	780.14	240.14
Dryas octopetala	Il Jalet	Alps	2009	123	8.40	655.47	265.47
			2010	–	–	–	–
	Calanda	Alps	2009	161	9.93	1250.70	620.70
			2010	–	–	–	–
	Bonistock	Alps	2009	164	8.77	1165.90	500.90
			2010	140	9.93	1102.43	547.43
	Baba Mare	Carpathians	2009	182	8.48	975.46	400.46
			2010	178	8.45	1081.97	441.97
Salix herbacea	Flüelapass	Alps	2009	125	8.15	366.82	141.82
			2010	–	–	–	–
	Blauberg	Alps	2009	104	7.71	339.31	119.31
			2010	102	8.09	655.61	250.61
	Lulip	Fennoscandia	2009	97	7.14	520.86	155.86
			2010	–	–	–	–
	Kåfjorddalen	Fennoscandia	2009	–	–	–	–
			2010	–	–	–	–
Vaccinium uliginosum	Gotthard	Alps	2009	157	7.44	997.62	327.62
			2010	132	8.80	1170.78	505.77
	Stillberg	Alps	2009	139	7.18	997.74	302.74
			2010	128	7.13	862.91	257.91
	Lulip	Fennoscandia	2009	106	6.00	497.86	82.86
			2010	–	–	–	–
	Steindalen	Fennoscandia	2009	–	–	–	–
			2010	–	–	–	–
Empetrum nigrum	Gotthard	Alps	2009	157	7.44	997.62	327.62
			2010	132	8.80	1170.78	505.77
	Piz Calmut	Alps	2009	162	8.46	1142.14	467.14
			2010	140	9.92	1120.70	555.70
	Torneträsk	Fennoscandia	2009	–	–	–	–
			2010	–	–	–	–
	Kåfjorddalen	Fennoscandia	2009	–	–	–	–
			2010	–	–	–	–

of rhizomes measured in two different control plots was 0.4 mm and 0.7 mm (C. Heid and C. Körner, unpublished). In the same experiment, the above-ground biomass production of *C. curvula* was found to be very sensitive to mineral nutrient additions, but showed no response to CO_2 enrichment (Schäppi and Körner 1996; Körner et al. 1997). Similarly, a presumably higher mineral nutrient availability in the early successional populations may have been the reason for a higher percentage of ramets producing new tillers found in *C. curvula* (44.3%) compared with late successional populations (28.6%). Clonal plants are expected to produce higher biomass and have larger annual horizontal growth rates in early successional sites than in late successional sites, because in pioneer habitats nutrient availability is usually better, and intra-specific competition is absent. Intra-specific competition has been reported to reduce genet and ramet dynamics, including horizontal growth, in late successional populations (Herben and Hara 1997; Suzuki and Hutchings 1997; van Kleunen et al. 2001).

The mean annual horizontal growth rates found here for dwarf shrubs are also consistent with previously published data. The calculated mean annual horizontal growth of *E. nigrum* (13 mm) is very similar to previously measured rates of 11 mm and 15 mm in Fennoscandia (Parsons et al. 1994; Shevtsova et al. 1997). Moreover, this value lies within the range published by Bliss (10–20 mm; 1956; 1971). However, faster growth rates of up to 100 mm

per year have also been recorded (Kojima et al. 1997; Wada et al. 2002). The mean annual horizontal growth found in early and late successional populations of *V. uliginosum* (9 mm) lies in the range of 6–20 mm recorded by Jacquemart (1996), but is lower than the value of 15 mm published by Parsons et al. (1994). The only growth rates previously published for *D. octopetala* were measured in northern England at low altitudes (280/530 m; Elkington 1971), and are much larger (13–39 mm) than the mean annual horizontal growth obtained in this study (8 mm) at much higher altitudes (2020–2130 m). Overall, the consistency of the results with previous findings increases the confidence in the methods used and ensures the precision of the genet age estimations performed based on the size and growth data (de Witte et al. 2012).

For *S. herbacea*, no horizontal growth rates have been previously published, but Wijk (1986) found that the relative shoot-length increment of annual stem segments decreased with increasing shoot age. This observation could be a good explanation for the high variability among the shoots that was found in this species and also in the other four species. In support of Wijk's hypothesis, a weak correlation of annual horizontal growth with genet size was found in *S. herbacea* and *V. uliginosum* growing in the pioneer sites, where the genet size is directly visible. It was not possible to determine visually the size of the genets growing in the late successional sites. This represents a weakness for the analysis of the variability of annual horizontal growth among genets of different size or age classes with respect to regional differences. However, because the population size and age structure in both of the regions was found to be similar for all the species except *D. octopetala* (de Witte et al. 2012), it can be assumed that there are no hidden regional differences in the annual horizontal growth measurements due to size or age differences.

In dwarf shrubs, including *E. nigrum*, great interannual variability has been found in biomass production (Shevtsova et al. 1997). Such variability among different years suggests that plant growth can differ among years due to variable climatic conditions, such as the length of the growing season. We recorded population means that more or less differed in accordance with the season length differences between the two subsequent years. Corresponding to the shorter length of the growing season in 2010 than in 2009, horizontal growth was generally smaller in many of the populations, especially in the early successional ones. However, the differences in horizontal growth in the late successional populations of most species between the two years were very small, suggesting rather stable growth rates in late successional vegetation despite variability in the season length and soil temperature.

The most striking result of this study was that the horizontal growth among populations did not differ within and among regions, despite the large differences in season length among regions. This finding suggests that the horizontal growth rate of species is not strongly affected by the different climatic regimes of distant geographical regions, and probably also not strongly affected by climate changes. There are some other studies in which no climatic effect on the growth of arctic-alpine plants was found. For example, climatic variables were found to contribute only very little to the total variation in the annual size increments of *S. herbacea* ramets (Wijk 1986). Furthermore, Bliss (1956) found no general effect of climate on growth rates, although he showed some correlations for a few plant taxa with either soil or air temperature or both.

In recent climate manipulation experiments, such as the International Tundra Experiment, vegetative horizontal growth was found to respond not at all or only weakly to the warming treatments in most of the studied species (Chapin and Shaver 1996; Arft et al. 1999; Molau 2001). Biomass production, however, was affected by various treatments. Some graminoids and evergreen shrubs, such as *E. nigrum*, produced more biomass, while deciduous shrubs, such as *V. uliginosum* had a reduced biomass production under experimental warming (Chapin and Shaver 1985; Shevtsova et al. 1997; Arft et al. 1999; Suzuki and Kudo 2000; Wada et al. 2002; Kudo and Suzuki 2003) or due to an extension of the season length (Kudo et al. 1999). In general, for the species studied here, climate manipulation experiments seem to indicate changes in biomass production due to climate warming but no clear responses in horizontal growth, consistent with our findings.

Arctic and alpine late successional vegetation types, such as grasslands and dwarf shrub heaths, have been found to be very stable communities that have not been much affected by past and current climate changes (Grabherr 2003). In contrast, an analysis of available observational data has demonstrated range expansions for several clonal species towards higher altitudes or latitudes), e.g. for *C. curvula Festuca alpina* and *Festuca halleri* in the Alps (Pauli et al. 1996; Walther et al. 2002) and *Colobanthus quitensis* and *Deschampsia antarctica* in the Antarctic (Smith 1994; Hughes 2000). The observed mechanism in these range expansions is an increased number of individuals per population as a result of greater seed germination and seedling survival, probably due to warmer air temperatures (Smith 1994). Moreover, recent findings of shrub expansions by clonal plants such as alder, willow and dwarf birch is an indication of vegetation change driven by vegetative growth (Tape et al. 2006). How the vegetation dominated by the species studied here will be affected by range shifts is unknown, but our results indicate that the response of existing vegetation dominated by long-lived clonal plants is slow.

Conclusions

The results of this study are strong evidence for a generally slow, but species-specific horizontal growth by clonal species of different life forms within and among arctic-alpine regions, despite large differences in climate parameters, such as season length or mean soil temperature among the geographical regions. Therefore, the reported annual horizontal growth for the five clonal species studied here represents a realistic approximation for use in studies of

life history and population dynamics. The obtained data are consistent with those of previous studies, including climate manipulation experiments, which demonstrate no correlation between annual horizontal growth and climate warming. This finding indicates that future climate changes might not strongly affect the horizontal growth in dominant clonal plants of cold environments.

As longevity of arctic-alpine plants is thought to enhance population persistence and, thereby, community stability and ecosystem resilience, arctic-alpine late successional vegetation dominated by the species studied here is likely to change slower in a warming climate, in comparison with other vegetation types.

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Chapter 5

Genet longevity and population age structure of the clonal pioneer species *Geum reptans* based on demographic field data and projection matrix modelling

Genet longevity and population age structure of the clonal pioneer species *Geum reptans* based on demographic field data and projection matrix modelling

Stáří genet a populační věková struktura pionýrského klonálního druhu *Geum reptans*, zjištěné pomocí demografických terénních dat a projekčních maticových modelů

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Dedicated to the memory of Leoš Klimeš

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Genet life span is a key demographic trait for understanding life history of plants. However, the longevity of clonal plants is hard to determine, especially when inter-ramet connections are short-lived and plants subsequently move independently of one another in space by means of an expansive growth strategy. In this study we estimated genet life span in the clonal pioneer species *Geum reptans*, living on glacier forelands, by using a projection matrix model based on demographic field data of ramets collected at two sites and in three subsequent years. We then calculated genet age structure at different population ages using multiple simulations, including a maximum carrying capacity and density-dependent mortality. Additionally, we estimated the age of the two field populations by comparing results from simulations with population structure recorded in the field. According to our simulations, more than half of the genets die within the first three decades. However, a considerable proportion survived more than 50 years and some genets even became immortal as they produced so many ramets that the risk of the entire genet becoming extinct was zero. Simulated genet age structures were strongly left skewed with many young and a few very old genets. The rather low carrying capacity was reached only after 350 years, after which density-dependent mortality started to influence genet age structure considerably. The age of the two field populations was estimated to be 250 and 450 years, respectively. Results indicate that in clonal plants, genet immortality can potentially lead to unlimited persistence of established populations. In the case of *G. reptans*, old populations may experience competition and increased mortality due to the ongoing succession in older parts of the glacier foreland that will prevent populations reaching their maximum carrying capacity. But due to the ability of this plant to colonize new sites and follow retreating ice on glacier forelands, populations of *G. reptans* can be very old as recorded here for the two field populations in the Swiss Alps.

Keywords: alpine vegetation, clonal reproduction, demography, genet age, glacier foreland, mortality risk, population persistence, recruitment, Swiss Alps

Introduction

Clonal life cycles and slow growth of individuals are among the most noticeable adaptations of plants in alpine habitats characterized by severe climatic conditions, strong natural fragmentation, sharp boundaries and a high frequency of disturbance (Hartmann 1957, Billings & Mooney 1968, Bliss 1971, Callaghan 1988, Klimeš et al. 1997). Clonality com-

pensates for the loss of parts of plants due to disturbance, and can thereby considerably enhance genet longevity and prolong population persistence over long periods of time (Cook 1979, Eriksson & Jerling 1990). In general, clonal plants strongly benefit from their capability to reproduce asexually as well as sexually, because the product of a single zygote can persist almost indefinitely as the mortality risk of genets is spread among their ramets (Eriksson & Jerling 1990), while sexual reproduction occurs only occasionally (Aarssen 2008).

Age structure and genet turnover in plant populations are determined by genet life span, a key demographic trait for understanding life history (Weiher et al. 1999), population dynamics (Harper 1977, Silvertown & Lovett Doust 1993) and evolutionary fitness (Silvertown 1991). Extended longevity of genets is known to slow down turnover rates of individuals and extend the persistence of populations, especially of clonal plants (Helm et al. 2006), and could play an important role in determining community stability and the vegetation responses to present and future climate change (Steinger et al. 1996, Eriksson 2000, Körner 2003, García et al. 2008, Morris et al. 2008). Even populations that have a negative population growth rate are able to persist for long periods of time due to the longevity and high survival rates of established genets. For example, Eriksson (1994) predicted, based on stochastic matrix models, that populations of *Potentilla anserina*, *Rubus saxatilis* and *Linnaea borealis* consisting of more than 250 ramets are able to persist much longer than 50 years despite a negative population growth rate, just by continuous vegetative reproduction and high adult survival. Often, clonal plants are even considered to be potentially immortal and the several extremely long life spans reported seem to confirm this (Thomas 2002, de Witte & Stöcklin 2010).

Unfortunately, there is little reliable data on genet life span and population age structure for clonal plants, because, in contrast to ramet age, genet age is difficult to measure (Dietz & Schweingruber 2002, de Witte & Stöcklin 2010). Due to the longevity of many clonal species, it is impractical to follow cohorts of ramets from birth until death of the genet. Moreover, there are two different types of clonal growth strategies in herbaceous plant species, for which the application of a common method for estimating age is not appropriate (de Witte & Stöcklin 2010). In clonal plants that form clearly delimited and dense patches and have long-lasting inter-ramet connections, genet age can be estimated using indirect methods based on genet size and increase in size over time. However, in spreading clonal plants with decaying inter-ramet connections, these methods cannot be used because genets become fragmented and genet size is poorly correlated with genet age. Currently for such plants, the only feasible alternative approach is a demographic analysis based on population growth models.

Some researchers have used population transition-matrix models to understand life history and investigate population dynamics and individual longevity in plants (Callaghan 1976, Hamilton et al. 1987, Cochran & Ellner 1992, Erschbamer 1994, Erschbamer & Winkler 1995, Molau 1997, Erschbamer et al. 1998, Barot et al. 2002, Diemer 2002, Ehrlén & Lehtilä 2002, Nicolè et al. 2005, Weppler et al. 2006). These models have increased the understanding of the structural and demographic properties of plant populations, especially for clonal species. For example, they allow us to study long-term population demography based on short periods of observation (Watkinson & Powell 1993). Moreover, they can be used to determine the age structure of populations rather than estimating the age of individuals, a crucial issue for understanding population demography

and viability. An example is the investigation of the life history of *Silene acaulis* inferred from size-based population projection matrices (Morris & Doak 1998). This study revealed a life expectancy of more than 300 years for 1.8% of the newborn of this herbaceous alpine plant, while 8.0% survived at least 50 years. Demographic data on long-lived clonal plants at the genet level is still scarce (Menges 2000) and there is a need for studies that use population models to investigate individual longevity and population persistence.

Here we present a study of genet longevity and population age structure of the herbaceus *Geum reptans* based on demographic modelling. Because of its well-known morphology (Conert et al. 1995, Pluess & Stöcklin 2005, Weppler & Stöcklin 2005, 2006), demography (Weppler et al. 2006), genetic constitution (Pluess & Stöcklin 2004) and dispersal ability (Pluess & Stöcklin 2004, Tackenberg & Stöcklin 2008) the alpine clonal pioneer species *G. reptans* is ideal for such a study. The projection-matrix models are based on empirical data collected on two glacier forelands in the Swiss Alps. Our particular objectives are (i) to assess genet longevity of *G. reptans*, (ii) to investigate the genet age structure in established populations and (iii) to estimate the age of the two field populations by comparing results from simulations with the genet stage structures recorded in the field. Finally, this study provides an opportunity to discuss the effect of clonality on life span, genet turnover and persistence of a plant during primary succession on glacier forelands.

Materials and methods

Study species

Geum reptans L. (*Rosaceae*) is a perennial clonal rosette plant widely distributed from the Central European Alps eastwards to the Carpathian Mountains. It is one of the first pioneer species to colonize protosoils left by retreating glaciers (Braun-Blanquet 1948) and occurs preferentially on moist moraines and alluvial soils of glacier forelands, screes and mountain ridges between 1950 and 3800 m a.s.l. (Conert et al. 1995). With ongoing succession the pioneer species is outcompeted by grasses and dwarf shrubs (Lüdi 1921). *Geum reptans* is hardly ever found in alpine grasslands (Rusterholz et al. 1993).

