Flower preformation in the nival plant *Ranunculus glacialis* L.: shoot architecture and impact of the growing season length on floral morphogenesis and developmental dynamics

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Abstract

Flower preformation is a widespread phenomenon in perennial plants from temperate and cold regions. An advanced preformation status reduces the prefloration period and thus increases the chance to mature seeds in time. Despite the particular importance of this strategy for high-mountain plants, studies are rare. Here we investigated how the length of the growing season impacts floral development, and to what extent floral development is synchronized with reproductive phenophases in the arctic-alpine species *Ranunculus glacialis* L. The study was carried out in the alpine-nival ecotone in the European Central Alps at sites with different snowmelt dates. Individuals were sampled at regular intervals throughout the growing season, and shoot architecture and changes in floral structures were analysed in detail using different microscopic techniques. *R. glacialis* individuals consist of a cluster of independent ramets, comprising 3 sympodia each. Floral initiation terminates the vegetative growth of each sympodium 2–3 years before flowers become functional. A specific feature is that basal and distal leaves on a sympodium mature in different years. The date of snowmelt did not affect the speed of development but flower size and the number of lateral flowers within an inflorescence. Belowground floral preformation is closely linked to aboveground reproductive processes, however, continues below the snow in case winter conditions set in too early. The staggered preformation of architectural units creates a permanent belowground reserve pool of floral structures which might be advantageous in the climatically harsh and unpredictable high-mountain environment.

Keywords Arctic · Alpine · Floral development · High-mountain · Phenophases · Reproduction

Introduction

Flower preformation, i.e. flowers are initiated one or more years before anthesis, is a widespread phenomenon in perennial plants from temperate and cold regions. Particularly in the arctic and alpine environments, where the growing season can be as short as a few weeks, an advanced preformation status and thus a short prefloration period (i.e. the time span between snowmelt and anthesis) increases the chance to mature seeds in time (e.g. Crawford and Smith 1997; Galen and Stanton 1991, 1995; Kudo 1991; Kudo and Suzuki 1999; Ladinig and Wagner 2005; Molau 1997; Wagner et al. 2010, 2012). Flower preformation has been documented for a

number of plant species in the arctic tundra (Sørensen 1941) and in the alpine (Billings and Mooney 1968; Bliss 1971; Mark 1970). Most studies recorded the developmental state of the flower buds at the end or at the beginning of the growing season, and only a few investigated the timing of floral development (Aydelotte and Diggle 1997; Diggle 1997; Ladinig and Wagner 2009; Larl and Wagner 2006; Meloche and Diggle 2001; Wagner et al. 2012). Thus, for most species, it is unknown when floral initiation takes place, and which impact site climate and season length exert on floral development and thus on flower abundance the year(s) after. It is generally known that flower frequency in the current year depends on the developmental conditions in the previous year (Abeli et al. 2012; Inouye et al. 2003; Semenchuk et al. 2013).

The high-mountain plants studied up to date mainly show a two-season strategy of flower preformation, i.e. flower primordia are initiated in the season before anthesis (Aydelotte and Diggle 1997; Ladinig and Wagner 2009; Larl

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and Wagner 2006). Only few species follow a one-season strategy, i.e. they initiate and develop the flowers in the year of anthesis. Among these species are *Saxifraga caesia* (inhabits only the alpine zone) and the subnival-nival species *Cerastium uniflorum* (Wagner et al. 2012). A multi-season strategy in alpine plants has been reported for *Acomastylis rossii* (Meloche and Diggle 2001) and *Polygonum viviparum* (Diggle 1997) so far.