As a hemicryptophyte, *G. reptans* produces clusters of rosettes sprouting from a monopodially and vertically growing rhizome (epigeogenous rhizome; Klimešová et al. 2011) in spring. Vegetative growth of a plant results in an increase in the number of side rosettes sprouting from the rhizome. The age at first reproduction is several years and depends on environmental conditions (Weppler et al. 2006). *Geum reptans* reproduces clonally by producing new rosettes at the end of above-ground stolons and sexually by seeds as an outbreeder via predominantly fly-pollinated flowers (Conert et al. 1995, Hess 2001). Both flower heads and stolons develop from axial leaf buds that were initiated in the previous year. Connections (stolons) between mother plant and daughter rosettes can be at least 1 m in length and decay within a year. Therefore, the size of genetic individuals (genets) cannot be distinguished by eye. Herbchronology revealed that single plants (ramets = cluster of rosettes attached to a single rhizome) are never older than about 40 years (J. Stöcklin, unpublished). Molecular analysis indicates that ramets that are > 4 m apart usually belong to different genets (Pluess & Stöcklin 2004). The seeds are adapted to wind dispersal (Tackenberg & Stöcklin 2008). Mechanistic modelling showed that seed

dispersal is limited, with only a small fraction being dispersed further than 10 m (0.03%; Tackenberg & Stöcklin 2008). *Geum reptans* does not have a persistent seed bank (Schwienbacher & Erschbamer 2002, Schwienbacher et al. 2010).

Empirical data for the simulation of demographic properties

The demographic data for *G. reptans* was collected over three years (two transitions) on two rocky glacier forelands in the Swiss Alps: Vadret da Porchabella and Furkapass (Weppler et al. 2006). The ramets were divided into five different life-cycle stages according to Weppler et al. (2006; Fig. 1): seedlings; juveniles; small adults; medium-sized adults; and large adults. Seeds germinate soon after snowmelt in the year following their production. Juveniles are young ramets that originate from sexual reproduction and are older than one year but not yet reproducing. Small, medium and large adults have increasing numbers of leaf rosettes and form three stages of reproducing ramets. The population growth rates (λ) of *G. reptans*, obtained from matrix modelling, varied between 0.999 and 1.074, and the variation among years was greater (up to 7%) than among populations (0.2%). The estimated population size was between 5000 and 20,000 plants on the glacier foreland where the study populations occurred (Weppler et al. 2006).

Modelling assumptions and simulation methods

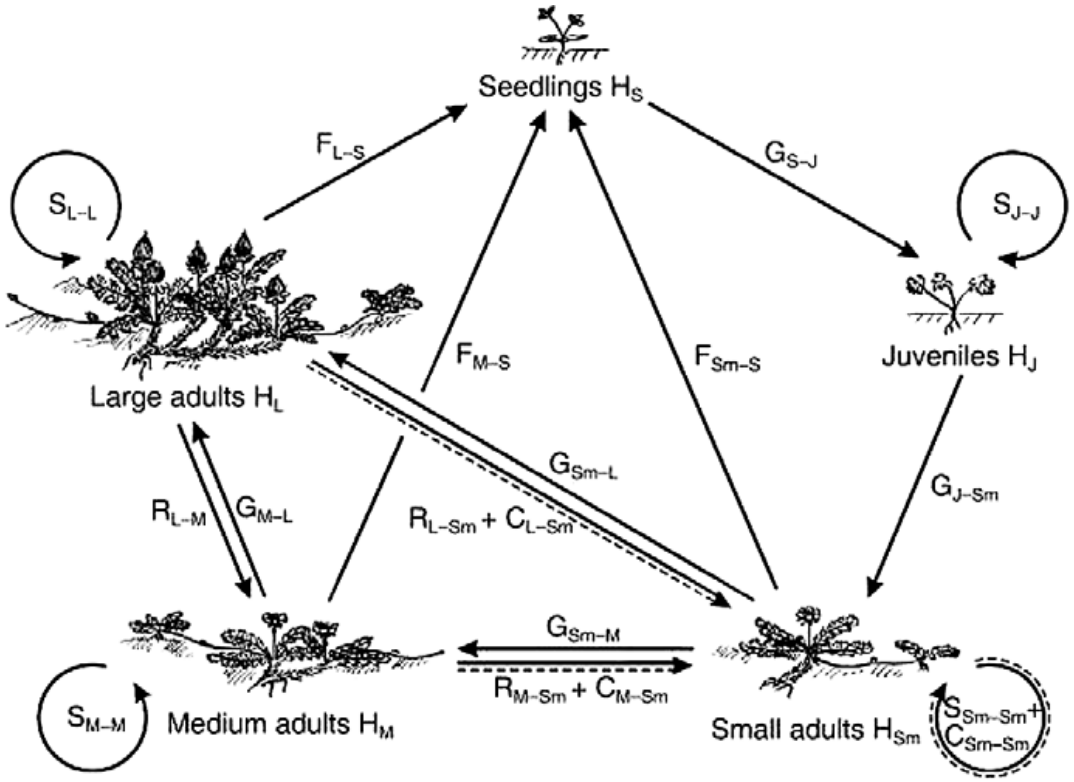

Fig. 1. – Life cycle of *Geum reptans* with five stages (H_S, seedlings; H_J, juveniles; H_{Sm}, small adults; H_M, medium adults; H_L, large adults). Transitions between stages represent: G, growth; S, stasis; R, retrogression; F, sexual reproduction. Clonal reproduction (C) is included in the transitions $S_{Sm–Sm}$, $R_{M–Sm}$ and $R_{L–Sm}$ and is represented by a dashed line. Note that the life cycle corresponds to the matrix population model (taken from Weppler et al. 2006, published with permission).

The small differences among populations allowed us to pool all the data from different years and the two sites (Table 1), resulting in a data set consisting of 1827 transitions of ramets. The model used for demographic simulations was a linear stage-classified population projection matrix model (Caswell 2011) of the form:

$$n_{t+1} = An_t,$$

where n_t is a vector of k stage classes and A is a square matrix of dimension k. The elements of A are stage-specific fecundities and/or transition probabilities that describe the generation of ramets in each class by ramets in all other classes from one year to the next. The geometric growth rate of the population when the population has reached a stable distribution of stages is the dominant eigenvalue of A, λ. The right eigenvector of A, w_m, describes the stage distribution of the population

$$Aw_m = \lambda_m w_m$$

and is the proportional representation of each stage class once the population has reached the equilibrium growth rate (Caswell 2001).

Table 1. – Average transition matrices of two populations of *Geum reptans* (Vadret da Porchabella, Furkapass) during a study period of 3 years. Transition matrices for each population contain the life-cycle stages (seedlings; juveniles; small, medium and large adults) and the probabilities of ramets remaining in the same stage or changing to another stage, respectively, within one year. Transition probabilities reflecting sexual reproduction (new genets) are given in italics. Probabilities of transitions to the stage of a small adult are based on values for survival of small adults or retrogression of medium or large adults from the previous year or production of new small adults via clonal reproduction (new ramets, second value in bold type). After Weppler et al. (2006).

	Seedlings	Juveniles	Small adults	Medium adults	Large adults
Vadret da Porchabella, 2000–2002 (λ=1.051)					
Seedlings	–	–	*0.02*	*0.08*	*0.22*
Juveniles	0.95	0.57	–	–	–
Small adults	–	0.32	0.89+**0.03**	0.2+**0.08**	0+**0.14**
Medium adults	–	–	0.08	0.67	0.07
Large adults	–	–	0.01	0.12	0.92
Furkapass, 2001–2003 (λ=1.038)					
Seedlings	–	–	*0.14*	*0.33*	*0.31*
Juveniles	0.90	0.60	–	–	–
Small adults	–	0.21	0.85+**0.02**	0.21+**0.22**	0.05+**0.05**
Medium adults	–	–	0.06	0.71	0.18
Large adults	–	–	0.01	0.08	0.73

The demographic parameters of a population used in the simulations are influenced by several factors. Demographic stochasticity (inherent variation in individual time of death and reproductive rates that are not due to differences in ecological condition; Goodman 1987) will introduce variation into the demography of a population in a given time period. Variation in environmental conditions influences the whole population, and the microclimatic changes and differences alter the survival probabilities and fecundities of single

ramets (environmental stochasticity; Tuljapurkar 1989). Additionally, it is possible that several elements of the transition matrix are not independent and covary (Gani 1987). Ideally, all these factors should be incorporated into the design of empirical studies and simulations of population demography. However, this is often impossible, and especially the covariation between different elements of the transition matrix is difficult to measure in long-lived plants.

In our models we accounted for demographic and environmental stochasticity. The demographic stochasticity was implemented by a multinomial distribution for transitions and a Poisson distribution for fecundities according to Akcakaya (1991). The environmental stochasticity was simulated by bootstrapping 50% of all ramets in each stage class in our dataset to parameterize a transition matrix for each year of a simulation. These transition matrices include both spatial and temporal variance, because we pooled data from different sites and years. To produce a realistic yearly dynamic for *G. reptans*, every time step (year) in the simulation included survival taking into account mortality during winter and growth by clonal reproduction in spring. This was followed by sexual reproduction in summer. Possible effects of intra- and interspecific competition were not simulated here because the influence of competition in open and nutrient-rich habitats such as glacier forelands is likely to be low. Nevertheless, the influence of competition is implicitly incorporated in our model by the fact that the demographic parameters were based on a study of the field populations where natural competition is assumed to occur.

Life span of genets

To estimate the life span of a genet under natural conditions we simulated the survival and clonal growth of genets based on the demography recorded in well-established populations (Weppler et al. 2006). Sexual reproduction was not included in these simulations, as this would produce new genets. We ran 1,000,000 simulations starting from either a 'seedling' (stage 1), a 'juvenile' (stage 2) or a 'small adult' (stage 3). Each genet was modelled separately assuming no restrictions on space, resources or competition, because the possible influences of these factors were already incorporated in the field data. Therefore these simulations considered genet development in an established population. At each time step each ramet of a genet either survived/grew or died based on a multinomial distribution. Additionally, at each time step, new ramets are produced by clonal growth based on a Poisson distribution. Simulations of a genet were run for a maximum of 2500 years or until all ramets of the genet were dead. Results are presented as a frequency distribution of modelled genet age from all simulations performed.

Genet age structure in established populations

To determine the theoretical age distribution of genets after 100, 250, 500, 1000 and 2500 years, we ran another type of simulation starting with a 'newly established' population of 10 'seedlings' (10 different genets). These scenarios were based on the demographic parameters recorded by Weppler et al. (2006) and growth conditions in established populations as no data on "virgin" habitats were available. These simulations included sexual and clonal reproduction. To prevent unrealistic population sizes we set a maximum carrying capacity of 10,000 ramets and applied a density function of the type 'ceiling' for ramets (Caswell 2001). Most observed populations had between 5000 and 20,000 ramets

depending on the size of the glacier foreland and therefore 10,000 ramets seemed reasonable since the field data suggest that natural populations rarely reach the carrying capacity because of the high dynamics of the habitat (glacial retreat and succession).

While we made no distinction between clonally and sexually produced plants for the total population size of ramets and the ramet carrying capacity, sexual reproduction created new genets every year. Based on the above simulations of genet life span we calculated the life span for each newly emerged genet and the resulting residence time in the population. But as the genet life span simulations were density-independent (no maximal carrying capacity) the number of genets was still increasing, even when the number of ramets in these simulations was already at the carrying capacity. To prevent the number of genets exceeding the number of ramets (which is impossible in nature) the carrying capacity for genets was defined separately. The carrying capacity for genets was set at 7500, which is 75% of the ramets carrying capacity. Therefore, at the carrying capacity of ramets at least 25% of the ramets need to be clones. We chose 75% as the data of Weppler et al. (2006) showed that clonal and sexual reproduction are almost similarly important for the population dynamics and therefore a rather large proportion of clonal offspring must be assumed.

To regulate the number of genets we tested two types of density-dependent mortality. The first type assumes age-independent mortality of genets ('random') with all genets having the same density-dependent mortality independent of their actual size when the threshold for genets was reached. The second type assumes older genets have a lower density-dependent mortality than younger ones ('age-dependent'), as genets with a large number of ramets are less likely to disappear. In our model, age-dependent mortality of genets means that if the carrying capacity is reached, no new genets (seedlings) can establish until genets disappear by density-independent mortality. We applied these two different density-dependent mortalities to determine whether and how strongly the models are affected by our assumptions. All models were implemented and evaluated with R 2.10.1 (R Development Core Team 2009).

Age estimation of field populations

Based on the average transition matrix of Weppler et al. (2006) we calculated the mean time spent in subadult classes ('seedlings' and 'juveniles') according to the simplified formula of Barot et al. (2002):

$$E\left(\tau_{seed}, s\right) = 1 + \sum_{i=1}^{s-1} \frac{1}{1 - p_i}$$

where τ_{seed} is the first stage, s the first adult stage and p is the survival probability in stage i. This formula was used to calculate the proportion of subadults among all ramets in our modelling results.

The comparison of the modelled subadult proportion with the proportion observed in field populations allowed us to roughly estimate their age. This is possible because subadult individuals must be "new" single-ramet genets, as subadult ramets cannot be produced by clonal growth or retrogression. Based on the assumption, that the proportion of "old" genets increases with time, the proportion of subadult ramets is a rough indicator of the age of a population.

Results

Life span of genets

The simulations of the life span of genets starting with 'seedlings' showed that 56% of the genets died before they reached reproductive age, which is on average 3.46 years (Fig. 2). The simulations starting with 'juveniles' gave similar results as 51% of the genets died before reproducing. When the models were started with 'small adults' (reproductive stage 3) they predicted a weaker peak in mortality in the first years, with more than 80% of the genets dying in the first 30 years, which is approximately the life span of large ramets (Weppler et al. 2006). In all simulations, however, a considerable proportion of genets survived for more than 50 years (6.6–13.2%), 100 years (4.3–8.7%), 500 years (3.0–6.1%) and even 2500 years (2.9–5.9%; Fig. 2). It is important to bear in mind that the model predicts the age of genets and not the age of ramets, which is rarely more than 50 years.

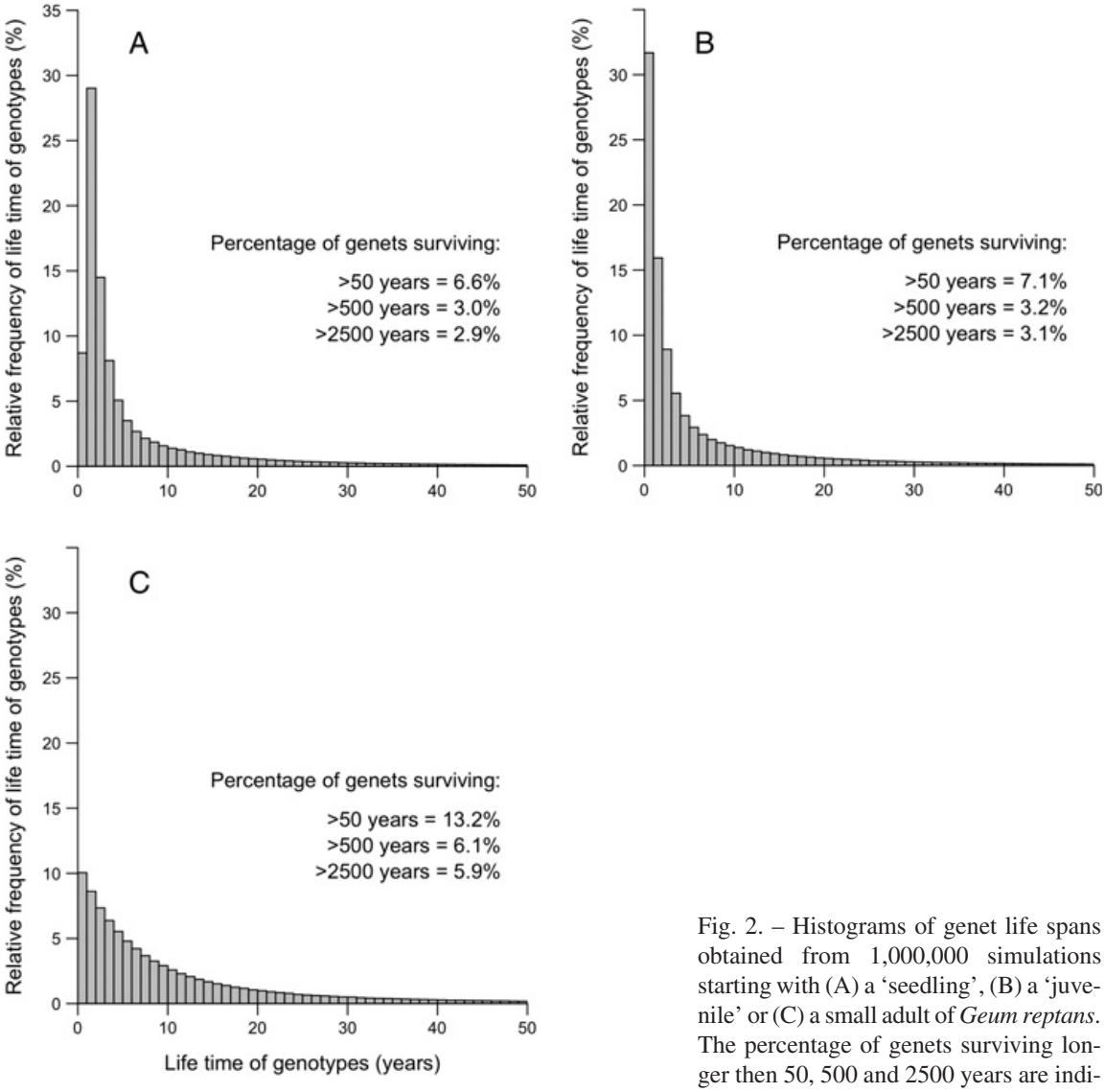

Fig. 2. – Histograms of genet life spans obtained from 1,000,000 simulations starting with (A) a 'seedling', (B) a 'juvenile' or (C) a small adult of *Geum reptans*. The percentage of genets surviving longer then 50, 500 and 2500 years are indicated within each panel.

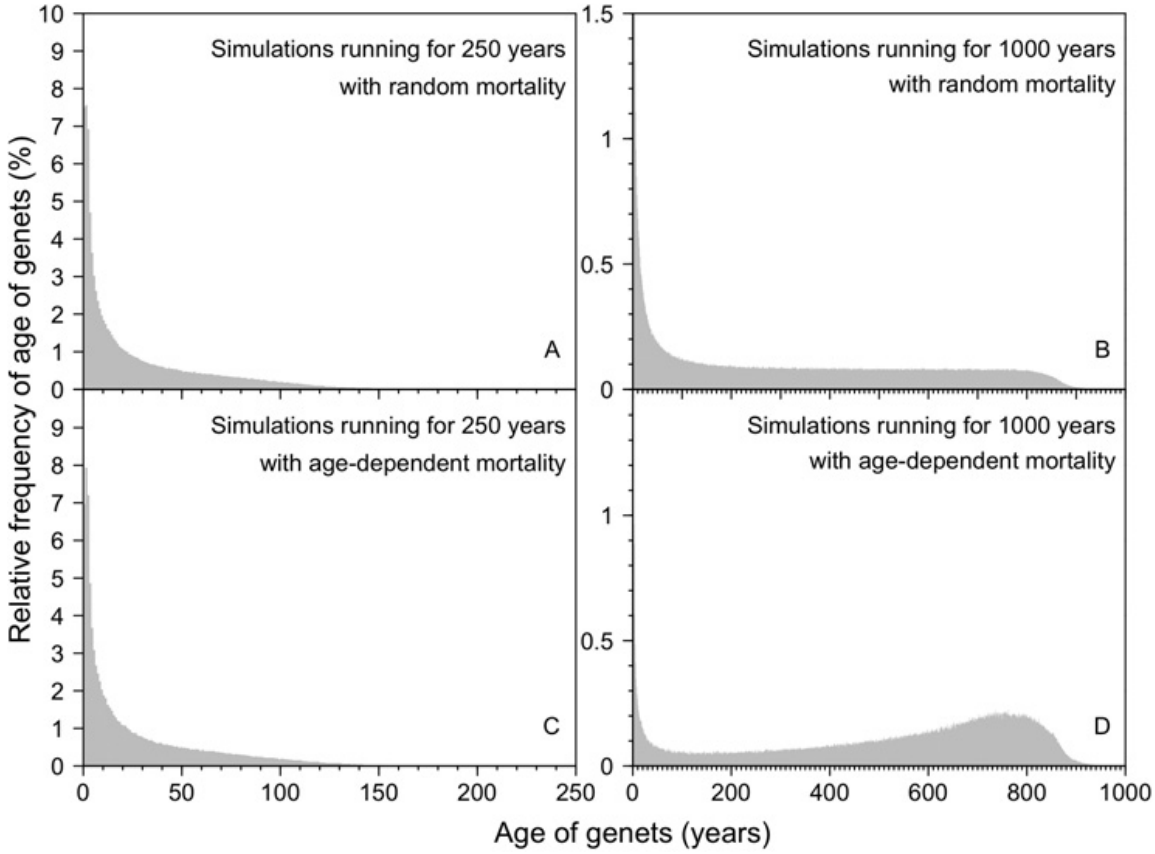

Fig. 3. – Genet age structure of *Geum reptans* populations emerged from 10 'seedlings' after 250 and 1000 years. A – genet age structure after 250 years assuming random mortality of genets due to density; B – same population as A after 1000 years; C – genet age structure after 250 years assuming strong age-dependent mortality of genets due to density; D – same population as C after 1000 years.

Genet age structure in established populations

The simulations predict no difference in the genet age structure of established populations with random or age-dependent mortality due to density effects in populations younger than 250 years (Fig. 3). In these "young" populations the age structure was always strongly left skewed, reflecting a large proportion of genets in subadult stage classes. This left-skewed age distribution is typical for the exponential growth phase of populations that have not yet reached carrying capacity, which took approximately 350 years in the simulations.

Density-dependent mortality started to influence population age structure only in populations older than 500 years. In simulations with 'random' density-dependent mortality a small but stable proportion of very old genets were predicted after 500 years, though the young genets still dominated the population (Fig. 3). Assuming 'age-dependent' mortality led to an increasing proportion of very old genets in populations older than 500 years, because after reaching the carrying capacity few new genets were able to establish.

Estimated age of field populations

Based on the average projection matrix the mean time spent in non-reproductive classes (stage 1 and stage 2) in the simulations was about 3.46 years (formula of Barot). By using this threshold in the simulations the proportion of subadult ramets (stage 1–2) among all

ramets (stage 1–5) in populations of different ages were obtained (Table 2). As there were two different types of density dependent mortality of genets (random mortality and age-dependent mortality) a (broad) range of proportions of subadults were obtained, especially if the model ran for long periods of time. However, in simulations that ran for less than 500 years all scenarios showed similar proportions of subadults. The two populations studied by Weppler et al. (2006) showed the subadult proportions of 20% at the Furkapass and 10% at Vadret da Porchabella. By comparing this field data with the simulation results (Table 2) a predicted age of a little more than 250 years for the population at Furkapass and 450 years for that at Vadret da Porchabella were obtained.

Table 2. – Simulation-based proportion of subadult ramets (stage 1–2) among all ramets (stage 1–5) in populations emerged from 10 seedlings after 100, 250, 500, 1000 and 2500 years, presented for two scenarios with regard to the type of mortality. Population age represents time since establishment. Means ± standard deviations of subadult proportions (%) are shown.

	Mortality type	
Population age (years)	Random	Age-dependent
100	44.8±6.7	45.3±4.9
250	24.2±3.0	24.4±5.6
500	15.2±1.0	7.9±0.9
1000	8.9±0.6	2.1±0.2
2500	4.1±0.2	0.1±0.1

Discussion

The results indicate that most genets of *Geum reptans*, despite their marked ability to reproduce clonally, die at young ages. But a small percentage of genets are very old and achieve immortality by producing many ramets and spreading the risk (Eriksson & Jerling 1990). The left-skewed frequency distribution of genet age in populations will only change when the carrying capacity is reached and density-dependent mortality becomes strong, which affects predominantly young and small genets. This is probably rare in many natural populations of pioneer plants living at high altitudes and growing slowly.

When discussing these results, it should be kept in mind, that there are a few caveats about the way the projection-matrix models were constructed and the simulations run. First, there may be some variation in transitions that were not detected with the sample sizes used to parameterize the model. Second, our estimated rates of population growth and genet or ramet survival are based on only four annual transitions, and it was assumed that the year-to-year variation is not higher than between these transitions. Thereby we might have excluded extreme events affecting germination rate, mortality and growth. Finally, we did not include any spatial structure in our simulations and assumed unlimited dispersal. This could have influenced the results by affecting parameters such as population growth rate and the mortality risks of genets. However, it is unlikely that these caveats affected the overall pattern of the results and had little effect on the quantitative predictions.

Life span of genets

Our results indicate that the initial mortality of *G. reptans* is rather high and decreases markedly with increasing age, implying that most genets die within the first three decades of their life. Some genets even seem to become immortal, probably because with increasing age the genets also increase in number of ramets. Thereby, the risk of mortality of a genet is reduced as it is spread among all the ramets produced during the genet's lifetime. Tanner (2001) reports an increasing 'expected remaining life span' (ERL), based on risk spreading, for other clonal species like *Potentilla anserina*, which has a similar growth strategy to *G. reptans*.

The frequency distributions of genet life spans obtained from simulations starting with three different stages are very similar (Fig. 2), except the initial mortality is low when the simulation started with already well-established ramets (small adults). But even in this case, only a fifth of the genets survived more than 30 years. The reason for this high initial mortality is the fact that only a small proportion of genets produce clonal offspring and many of them die before they reproduce vegetatively for the first time. The extreme longevity of a few genets, together with a strongly left-skewed genet age structure, are also found in other alpine clonal plants such as the tussock grass *Carex curvula* and the dwarf shrubs *Rhododendron ferrugineum*, *Vaccinium uliginosum*, *Salix herbacea* and *Dryas octopetala* (Steinger et al. 1996, Pornon et al. 2000, L. C. de Witte et al., unpublished). Comparison with these species should, however, be treated with care, as these species have a completely different growth strategy and usually form large and dense patches that dominate late-successional vegetation. In addition, the genet age of such species is estimated based on growth-ring analysis or indirect methods using size and growth-rate measurements (de Witte & Stöcklin 2010). This method of ageing genets cannot be used for species like *G. reptans*, because its genets consist of independent ramets and do not form clearly delimited dense patches of which the size and yearly expansion can be measured. In *G. reptans*, new daughter ramets can easily grow one metre away from the mother plant and the inter-ramet connections (stolons) are lost within one year. Therefore, the only possible method of estimating genet age in *G. reptans* is to simulate genet longevity based on demographic data and a matrix model, as in this study. Nevertheless, it is noteworthy that the pattern of a left-skewed frequency distribution of genet age in a clonal plant with a growth strategy consisting of short-lived above ground stolons, is similar to distributions of genet age in clonal plants that grow in large and dense homogeneous populations and other perennial plants measured in the field. This may indicate that the basic demographic properties of slow-growing clonal plants are very similar, irrespective of their growth strategies. A similar left-skewed frequency distribution of genet age is also recorded for the clonal species *Carex curvula*, *Dryas octopetala*, *Salix herbacea* and *Vaccinium uliginosum* (L. C. de Witte et al., unpublished). Also the study of Colling & Matthies (2006) on the non-clonal perennial *Scorzonera humilis* revealed a low mortality of adults, a life expectancy of several decades and a left-skewed frequency distribution of genet age at nutrient-poor sites where populations had a positive growth rate. In contrast for populations at nutrient-rich sites with low recruitment rates and negative population growth they record a right-skewed frequency distribution.

Despite the low frequency of old genets, our simulations indicate that in clonal plants with the capacity to potentially increase genet size indefinitely by producing new ramets, a small proportion of genets are practically immortal. To our knowledge, this is the first

time that the potential immortality of a clonal plant has been demonstrated based on field measurements and matrix modelling. An important consequence of such immortality is the practically unlimited persistence of a once established population of a clonal plant, when no extrinsic factors are destroying the population. In the case of *G. reptans*, populations are therefore likely to persist forever, if the ongoing succession does not displace pioneer stages on the glacier foreland resulting in alpine grasslands or dwarf shrub heaths. It is noteworthy that *G. reptans* occurs at altitudes where succession progresses very slowly or sometimes not at all.

Genet age structure in established populations

Genet age structure is not influenced by density- or age-dependent mortality, as long as the population does not reach carrying capacity. The carrying capacity used in our model was based on measurements of population size at field sites. As the vegetation on glacier forelands where *G. reptans* occurs is usually still open and at an early stage of succession, the carrying capacity we used may be considered as too low. Nevertheless, in the simulation, the carrying capacity of genets is first reached after 350 years. Only then does density-dependent mortality start to have a considerable affect on genet age structure, depending on the type of mortality (Fig. 3C, D). Random mortality, in which genets die independent of their age, results in the high mortality of young genets and more genets with a high ERL, which remains constant over the whole simulation period. However, age-dependent mortality results in an increase in the number of older genets each represented by many ramets, which reduces the risk of genets becoming extinct and results in a strong increase in genets with a high ERL. This implies that young genets are unlikely to survive in populations approaching the carrying capacity. Clearly, in natural populations approaching the carrying capacity, age-dependent mortality of genets is more realistic than random mortality, as old genets consist of more ramets and survive even when some ramets die. The simulations predict that very old populations of *G. reptans* are likely to be composed of mostly old genets and the establishment of new ones is prevented as long as density-dependent mortality or other disturbances do not create new open spaces for seedlings. Interestingly, however, the carrying capacity is reached only when the population is several hundred years old. Such old populations of *G. reptans* will probably occur only in older parts of glacier forelands where they are already exposed to competition and a high risk of mortality due to ongoing succession. The results thus indicate that populations of *G. reptans* on glacier forelands are usually not subjected to strong density-dependent mortality and exhibit left-skewed genet age distributions with a few very old and potentially immortal genets. This conclusion is consistent with the observation that *G. reptans* mostly occurs at pioneer sites where there is little vegetation cover and hence little competition.

Estimated ages of field populations

If the proportion of subadults predicted by simulations with age-dependent mortality are used to estimate the age of the field populations studied in the Swiss Alps by Weppler et al., then they are a few hundred years old. This seems quite realistic for a pioneer species on glacier forelands above 2000 m a.s.l. *Geum reptans* is able to colonize new soil, e.g. left by retreating glaciers, by means of wind-dispersed seeds or even by vegetative growth, as stolons easily grow up to 1 m in length. It is a reasonable assumption that the colonization

capacity of *G. reptans* is sufficient to escape competition resulting from primary succession and to colonize freshly deglaciated bedrock and soil, provided there is sufficient moisture. The field populations, the demographic measurements of which were used in the simulations, are according to the estimates, either the remains of a more widespread occurrence of *G. reptans* during the postglacial period when glaciers started to retreat, or from the Little Ice Age with several cooler periods and glacial expansions between 1350 and 1820 when temperatures in the Alps dropped considerably (maximal –4.5°C; Büntgen et al. 2006). From 1850 onwards, glaciers in the Alps were retreating steadily, creating new space for population expansion. Thus, today we see populations on glacier forelands that have expanded steadily for more than 150 years. Clearly, at this age, density-dependent mortality has an insignificant effect on the genet age structure, as revealed by the simulations.