In high mountains, the start of the growing season strongly depends on the topography of the terrain. Snowmelt dates between exposed sites with shallow snow accumulation and hollows where huge snowpacks accumulate during winter may differ for 1–2 months at the same elevation (e.g. Inouye et al. 2002; Körner 2003; Kudo 1991; Ladinig and Wagner 2005, 2007; Liberati et al. 2019; Molau et al. 2005; Pickering et al. 2014; Wagner and Reichegger 1997). Depending on the melting date, plants experience different temperature conditions and daylengths, which affects the speed of growth, development, and reproduction. Early-emerging individuals of the same species usually develop slower than individuals that become snow-free in summer when higher temperatures accelerate development (Galen and Stanton 1991; Gugger et al. 2015; Huelber et al. 2006; Kudo 1991; Ladinig and Wagner 2007; Totland and Alatalo 2002; Wagner and Reichegger 1997; Wipf et al. 2009). Unlike most of the alpine species studied, the arctic-alpine pioneer species *Ranunculus glacialis* L. hardly seems to respond to variable environmental conditions in its high elevation habitat in the European and Scandinavian mountains. The perennial herbaceous rhizome plant persists belowground most of the year. Leaves and flowering shoots appear aboveground only for a few weeks. Once seeds have matured, all aboveground structures senesce. Several studies on *R. glacialis* have shown that neither the melting date nor temperature significantly affect the speed of development and the reproductive output (Keller and Körner 2003; Prock and Körner 1996; Totland and Alatalo 2002). In an earlier study on *R. glacialis* in the European Alps, we could confirm that there is little variation in the length of reproductive phases in individuals growing in the alpine-nival ecotone (Wagner et al. 2010). In the nival zone, however, reproductive phases were markedly prolonged. Because of the lower temperatures, and about 50% of days with snow and frost, the whole reproductive cycle lasted nearly the double of time and seed output was significantly reduced.

In contrast to postfloral development and reproductive success, hardly anything is known about the floral development in this dominant species of the subnival and nival flora of the European Central Alps. The only information available is that flower buds pass winter in an advanced developmental stage (Körner 2003; Sørensen 1941). However, there are no reports about the pattern and timing of floral development. We, therefore, asked the following questions:

(1) what is the shoot architecture, and where and when are flowers initiated, (2) to what extent is floral preformation synchronised with flowering and fruiting in the current year, and (3) what influence exerts the date of snowmelt on the timing of floral development, the size of floral structures, and the flowering frequency in the year to come?

To answer these questions, individuals of *R. glacialis* were sampled at regular intervals throughout the whole growing season, and shoot architecture and developmental state were microscopically analysed. To reveal the impact of the snowmelt date on the course of floral development, sampling took place at an early, mid and late melting site in an alpine-nival ecotone in the Austrian Central Alps.

Materials and methods

Species description

Ranunculus glacialis L. is a perennial herbaceous cryptophyte. The shoot system is a belowground sympodial rhizome, consisting of individually rooted ramets with alternate rosette-like arranged leaves with a sessile, sheathing petiole, and a palmatisect lamina. Sympodia are either vegetative (younger ramets) or end in a uni- to multiflorous inflorescence. The perianth of the radially symmetric flower comprises 5 “sepals” (evolutionary tepals), densely covered with brown villous hairs, and 5 “petals” (evolutionary staminodia with a nectary pit at the basis) which are coloured white during anthesis and turn to pink during fruit development (Svalbardflora.net, 1998–2020 <http://svalbardflora.no/?id=819>). Fertile flower organs consist of numerous stamens and free uniovulate carpels arranged in a spiral (see Fig. 5m). Carpels mature into an aggregate fruit consisting of single-seeded achenes, which disperse independently. *Ranunculus glacialis* is diploid ($n = 16$; CCDB chromosome counts database, <http://ccdb.tau.ac.il/Angiosperms/Ranunculaceae/>), propagates sexually (Hörandl et al. 2011), and is predominately outcrossing (Totland and Alatalo 2002; Wagner et al. 2010).

The arctic-alpine pioneer species grows in snow-beds and sparsely vegetated alpine fell-fields at higher elevations in the European and Scandinavian mountains, and at sea level in the circumpolar High Arctic (Rønning 1996). *R. glacialis* occurs in genetically different geographical groups (Ronikier et al. 2012; Schönschwetter et al. 2004). In the European Alps, the species inhabits the subnival (i.e. the alpine-nival ecotone) and the nival zone on siliceous bedrock. The main distribution range is between 2400 and 3200 m a.s.l.; in climatically favourable microhabitats, the species ascends over 4000 m a.s.l. (Zimmermann 1975). *R. glacialis* is indifferent with regard to the habitat structure and grows equally on solid rock, skeletal soil, stable and unstable scree (Pauli et al. 1999), but prefers moist sites with sufficient snow cover in

winter. Sprouting and flowering occur when the meltwater runoff at the growing sites is highest.

Study sites

Main fieldwork and sampling had been carried out by JW in the growing season 2001 in the Zillertal Alps (Austrian Central Alps). The flowering frequency was assessed in the following growing season 2002. The study sites were located in the north-east-facing forelands of the Hintertux Glacier (2650 m a.s.l., 47° 04' N, 11° 39' E) which had been deglaciated about 60–80 years ago. In the highly disturbed subnival scree habitat the coverage of vascular plants is medium to low and shows typical elements of a subnival association (*Androsace alpina*, *Cerastium uniflorum*, *Geum reptans*, *Oxyria digyna*, *R. glacialis*, *Saxifraga bryoides*). Three sites with different snow accumulation in winter and thus different melting dates were selected: the early-thawing site (E-site) became snow-free in early June, the mid-thawing site (M-site) at the beginning of July, and the late-thawing site (L-site) in a NW-facing hollow at the end of July. The growing season 2001 was particularly short and ended with heavy snowfalls in the first week of September.

Field observations and samplings

In the growing season 2001, *R. glacialis* individuals ($n=60$ E-site, 60 M-site, 30 L-site) were marked with coloured plastic pickers just as they emerged from the winter-snow at the edge of snowfields (ESM Fig. 2a). Aboveground phenology was regularly monitored. For microscopic investigations, whole individuals ($n=3–10$ per date and site) were excavated and fixed in FPA 50 (propionic acid, formalin, 50% ethanol, 5:5:90) at intervals of about 10 days from the date of snowmelt until mid-October. Samples were kept in the dark at room temperature until later processing.

In the following growing season 2002, the number of flowers per inflorescence was determined in 10–15 individuals during peak flowering at each site. The percentage of inflorescences with different flower numbers was calculated; differences among sites were tested for statistical significance by Chi-square test. In the same individuals, the number of carpels per terminal flower was counted; site-differences were tested by one-way ANOVA followed by Duncan's post hoc test ($\alpha=0.05$; SPSS, IBM, NY, USA).

Microscopic analyses

Individuals were divided into ramets which were further dissected under a binocular microscope in 70% ethanol. The number of sympodia (S) per ramet was assessed from the basis to the top (hereinafter referred to as S1, S2, S3), and the number of developing and mature leaves per ramet was

determined. To investigate flower development, shoot tips were excised and placed on a microscopic slide in a drop of clearing solution composed of lactic acid (85%), chloral hydrate, clove oil, and xylene (2:2:2:1, by weight), following Herr (1971). The clearing solution homogenises the refractive index in the tissues, making them translucent. This allows the microscopic observation of structural details at different focal planes. We used differential interference contrast microscopy (Olympus BX50, Japan) to determine the stage of floral development, and, within anthers and ovules, the timing of sporogenesis. The length of floral structures (stamens, anthers, carpels, ovules) of terminal flowers were measured using image analysing software (Optimas 6.5, Optimas Corp., Seattle, WA, USA). The mean numbers of measurements per structure type, site, and date were: M-site $n=37$ out of 3–7 flowers; L-site $n=21$ out of 3–4 flowers. Statistical differences between M-site and L-site individuals for a given structure and date were tested by *t* test ($\alpha=0.05$). The data set for length measurement in E-site individuals was incomplete at the beginning of the growing season and therefore not considered.