Ramet and genet demography

Currently there are only a few studies explicitly on both ramet- and genet-level dynamics (e.g. Hartnett & Bazzaz 1985, Eriksson 1986, 1988, Karlson 1991, Eriksson 1994, Damman & Cain 1998). Most ecological studies do not consider the genet level but take ramets as the individual unit and look at the demographic properties of populations without regard to genetic identity. Consequently, matrix models or other types of models have hardly been used to investigate genet longevity and population age structure in long-lived clonal plants (Menges 2000). A notable exception is the matrix model study of the endangered herbaceous plant, *Scorzonera humilis*, by Colling & Matthies (2006). As in this study they also record a high ERL of adult genets. Ehrlén & Lehtilä (2002) review the use of population matrix models to determine the longevity of 71 species of herbaceous perennials. They report ramet life spans ranging from 4 to almost 1000 years, with more than half of the species studied having a ramet life expectancy of more than 35 years. But even in this study, genet life span was not investigated. The reason for this is the great difficulty in identifying genets, measuring genet sizes and estimating genet life spans of clonal plants.

A future task will be to use demographic techniques on genet data obtained by molecular genotyping studies to make more accurate predictions at the genet level. For example, a combined demographic-molecular approach is likely to reveal spatial and temporal patterns at the genet level and thus characteristics particularly relevant to clonal life histories and population viability (e.g. de Steven 1989, Torimaru & Tomaru 2005, Araki et al. 2009).

Conclusions

The results demonstrate that predicting life-history parameters, such as genet longevity or genet age structure of populations, using simulations based on projection matrix models is a promising way of reaching a better understanding of the dynamics of long-lived clonal plants such as *Geum reptans*. Especially for clonal plants with a growth strategy that includes a great spacing out of ramets and decaying connections, this demographic approach to longevity is a valuable achievement as other methods are not suitable for estimating genet age. Moreover, this approach enables the analysis of the age structure of whole populations and, therefore, to investigate population structure and demography. We hope that such an approach will be applied to other populations and species in the future, enabling comparisons between species and a better insight into the life history of clonal plants in general.

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Souhrn

Délka života genety je klíčovým demografickým parametrem, u rostlin s klonálním růstem se však určuje obtížně, zejména když spojení mezi rametami přetrvává krátce a rostliny poté obsazují prostor nezávisle na sobě. V práci jsme pomocí projekčních maticových modelů, založených na demografických datech sbíraných na dvou stanovištích po dobu tří let, studovali délku života genety klonálního druhu *Geum reptans*, který je pionýrským druhem sukcese na předpolí ledovce ve Švýcarských Alpách. Pomocí počítačových simulací, zahrnujících maximální nosnou kapacitu prostředí a na hustotě závislou mortalitu, jsme spočítali věkovou strukturu genet v různě starých populacích a srovnáním těchto simulací s terénními daty jsme odhadli skutečné stáří dvou populací v terénu. Simulace ukázaly, že více než polovina genet uhyne během prvních tří desetiletí, značná část však přežívá více než 50 let a některé jsou v podstatě nesmrtelné, protože při akumulaci dostatečného počtu ramet je riziko úhynu nulové. Simulovaná věková struktura genet měla výrazně šikmé rozdělení, populaci tvořil velký počet mladých a několik málo velmi starých genet. Poměrně nízké nosné kapacity prostředí je dosaženo po pouhých 350 letech, poté začíná být věková struktura výrazně ovlivněna na hustotě závislou mortalitou. Populace *G. reptans* dosahují ve Švýcarských Alpách značného stáří, pro dvě v terénu studované populace bylo odhadnuto na 250 a 450 let. Naše výsledky ukazují, že populace klonálně rostoucích rostlin mohou díky prakticky nesmrtelným genetám přetrvávat v terénu bez omezení. V případě *G. reptans* dochází u starých populací ke kompetici a zvýšené mortalitě díky sukcesi probíhající ve starších částech předpolí ledovce, což populacím brání dosáhnout maximální nosné kapacity prostředí. Populace tohoto druhu jsou však schopny kolonizovat nová místa, a sledovat tak ustupující čelo ledovce.

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Chapter 6

General Summary and Conclusions

General Summary and Conclusions

Summary

This thesis addressed several important aspects of longevity of clonal plants including its positive effect on population persistence, community stability and ecosystem resilience in arctic-alpine regions. The main objectives were to assess the present knowledge on longevity of clonal plants and the methods used to measure it (Chapter 2), as well as to generate high-quality lifespan data for clonal plant species that dominate late-successional alpine-arctic vegetation based on genet size data and annual horizontal growth measurements (Chapter 3). Moreover, the results were used to assess the persistence of clonal plant populations under past and future climate change. The annual horizontal growth measured in the field was compared among species and between successional stages, regions and years (Chapter 4). Also the effect of geographically or temporally variable climate parameters on horizontal growth was investigated. Additionally, the longevity of an early-successional species living on glacier forelands in the Swiss Alps was investigated using a new indirect matrix modelling approach (Chapter 5).

The review on clonal plant longevity revealed that our empirical knowledge on the lifespan of clonal species has increased considerably in the last few years. The previously published age estimates suggest extreme longevity for some clonal plants and are an indicator of long population persistence (e.g. Steinger et al. 1996, Pornon et al. 2000, Morris et al. 2008). However, such data are not sufficient to evaluate turnover rates and the ability of long-lived clonal plants to enhance community stability and ecosystem resilience. In order to better understand the dynamics of clonal populations, it is necessary to measure genet size and age structure, not only the lifespan of single individuals.

For many clonal plants, direct age estimates are not adequate due to the difficult genet identification. This is also the case for many arctic-alpine species that are dominating late-successional vegetation in arctic and alpine regions. Therefore, an indirect method, based on genet size and annual horizontal growth measurements, was applied to the five species selected for this study: *Carex curvula*, *Dryas octopetala*, *Salix herbacea*, *Vaccinium uliginosum* and *Empetrum nigrum*. Fortunately, such methods allow also the investigation of size and age structure of whole populations, a fundamental basis to understand their dynamics and persistence. The obtained fingerprint markers for the species *Empetrum nigrum* were not sufficiently polymorphic for the genotype assignment. But in the other four species, genoype assignment was successful and high genet diversity as well as a strongly left-skewed frequency distribution of genet age with a dominance of young and a low number of old individuals was found. The largest *Carex curvula* genet had an estimated minimum age of ca. 4,100 years and the oldest genets of *Dryas octopetala*, *Salix herbacea* and *Vaccinium uliginosum* were found to be at least 500, 450, and 1,400 years old, respectively. However, in all four species, most genets were found to be young. In *Carex curvula*, for example, 85% of the genets were less than 200 years old. The presence of genets in all size classes and the dominance of presumably young individuals suggest continuous recruitment over time, a prerequisite for adaptation to changing environmental conditions. These results indicate that the populations have experienced and survived pronounced climatic changes in the past such as the Little Ice Age or the post-industrial warming. Moreover, the results suggest that long-lived clonal plants in arctic-alpine ecosystems will persist despite future climate warming, unless future changes are substantially greater than those changes that the studied species have experienced during the past few hundred or thousand years.

The in-situ annual horizontal growth measurements used for the indirect age estimates represent a realistic approximation ready to use in studies of life history and population dynamics, because annual horizontal growth was found to be species-specific and not affected by climatic variability occurring among geographically distant regions. These results are consistent with data obtained, for instance, from temperature manipulation experiments (Chapin &

Shaver 1996, Arft et al. 1999, Molau 2001), and therefore, indicate that a warming climate may have no fundamental effect on the horizontal growth of the arctic-alpine clonal plants studied.

For species in which size and age is weakly correlated or their ageing is poorly understood, alternative methods to investigate genet life span, such as demographic approaches or the use of mutation rates, can be applied (e.g. Cochran & Ellner 1992, Morris & Doak 1998, Barot et al. 2002, Heinze & Fussi 2008). Here, a new demographic approach based on a stage-based projection matrix model was applied to the typical pioneer species *Geum reptans* living on recently deglaciated areas. Simulation of population establishment including carrying-capacity threshold and density- or age-dependent mortality revealed that genet age structure is strongly left-skewed with many young and a few very old genets after several hundred years. This population age structure is very similar to that of the four late-successional species. According to the simulations, more than half of the genets died within the first three decades, but a remarkable proportion survived more than 50 years. Some genets even became immortal as they accumulated a sufficiently high number of ramets that reduced the mortality risk of the entire genet to zero. The age of the two field populations was estimated to be 250 years and 450 years, respectively. This led to the conclusion that even in clonal plants that live mainly in pioneer habitats, genet longevity can potentially lead to unlimited persistence of established populations. In the case of *Geum reptans*, old populations may experience competition and increased mortality due to the ongoing succession in older parts of the glacier foreland. But due to the pronounced ability of the genets to reproduce clonally and thereby to colonise new sites and to follow the retreating ice on glacier forelands, populations of *Geum reptans* can persist.

Conclusions

Given the heterogeneity of habitats and the harsh environmental conditions in arctic and alpine regions, many plant populations are small and spatially isolated from each other and, therefore, vulnerable to changing abiotic conditions and stochastic processes. The species chosen within this study, however, occur in large populations and usually are dominating their community. This already is an indication for their outstanding evolutionary potential and robustness in the face of harsh environments and disturbances. The lifespan investigations conducted during this thesis revealed extreme longevity for several clonal species dominating late-successional vegetation as well as pioneer sites. With the applied indirect methods it was possible to observe genet age structures of whole populations that indicated high turn over rates and population dynamics that allow adaptation to changing climates. Together, clonality, longevity and continuous genet turnover form the combined strategy of arctic-alpine clonal plants to cope with their environment. In the meantime, they enhance community stability and ensure maximum ecosystem resilience. Therefore, it can be predicted that populations of clonal and long-lived plants will persist despite future climate changes, at least as long as the changes are not too fast and do not overrule the potential for adaptation.

Outlook

The results of this thesis help to improve predictions of species distributions and vegetation patterns under future climate change. But instantly, new questions emerge that need to be answered to further enhance our understanding of population dynamics and ecosystem functioning. Here, I briefly outline four ideas for future research:

(i) It is widely accepted that genetic and demographic factors synergistically determine the dynamics of a plant population and that both factors have to interact to avoid extinction (Till-Bottraud & Gaudeul, 2002). Longevity of plants may not be the only prerequisite for population persistence, as the genetic constitution of a population will determine the ability to respond to environmental variability. What is exactly the genetic precondition that plants need to form persisting populations? What is the importance of genetic diversity for population persistence? Do phenotypic plasticity or even genetic mutations play a role?

(ii) Since the size of a genet might not be

related to its age due to physical or biotic interactions, alternative estimators for genet lifespan are searched for. The accumulation of mutations at microsatellite loci for example was used to estimate genet age in trembling aspen (*Populus tremuloides*; Mock et al. 2008, Ally et al. 2008). During this thesis, small genetic distances within multilocus lineages were found, suggesting the occurrence of genotyping errors and somatic mutations. However, it was not possible to distinguish between them. Will this be possible in the future? Can somatic mutation rates be investigated and used for studies of clonal population dynamcis and lifespan in arctic-alpine clonal plants?

(iii) In this thesis, the observed genet size and age structure suggests high turn-over rates within the persisting populations. How is repeated offspring recruitment maintained in such homogenous populations where practically no seedlings have been observed? Under which environmental conditions does sexual reproduction and seedling establishment in such persistent populations take place?

(iv) Indirect methods using demographic data have been applied only to very few species. Can they be applied to other species? And how? Do stage-based projection matrix models confirm the results of indirect methods based on size data and vice versa?

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Chapter 7

Appendix

A.1

This is the supplementary material to Chapter 3 "AFLP markers reveal high clonal diversity and extreme longevity in four arctic-alpine key species".

Figure 7.1: Map showing the spatial distribution of the 77 *Carex curvula* genets in the population "Biedmer". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

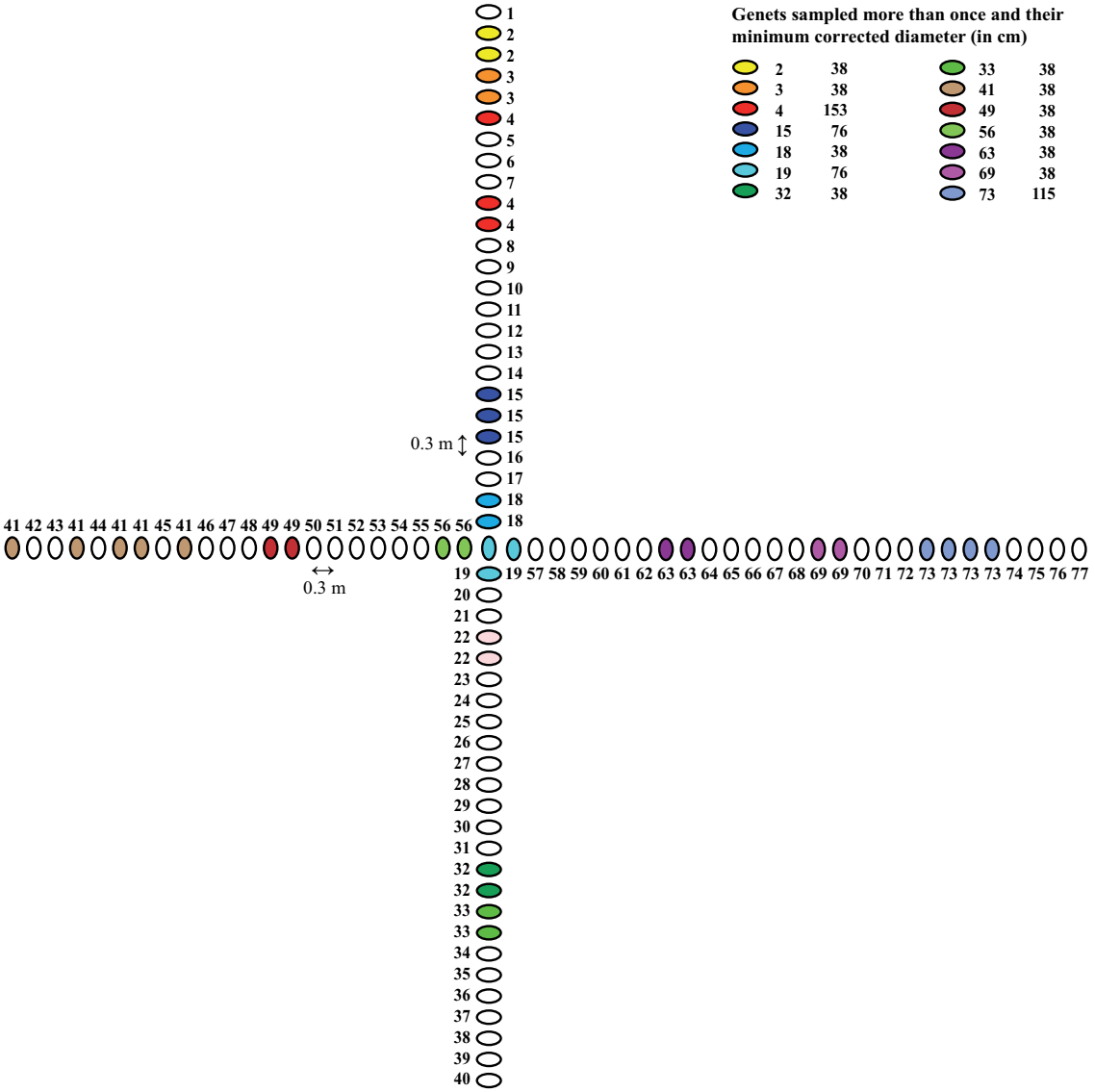


Figure 7.2: Map showing the spatial distribution of the 68 *Carex curvula* genets in the population "Valetta Schlattain". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