For better visualisation of the different floral stages, scanning electron microscopy (SEM) was used. Dissected shoot tips were collected in 70% ethanol which was then replaced by amyl acetate (two changes), wherein the samples were stored for at least 24 h. Shoot tips were critical-point dried using liquid CO₂, mounted on aluminium stubs with adhesive paint, sputter-coated with 80% gold and 20% palladium (layer thickness 15 nm) and examined with a PHILIPS XL 20 Scanning Electron Microscope.

Results

Shoot architecture

Morphology and shoot architecture of *Ranunculus glacialis* is basically the same as presented by Aydelotte and Diggle (1997) for *Caltha leptosepala* (Ranunculaceae). The shoot consists of successive sympodia, each terminating in a uni- or multiflorous inflorescence (Fig. 1). Each sympodium derives from the axillary meristem of the leaf at the basis of the inflorescence of the previous sympodium. The apical meristem first forms a variable number of stem units and leaves before it becomes floral. As with *C. leptosepala*, structures of a sympodium do not mature in the same growing season. The aboveground foliage in a given growing season consists of a variable number of leaves from the distal end of the flowering sympodium of the current year (S1) that had begun to preform 2 years before, and the basal leaves of the next sympodium (S2) which will flower in the following year. Concomitantly with the preformation of the next year inflorescence (IF2) on S2, the S3 sympodium starts to

Fig. 1 Architectural diagram of the sympodial ramet structure of *R. glacialis* shown as extended axis following the presentation style of Aydelotte and Diggle (1997). S1, S2, S3 sympodium 1, 2, 3, respectively; IF1, IF2 inflorescence of the sympodium 1 and 2 respectively, the terminal apex of S3 is depicted as vegetative; R1 adventitious roots of S1. Only leaves and inflorescences shaded in full black mature and appear aboveground in a given growing season, younger structures above in grey are preforming belowground. WB winter bud; the bracket comprises the structures of the overwintering belowground ramet shown in Fig. 3

develop. Each S1 sympodium forms stem-borne roots while the previous sympodium (proximal to S1) degrades. Thus, during the growing season, older plant individuals consist of a cluster of largely independent ramets (Fig. 2a), comprising 3 sympodia each.

Sequence of shoot and flower development

Overwintering belowground ramets are budlike and contain all structures that mature and function in the current growing season plus preformed structures for the next two years (see WB in Fig. 1; Fig. 3). Buds are enclosed by scale-like leaves which arise from the middle part of each sympodium. These scales do not appear aboveground, are still present at the time of anthesis (Fig. 2b), and senesce shortly after. Immediately after snowmelt one to two photosynthetically active leaves at the distal end of the belowground part of sympodium (S1) expand. During the prefloration period the flowering shoot, bearing a variable number of stem leaves, elongates and flowers complete development. At the same time up to 4 basal leaves of the next sympodium (S2) emerge (L2 in Fig. 2b, c). Anthesis starts 2–3 weeks after snowmelt which is the second half of July at sites that become snow-free at the beginning of July (Fig. 4). In parallel, within the reproductive terminal bud of S2, the inflorescence for the subsequent year (IF2) continues preformation belowground wrapped by undeveloped foliage leaves and bud scales. Furthermore, sympodium S3 develops within the S2 bud in the

Fig. 2 Morphological status of *R. glacialis* at the time of anthesis. **a** individual consisting of two individually rooted reproductive ramets and two young, still vegetative daughter ramets (DR) which have originated from lateral buds of the previous sympodium. **b** Single reproductive ramet from **a**; most structures depicted belong to the currently flowering sympodium S1 with scale-like leaves (Sc1), two foliage leaves (L1), adventitious roots (R1), and the branched inflorescence shoot (IF1) with a number of stem leaves; the lowest inflorescence branch (red arrow) in the axil of the lowest stem leaf did not sprout. The two L2 leaves in the axil of the left L1 leaf are the basal leaves of the belowground renewal shoot S2 (not visible in this picture). **c** same ramet as in **b** with the scale-like leaves and the L1 leaves removed. Between the L2 leaves, the bud for the S2 shoot (blue arrow) is visible; the S2 bud is protected by juvenile bud scales which enclose underdeveloped foliage leaves, a preforming inflorescence which will mature in the following year, and the lateral bud primordium for the renewal shoot S3