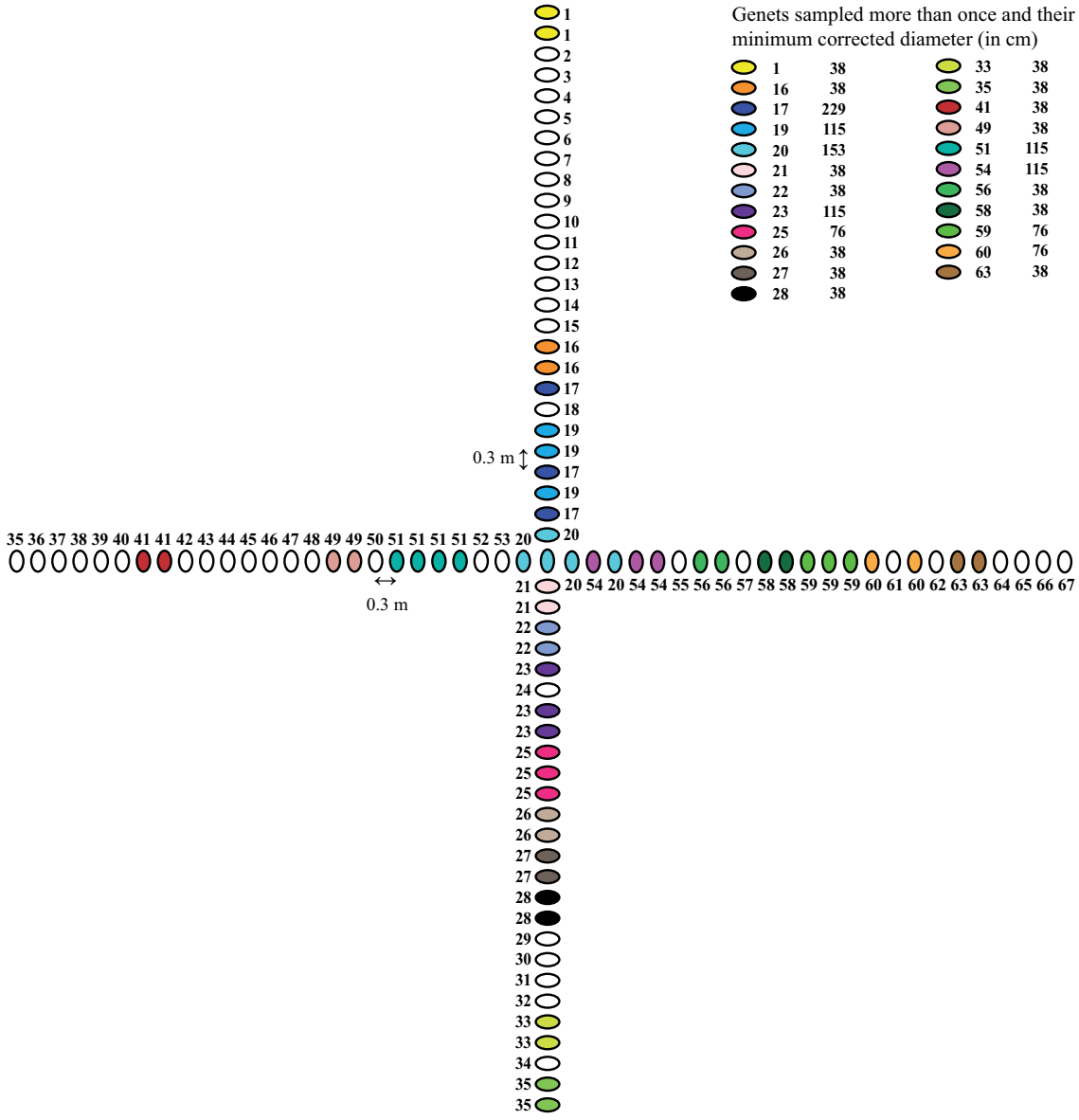


Figure 7.3: Map showing the spatial distribution of the 37 *Carex curvula* genets in the population "Vf. Paltinului". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

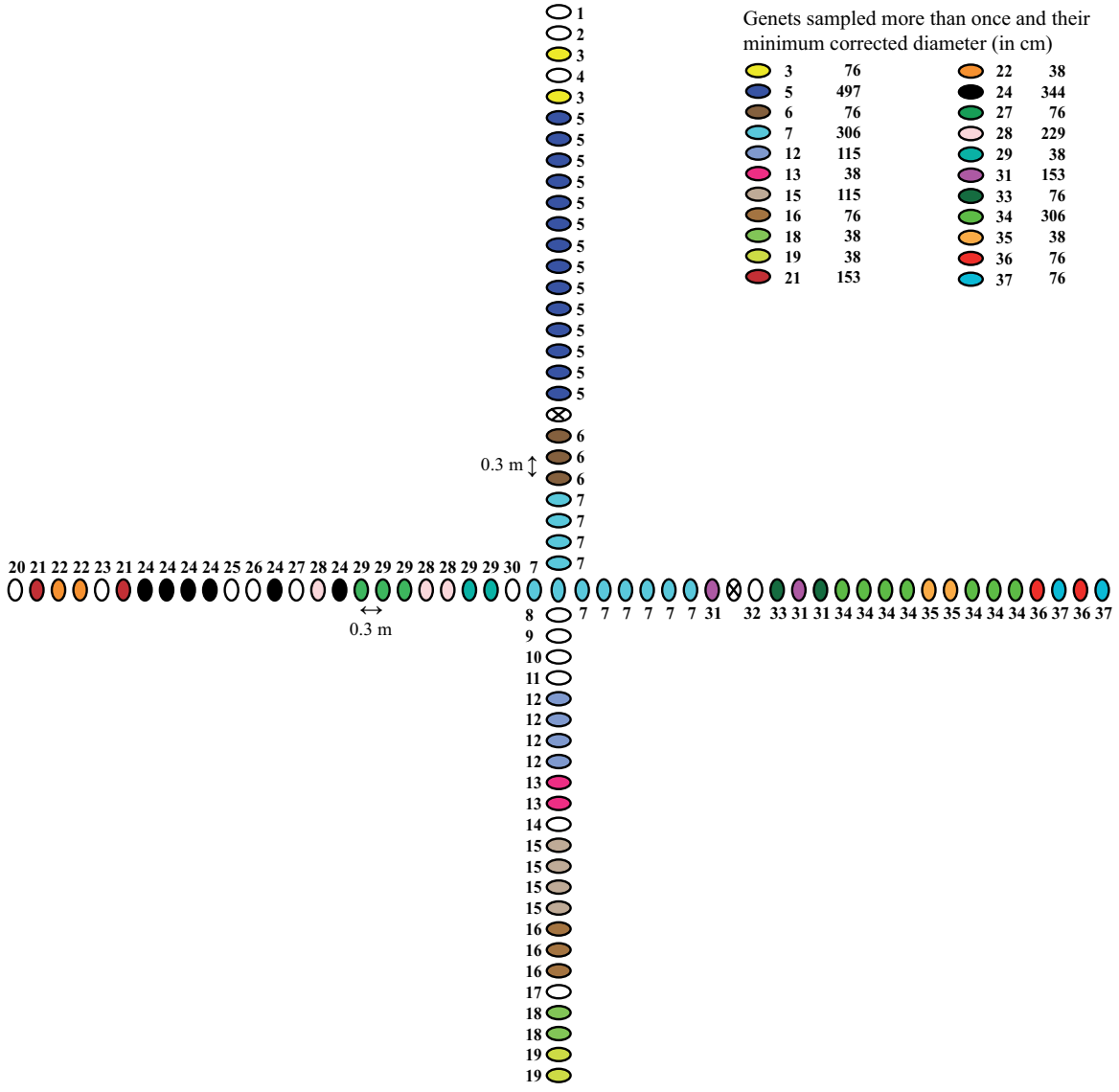


Figure 7.4: Map showing the spatial distribution of the 62 *Carex curvula* genets in the population "Vf. Pietrosu". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

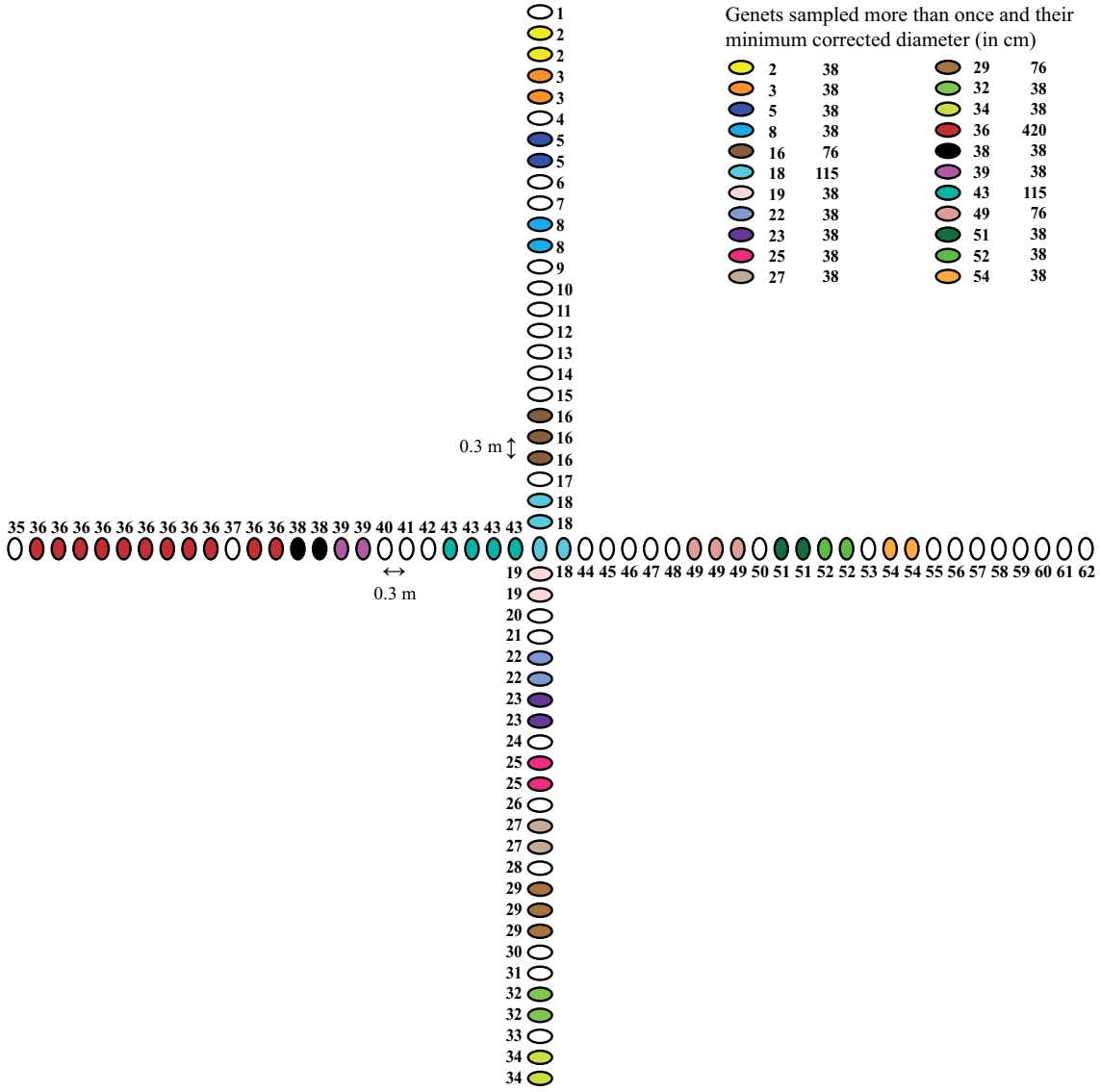


Figure 7.5: Map showing the spatial distribution of the 78 *Dryas octopetala* genets in the population "Il Jalet". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

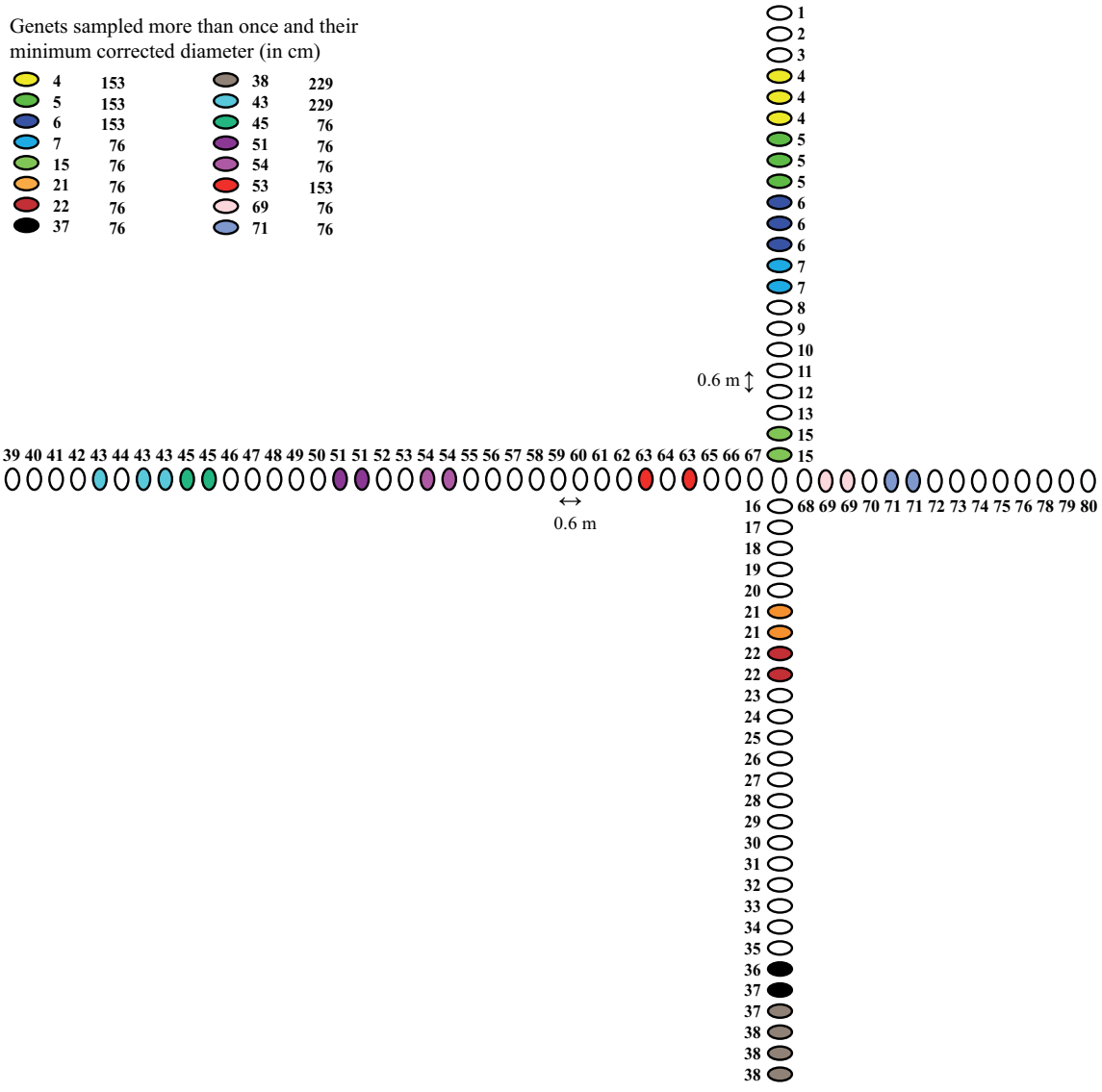


Figure 7.6: Map showing the spatial distribution of the 91 *Dryas octopetala* genets in the population "Calanda". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

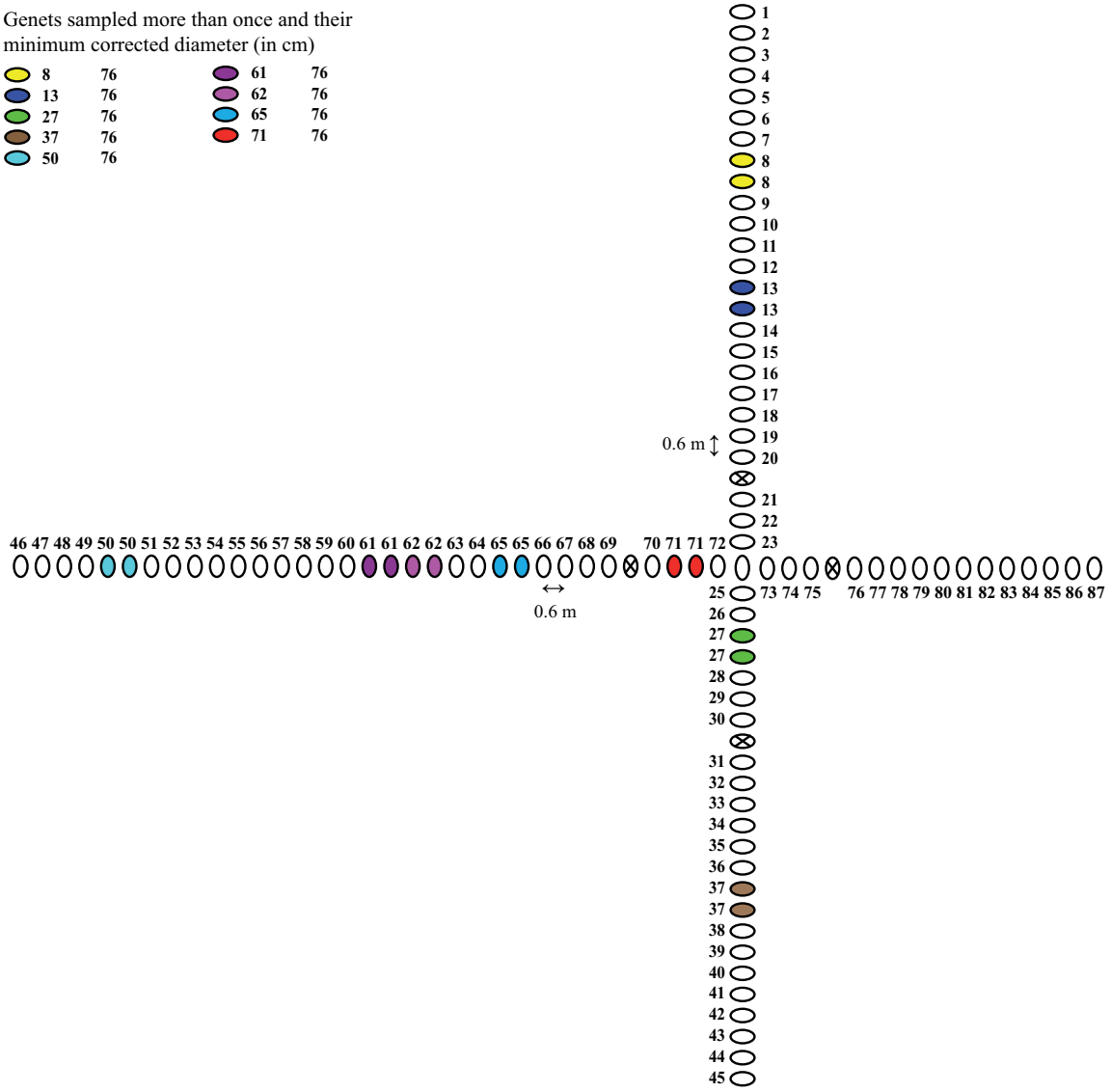


Figure 7.7: Map showing the spatial distribution of the 75 *Dryas octopetala* genets in the population "Bonistock". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

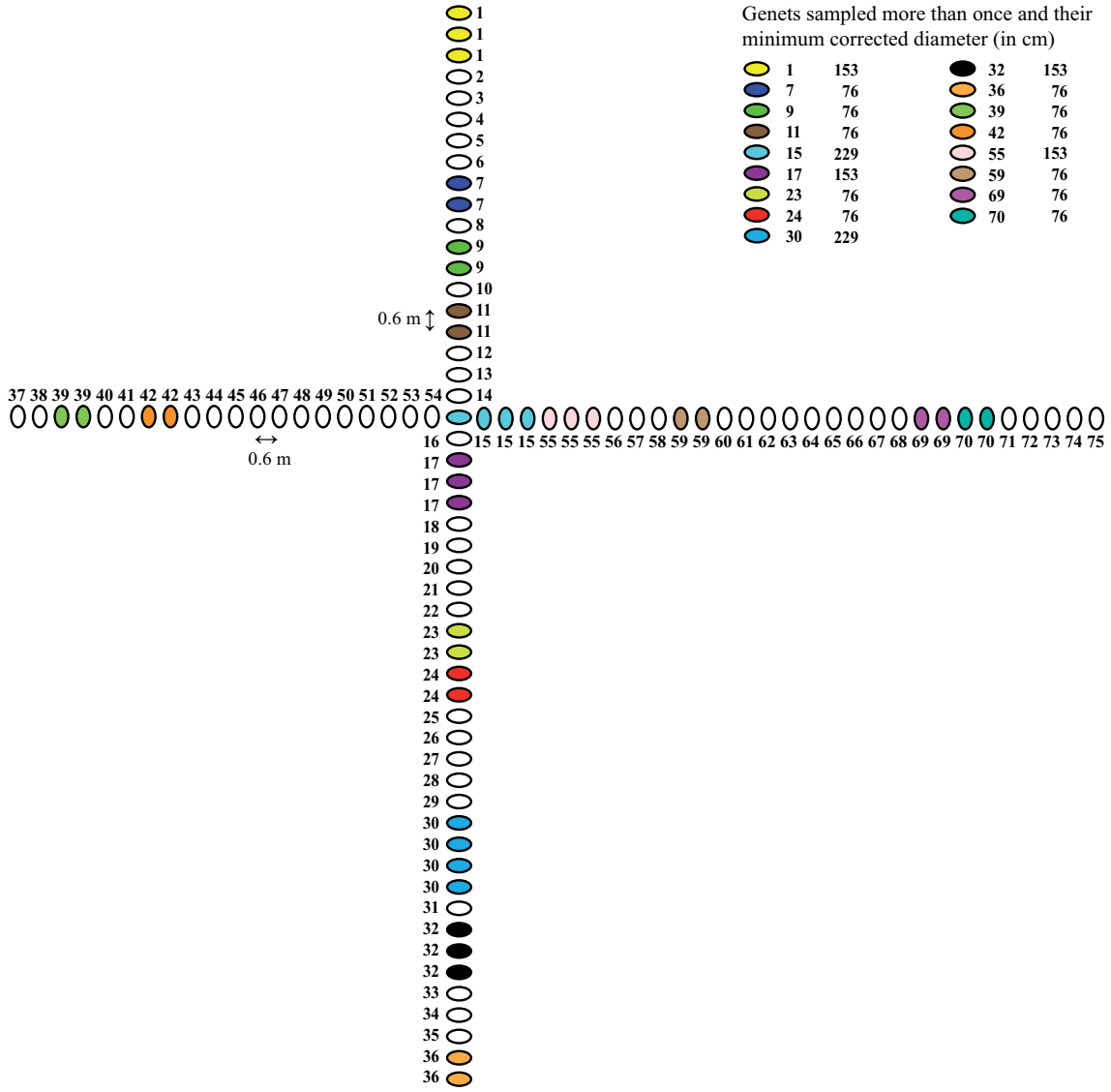


Figure 7.8: Map showing the spatial distribution of the 76 *Dryas octopetala* genets in the population "Baba Mare". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

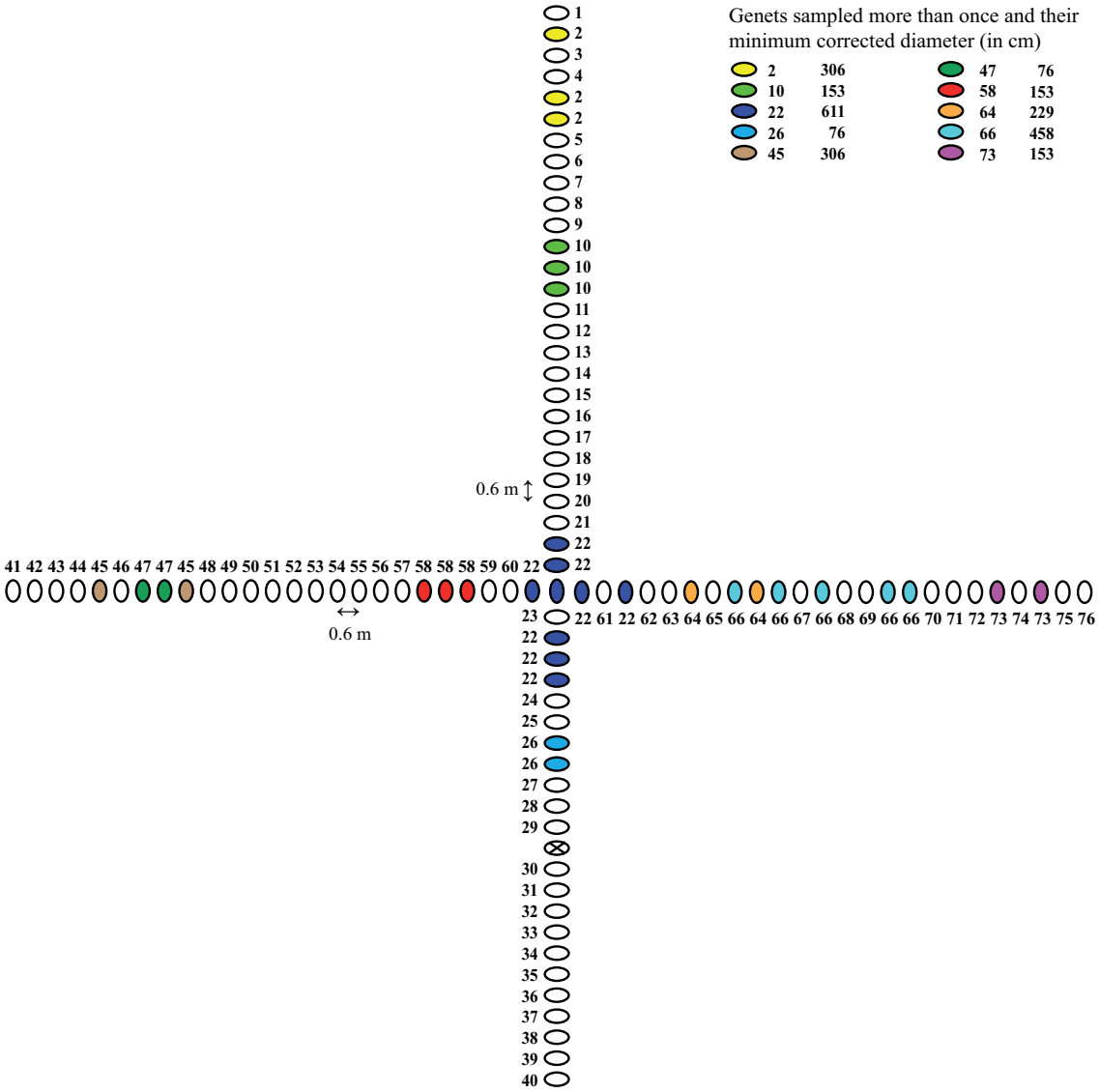


Figure 7.9: Map showing the spatial distribution of the 79 *Salix herbacea* genets in the population "Blauberg". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

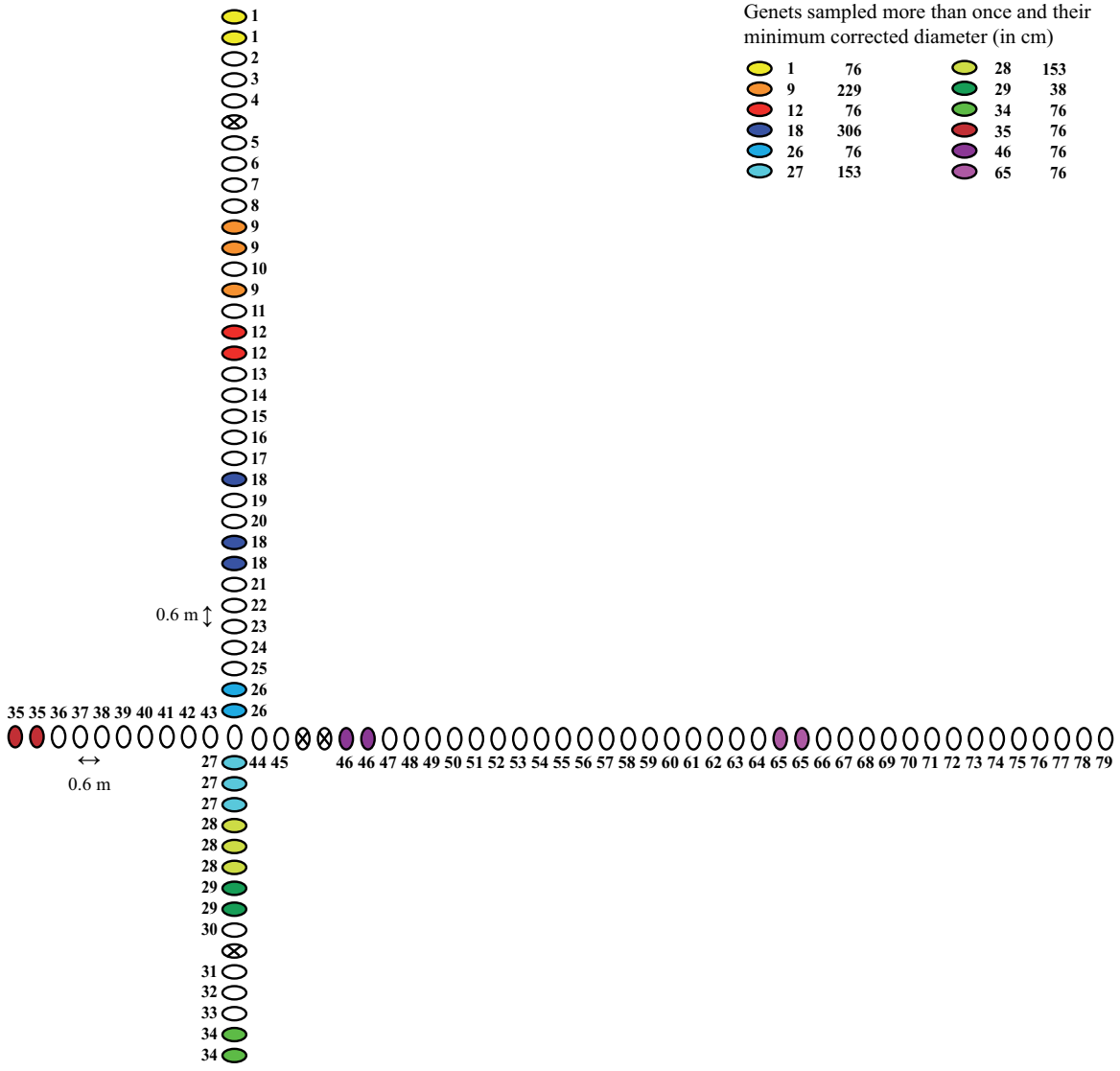


Figure 7.10: Map showing the spatial distribution of the 76 *Salix herbacea* genets in the population "Flüelapass". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