Fig. 3 Overwintering belowground bud of *R. glacialis*. For the winter bud (WB) architecture see Fig. 1. **a** closed bud with view on L1 leaves; scales had been removed; R1 adventitious roots. **b** dissected bud with L1 leaves and, behind the lamina, the IF1 inflorescence (red arrowhead) which will mature and function in the coming growing season; sepals of the flower buds are covered with dark brown

trichomes which are partly visible; on the right side of IF1, the S2 symposium had formed in the axil of the L1 leaf. **c** dissected S2 symposium from picture **b**; the two L2 leaves at the basis will mature in the same year as the L1 leaves; they enclose the terminal bud of the S2 shoot with underdeveloped leaves and an already floral apex inside

Fig. 4 Sequence of flower and shoot development, and functional changes of structures in *R. glacialis* in the course of the growing season. For reasons of clarity, root symbols are not drawn. For general information on the shoot architecture and symbols see Fig. 1

axil of the uppermost still unemerged foliage leaf at the basis of the S2-inflorescence. S2-inflorescence primordia appear uniflorous at the beginning of the growing season but may form a monochasial cyme later. The apex of the youngest sympodium S3 remains vegetative during the whole growing season and continually forms new leaf primordia which will mature in the next year (basal leaves) and in the year after (distal leaves). At the end of the growing season, some S3-apices become reproductive and overwinter in an early floral state. Soon after seed dispersal, all aboveground structures senesce.

Stages of floral development

Seven main stages of flower bud differentiation were distinguished (Fig. 5). Stage 0 defines the vegetative shoot apex with alternately arranged leaf primordia (Fig. 5a). Stage 1 marks the transition from the vegetative to the reproductive apex which becomes clearly identifiable by changes in shape and size of the apical meristem and the appearance of primordial stem leaves (Fig. 5b) and later first sepal bulges. In stage 2 sepal and petal primordia emerge (Fig. 5c). In stage 3 stamen primordia appear as clear humps in a spiral arrangement; sepal primordia further enlarge (Fig. 5d, e). In stage 4 carpel primordia become visible at the top of the floral apex (Fig. 5f). In stage 5 stamen primordia differentiate into filaments and pollen sacs with archesporial tissue (ESM Fig. 1b); within each peltate carpel, a single ovule primordium forms (ESM Fig. 1c); the enlarged sepals begin to conceal the other floral organs within (Fig. 5g). Stage 5 defines the development status at the onset of winter dormancy; plants pass winter belowground without further development. Stage 6 characterizes the prefloration period, i.e. the time span between the melting of the winter snow and anthesis during which the floral organs further enlarge and mature (Fig. 5h, i). Floral development resumes when the belowground buds begin to sprout below the thinning snow cover (ESM Fig. 2b). Within the anthers, the archesporial tissue differentiates into sporogenic tissue (ESM Fig. 2c). Ovules enlarge but remain still in a premeiotic state (ESM Fig. 2d). In flower buds sampled 12 days after snowmelt (ESM Fig. 3a), male and female meiosis was completed: pollen sacs contained uninuclear pollen grains (ESM Fig. 3b), and embryo sac development had started (ESM Fig. 3c). Within an inflorescence, depending on the position in the inflorescence, the development of lateral flowers lags one and more stages behind the terminal flower (Fig. 5j, k). In the weakly protandrous flowers, anthers of the outermost whorl open on the first day of anthesis (Fig. 5m). On day one stigma lobes are still short but already receptive at the tips. Full stigma receptivity occurs from day 3 on (Steinacher and Wagner 2010).