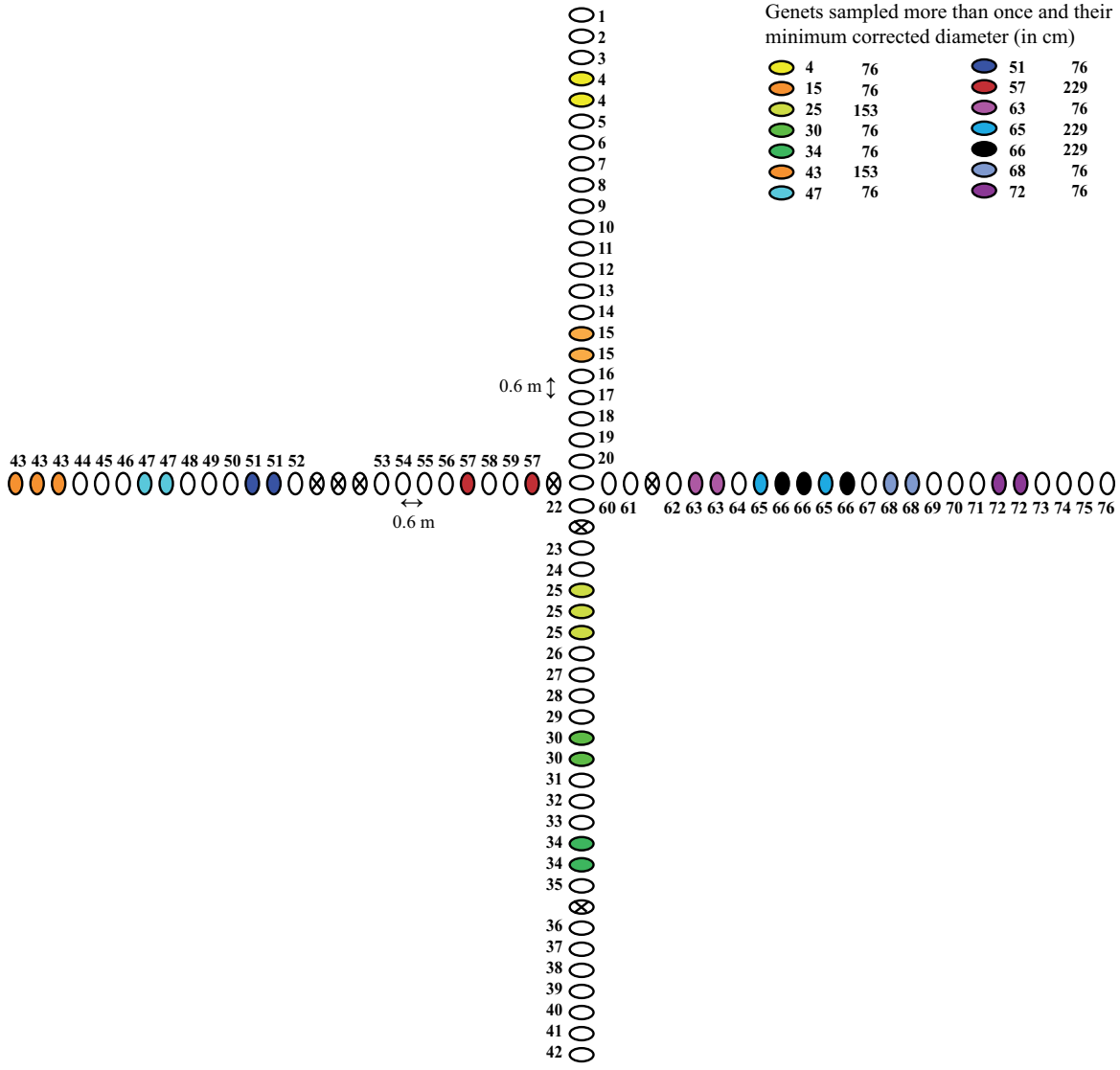


Figure 7.11: Map showing the spatial distribution of the 92 *Salix herbacea* genets in the population "Lulip". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

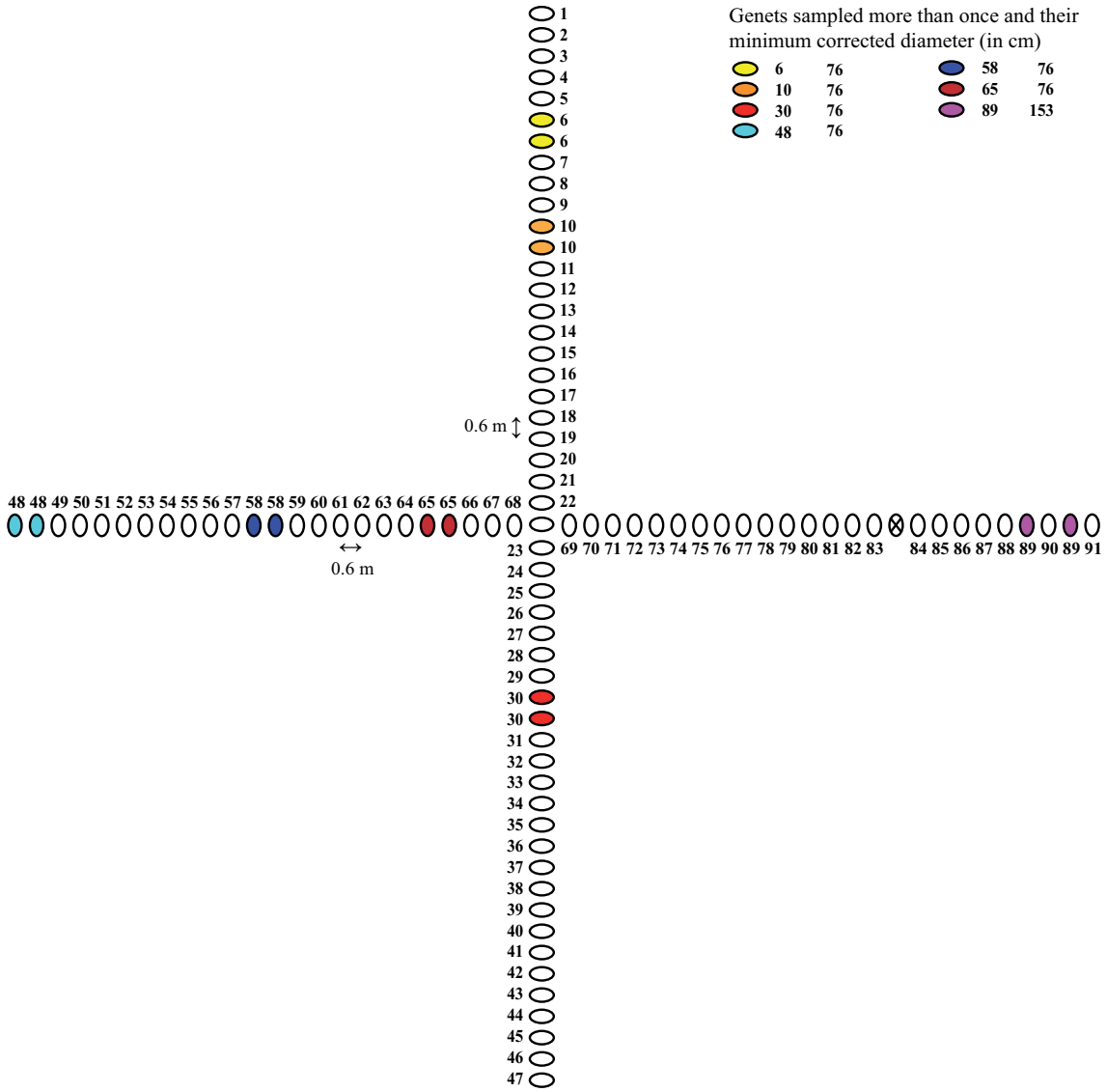


Figure 7.12: Map showing the spatial distribution of the 91 *Salix herbacea* genets in the population "Kåfjorddalen". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

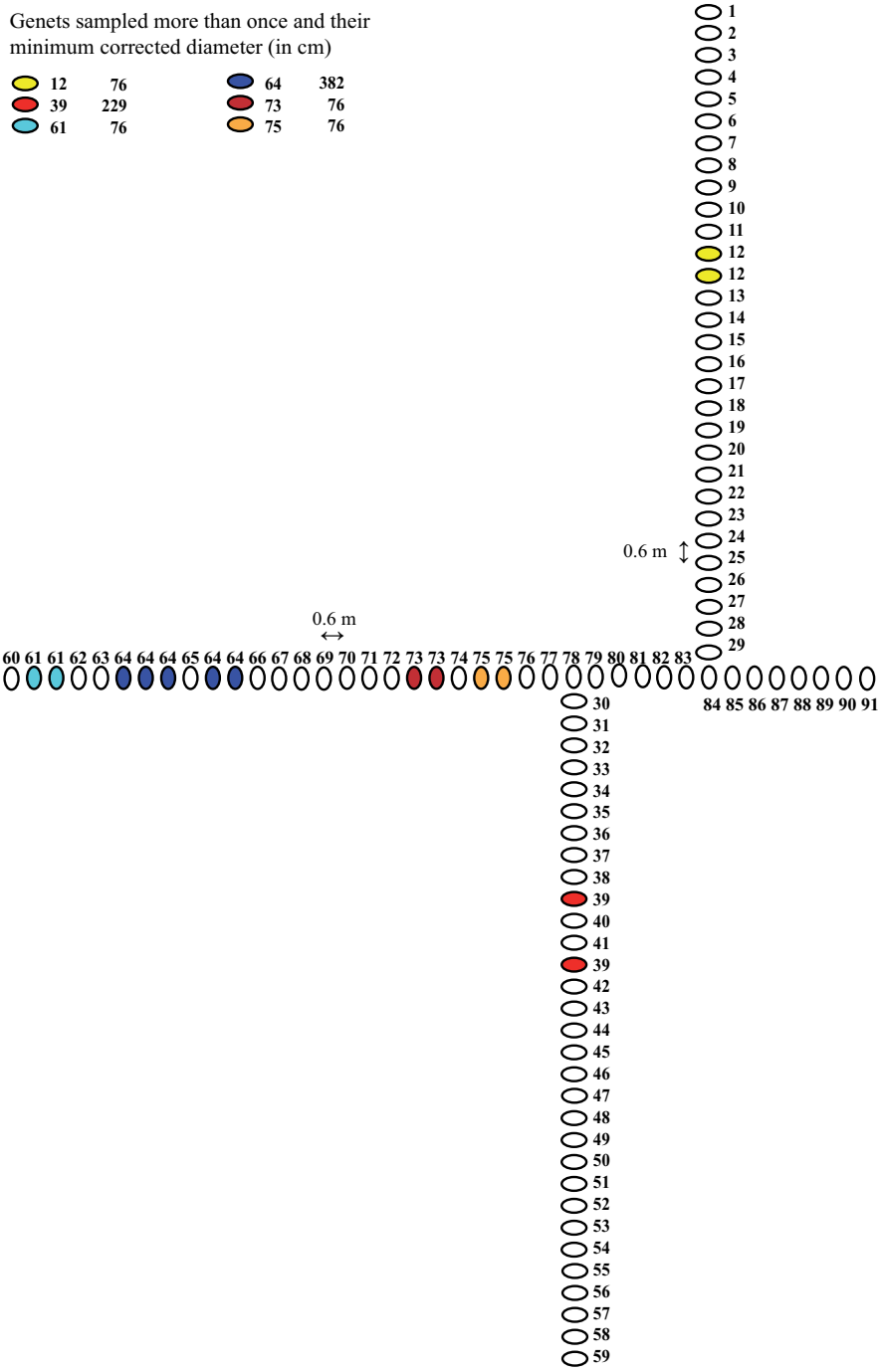


Figure 7.13: Map showing the spatial distribution of the 17 *Vaccinium uliginosum* genets in the population "Stillberg". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

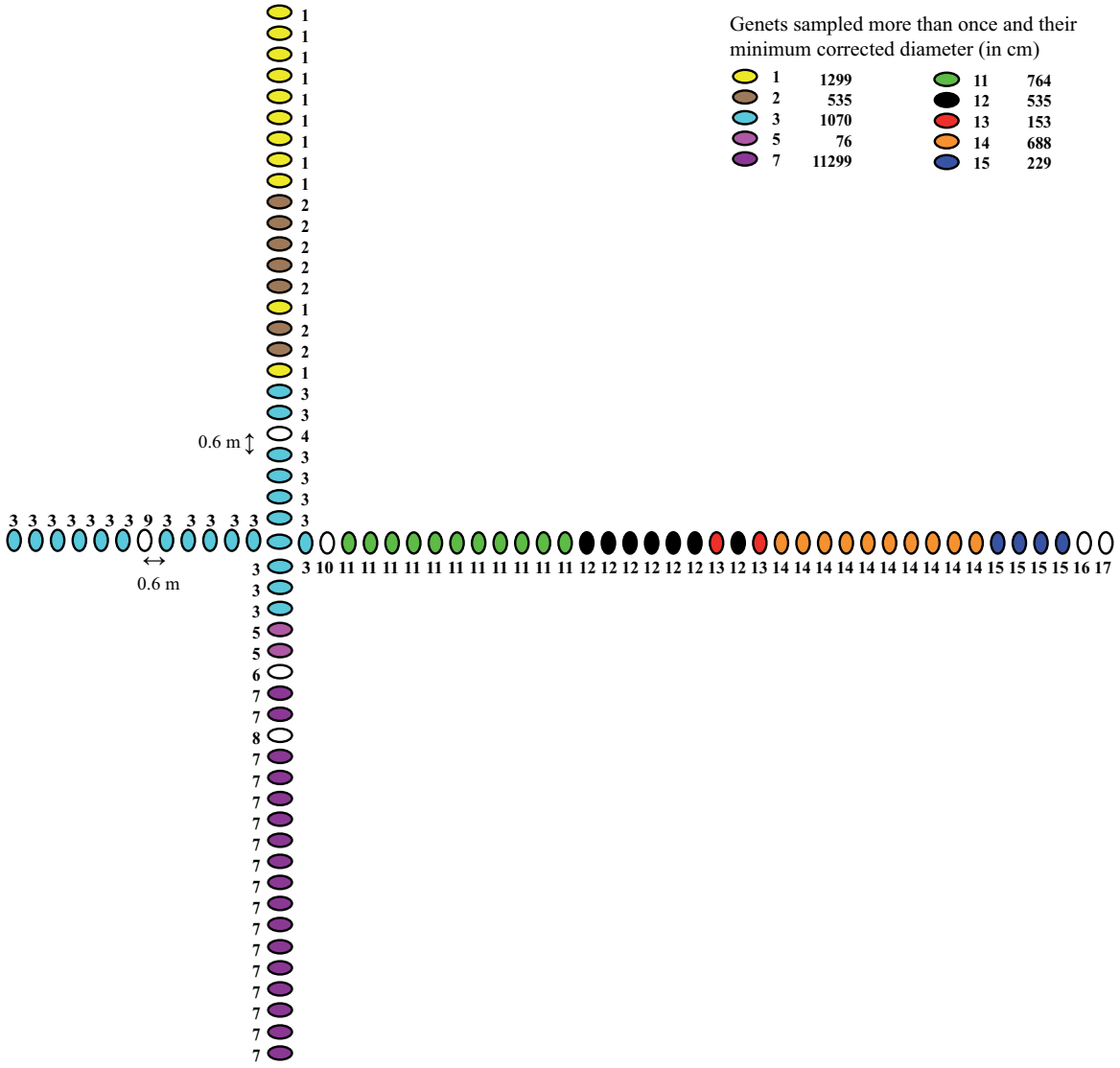


Figure 7.14: Map showing the spatial distribution of the 20 *Vaccinium uliginosum* genets in the population "Lulip". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.

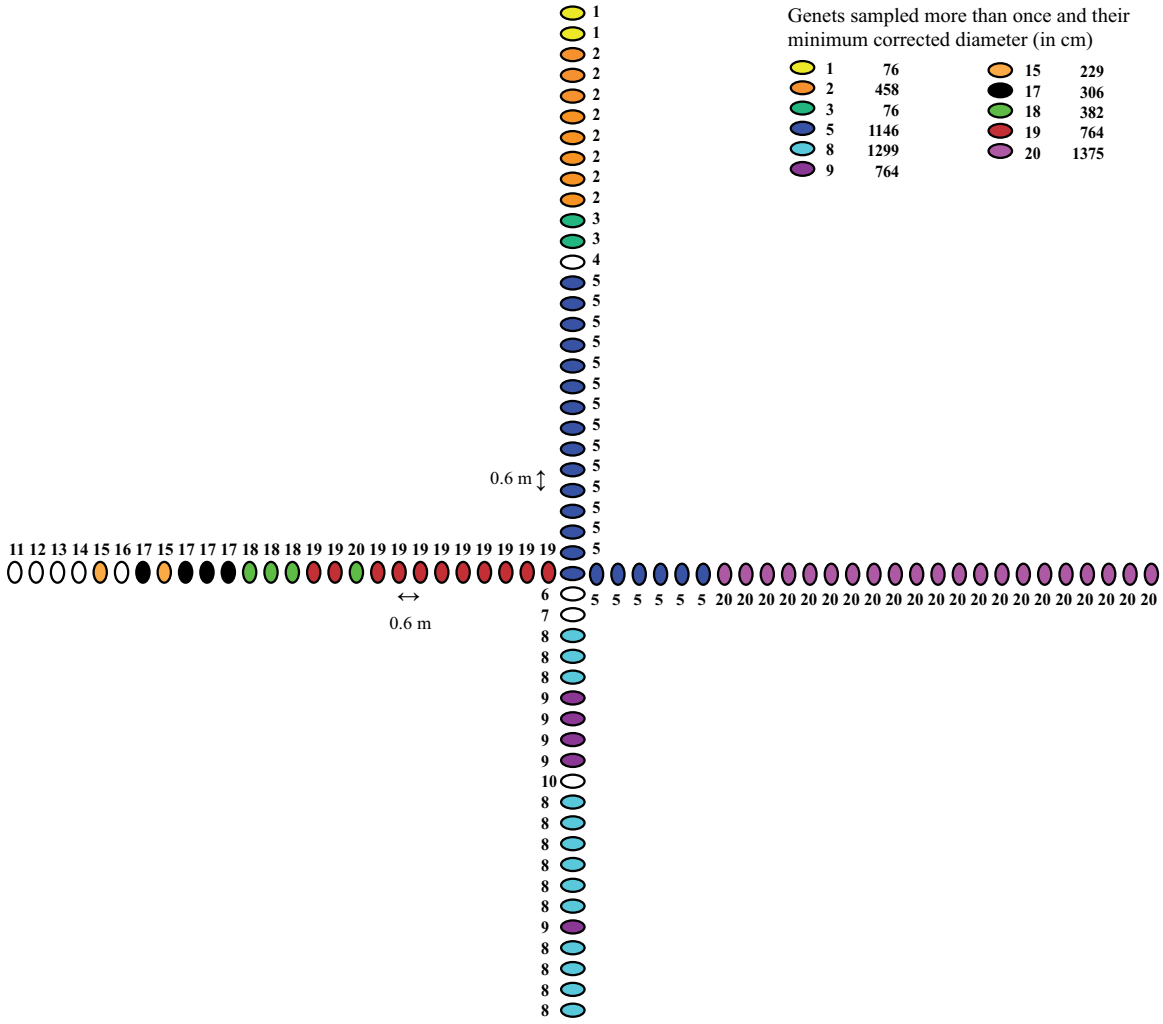
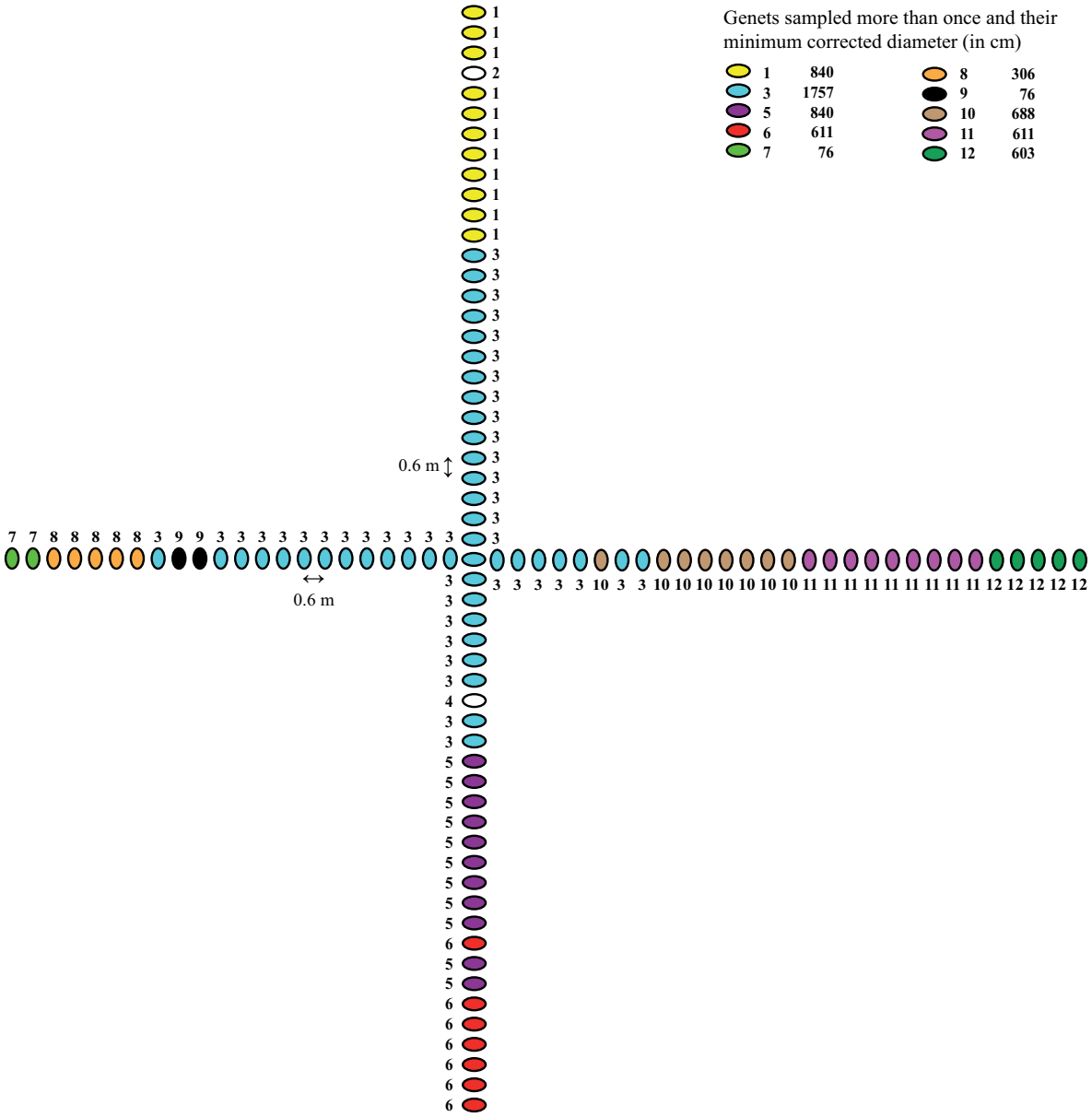


Figure 7.15: Map showing the spatial distribution of the 20 *Vaccinium uliginosum* genets in the population "Steindalen". Genets sampled only once are shown without colour filling, while genets sampled more than once are shown in different colours.



A.2

Curriculum Vitae

Curriculum Vitae

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OBJECTIVE

As a dynamic and motivated scientist with experience in carrying out research projects especially in plant taxonomy, population genetics and ecology, I would like to apply my knowledge in biodiversity research and thereby contribute to nature conservation.

EDUCATION

PhD (2008–2011)
Institute of Botany, University of Basel, supervision: Prof Jürg Stöcklin
Topic: Alpine-arctic clonal plants and ecosystem resilience
Work package leader within the project "EcoChange, Biodiversity and Ecosystem Changes in Europe"
Funded by: 6th framework programme of the European Union, FP6-036866

Master of Science in Biology (2006–2007)
Nationaal Herbarium Netherland and Faculty of Mathematics and Natural Sciences, Leiden University (NL)
Topic: Biodiversity in Time and Space
Minor MSc project : A taxonomic revision of *Bulbophyllum* species (Orchidaceae), supervision: Dr JJ Vermeulen (NHN)
Major MSc project: Homeotic mutations are causing speciation in orchids, supervision: Dr B Gravendeel (NHN), Dr EM Kramer (OEB – Harvard University)

Bachelor of Science and Major in Integrative Biology (2002–2005)
Philosophisch-Naturwissenschaftliche Fakultät, University of Basel

PROFESSIONAL EXPERIENCE

Since 2011	Curator of the Swiss Orchid Foundation at the Herbarium Jany Renz, University of Basel (CH)
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2008-2011	Teaching assistant at the Botanical Institute of the University of Basel (CH)
2006-2007	Management of orchid collections Nationaal Herbarium Nederland (NL)
2005	Teacher in Biology, Geography and Informatics at secondary school in Gelterkinden (CH)
2004-2005	Data baser at Herbarium Jany Renz Orchid herbarium located at the University of Basel (CH)

OTHER RELEVANT EXPERIENCE

Transferable skills
- Grant proposal writing (Zürich-Basel Plant Science Center certificate)
- Scientific presentations (PSC certificate)

Molecular techniques
- Collecting and preserving plant material
- DNA & RNA isolations
- mRNA & cDNA construction
- (RT-)PCR
- Cloning
- DNA sequencing

- Primer & probe design
- AFLP fingerprint analysis
- Microsatellite analysis
- Preparing anatomical sections
- Light microscopy & Scanning electronic microscopy

Statistical and phylogenetic analyses , databases and software
- R syntax, JMP
- Bayesian Analysis (MrBayes), Maximum Parsimony & Maximum Likelihood (PAUP 4.0b1)
- Genotyping (Genemapper, Genographer, Genodive)
- Aligning (Sequencher, MacClade, MacVector)
- Taxonomic databasing (Access, BRAHMS, Linnaeus II)

Fieldwork
- Functional ecology of arctic-alpine clonal plants in the Swiss Alps, Lapland and Carpathians (2008–2010)
- Ecology and evolution of subtropical island vegetation, Tenerife, ES (1 week, 2009)
- Dutch flora, inventories for FLORON (2006–2007) and pollinator studies with various orchids (2007)
- Tropical Biology Association field course in the Usambara mountains, Tanzania (4 weeks, 2005)
- Mediterranean flora, Languedoc-Roussillon/Hérault, F (1 week, 2004)
- Mediterranean ecosystems, Samos, GR (1 week, 2003)

PUBLICATIONS

- **In prep**. Gravendeel B, de Witte LC, Eurlings MCM, van Heuven BJ, Vogel A, Johansen BB, Kramer EM: Differential expression of MADS-box B gene lineages correlates with changes in petal shape of orchid flowers.
- **In press**. **LC de Witte**, J Stöcklin. Horizontal growth in arctic-alpine clonal plants is not affected by climatic variability among regions. *Plant Ecology and Diversity*
- **2012**. **LC de Witte**, GFJ Armbruster, L Gielly, P Taberlet, J Stöcklin. AFLP markers reveal high clonal diversity, repeated recruitment and extreme longevity of four arctic-alpine key species. *Molecular Ecology* 21:1081–1097.
- **2011**. **LC de Witte**, D Scherrer, J Stöcklin. Genet lifespan and population age structure in the clonal pioneer species *Geum reptans* based on demographic field data and projection matrix modelling. *Preslia* 83: 371–386
- **2010**. **LC de Witte** & J Stöcklin. Longevity in clonal plants: why it matters and how to measure it. A review. *Annals of Botany* 106: 859–870
- **2010**. **LC de Witte** & JJ Vermeulen. The Bulbophyllum species attributed to section *Hymenobractea* (*Orchidaceae*). *Blumea* 55: 80–85.
- **2005**. **LC de Witte** & W Dawson. Does plant species composition vary beneath *Cinnamomum camphora* plantation trees, compared to natural forest in Amani Nature Reserve? Online EAMCEF report, http://easternarc.or.tz/

CONFERENCES

- **25th Plant Population Biology Conference (GfÖ), Zürich** (Mai 2012) *What makes a homeotic orchid mutant successful?* (Poster)
- **15th European Orchid Congress, Budapest** (2012 April) *The World Orchid Iconography* (presentation)
- **20th World Orchid Conference, Singapore** (2011 November) *The World Orchid Iconography* (presentation)
- **2nd International GMBA-DIVERSITAS Conference, Chandolin** (2010 July 27) *Longevity of arctic-alpine clonal plants and ecosystem resilience* (presentation)
- **23th Plant Population Biology Conference (GfÖ), Nijmegen** (2010 May 15) *Size structure and longevity of arctic-alpine clonal plants* (presentation)
- **4th EcoChange Meeting, Birmensdorf** (2010 May 4) *Longevity of arctic-alpine clonal plants and ecosystem resilience* (presentation as work package leader)
- **Orchiade 2010, Leiden** (2010 April 2) *Hopeloos geval of monster met toekomst - soortvorming bij tropische orchideeën* (invited speaker)
- **2nd annual EcoChange Meeting, Cluj** (2008 May 20) *Longevity of arctic-alpine clonal plants and ecosystem resilience* (presentation as work package leader)
- **6th Biennial Conference of the Systematics Association, Edinburgh** (2007 August 31) *MADS-box B gene expression in flowers of a homeotic orchid mutant* (presentation and poster)

FUNDING

- Stiftung zur Förderung der Pflanzenkenntnis, Basel
- Swiss Orchid Research Award 2011 by the Swiss Orchid Foundation at the Herbarium Jany Renz
- 6th framework programme of the European Union: FP6-036866
- The Systematics Association
- The Tropical Biology Association

LANGUAGES

German / Dutch:	Mother languages
English:	Fluent
French:	Basic knowledge

DRIVERS LICENSE

Swiss Kategory B and D1E since 2001

MEMBER OF

- Schweizerische Botanische Gesellschaft
- Basler Botanische Gesellschaft
- Verein Botanischer Garten beim Spalentor, Universität Basel
- Stiftung Dr.h.c. Erich Nelson, Bern (vice chair)

PERSONAL INTERESTS

In my free time I love to go outside to explore nature and to do sports such as nordic skiing, triathlon, biking and I play music (clarinet) in a wind band

REFERENCES

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- Prof Jürg Stöcklin, Institute of Botany, University of Basel, +41 61 267 35 01