Timing of floral development at different lengths of the growing season

At the early-thawing site (E-site), sampling began only at the onset of anthesis in the second half of June. At that time, flower preformation in the terminal flowers of the next year inflorescences (IF2) was in stage 1–2 and reached stage 3 at the end of anthesis (Fig. 6 left). Stage 4 was attained at the time of seed dispersal in early August. While the aboveground structures senesced, flower preformation continued belowground. IF2 flower buds entered winter dormancy in stage 5 in mid-August, two weeks before winter conditions began. Development in lateral flowers varied greatly, depending on the position within the inflorescence. Lateral flower buds of the first order, though developing slower than terminal flowers, reached the same final stage 5 as terminal flowers before the onset of winter. Flowers of the second and higher order more strongly lagged behind and stopped development in an earlier stage (Fig. 6 right). At the beginning of September, a part of the apical meristems of sympodium 3 (which will continue in the coming year as sympodium 2) had become floral and was in stage 1.

At the mid-thawing site (M-site), which became snow-free at the time when the E-site individuals flowered, the prefloration period lasted about 2 weeks. During this period IF1 inflorescences expanded and matured, whereas IF2 apices remained in the floral stage 1 all the time. Further IF2 development only began with anthesis of IF1 and continued at about the same speed as at the E-site. IF1 flowers matured seeds just in time before snow-falls in early September began. Terminal IF2 flower buds were still in stage 4 but continued development below the snow and reached stage 5 at the beginning of October. Preformation of lateral flowers strongly lagged behind and was in stage 3 before the September snow-falls began. Like in the terminal flower buds, development progressed below the snow and attained stage 5 in the lateral flowers of first order until October. Floral IF3 apices in sympodium 3 were observed from early September on.

For the individuals at the late-thawing site (L-site) the growing season lasted only 1 month from the end of July until the end of August. When the plants emerged from the snow, leaves and inflorescence shoots of S1 had elongated (but still lacked chlorophyll), and terminal IF1 flowers were mostly in stage 6, shortly before anthesis. Due to subnivean development, the prefloration period was somewhat shorter than in E- and M-site individuals. Nevertheless, seeds of IF1 flowers did not mature in time and decayed below the snow in September. Belowground flower preformation continued until October but stopped in stage 4 in most flower buds at

Fig. 5 Stages of floral development in *R. glacialis*. **a** Stage 0: vegetative shoot apex (VA) with leaf primordia (L). **b** Early stage 1: floral apex (FA) with stem leaf primordia (SL). **c** Stage 2: sepal (s) and petal primordia (p) arise. **d** Early stage 3: stamen primordia (st) appear in several whorls. **e** Late Stage 3: stamen primordia as clear humps visible. **f** Stage 4: carpel primordia (c) emerge at the top of the apex. **g** Stage 5: stamens begin to differentiate into filaments and anthers, carpel primordia enlarge. **h** Stage 6: prefloration period; pollen sacs and emerging stigma lobes are clearly visible. **i** Petals have

significantly enlarged and stigmas (arrowhead) have further elongated. **j** Within the determinate inflorescence, the development of lateral flowers (LF) initially lags behind the terminal flower (TF). **k** Later inflorescence stage with TF and adjacent LF of the first order in stage 5; carpels are horse-shoe shaped and still open (circle); filamentous structures between the flowers are trichomes on the surface of the sepals. **m** View into an open flower at the beginning of the male phase of anthesis (first outer anthers open)

the transition to winter dormancy. Many inflorescences were uniflorous and, in the case lateral flowers had formed, they

Fig. 6 Dynamics of floral development in *R. glacialis* at the early, mid and late melting site. Values represent floral stages in single individuals (for staging see Fig. 5) at different times during the growing season for terminal and lateral flowers of the S1 sympodium (flowering in the current year, black filled circles), the S2 sympodium (flowering in the following year, grey filled circles) and the S3 sympodium

whose flowers mature in the year after next (open symbols). Circles with a cross stand for the same value determined several times on the same date. Open triangles signify a vegetative apex (stage 0) in the S3 sympodium. Black trapeziums mark the period of anthesis in the current year. **m** Seed maturity; **d** aboveground structures partly (broken line) or fully (solid line) died off. Dotted area: snow cover

were in an early stage. Irrespective of the developmental lag in S2, few S3 sympodia had become floral.

Size of fertile floral structures and flowering frequency

At snowmelt (SM), stamens, anthers, carpels and ovules of terminal S1 flower buds were significantly larger in L-site individuals than in M-site individuals ($p \leq 0.002$, t test); (Fig. 7a). Conversely, structures in S1 flowers at the beginning of anthesis (A) and in belowground S2 flower buds at the onset of winter (W) were significantly larger in M-site individuals than in L-site individuals ($p \leq 0.027$).

Furthermore, ovules were not yet initiated in S2 buds of L-site individuals before winter. Data for E-site individuals are not shown because of a lack of data for snowmelt and early anthesis.

The number of flowers per flowering shoot differed significantly among sites in the year following the main investigation year in which the growing season was particularly short (E-site > M-site, L-site, $p \leq 0.002$, Chi-square test); (Fig. 7b). Only E-site individuals formed up to 3 lateral flowers per shoot, whereas flowering shoots of L-site individuals were mostly uniflorous. Moreover, the mean number of carpels per terminal flower was significantly higher ($p \leq 0.014$, one-way ANOVA) in early and mid flowering individuals

Fig. 7 **a** Length of fertile floral structures at the mid and late melting site in S1 flowers at the time of snowmelt (SM) and anthesis (A), and in belowground S2 flower buds at the beginning of winter (W); values are means $\pm$ SE; asterisks indicate significant differences between mid and late for a specific structure at SM, A and W (t test). **b** Percent

flowering shoots with 1 (white), 2 (light grey), 3 (dark grey) and 4 (black) flowers per inflorescence in early, mid and late-flowering individuals in the following growing season; asterisks indicate significant differences among the sites (Chi-square test)

($n = 136 \pm 62$ standard deviation, SD) than in late flowering individuals ($n = 79 \pm 20$ SD).

Discussion

Shoot architecture of *R. glacialis* is similar to that described for other Ranunculaceae such as *Caltha leptosepala* (Aydelotte and Diggle 1997) and *Ranunculus alpestris* (Mauracher 2017), and thus shows a strong phylogenetic conservatism. The rhizome system disintegrates into single ramets which consist of three sympodia each: (1) the functional sympodium which ends in a terminal inflorescence flowering in the current growing season, (2) the following sympodium whose basal foliage leaves become functional in the current growing season, whereas the rest of the sympodium forms a belowground bud which sprouts and flowers in the next growing season, and (3) the next but one primordial sympodium which—after a vegetative phase—may become floral at the end of the growing season or in the following spring. Hence, an older *R. glacialis* individual consists of a cluster of independent individually rooted ramets. This plant architecture is beneficial for the colonization of higher mountain areas. As pioneer species, *R. glacialis* often grows on unstable scree (Pauli et al. 1999). Small autonomous ramets might cope better with the mechanical stress on the unstable ground than complex shoot systems. A further advantage of small units is the short transport distances for assimilates from leaves or rhizoms to the growing parts which might enhance growth and maturation of

plant structures—similar as in bulb geophytes with a short aboveground growth period (Khodorova and Boitel-Conti 2013). The continuous emergence and maturation of leaves suggest the following allocation pattern in *R. glacialis*: leaves in the distal region of the lowest sympodium emerge first after snowmelt and feed mainly flowers and fruits of the current year inflorescence; later expanding basal leaves of the next sympodium partly supply flower preformation and partly deposit storage compounds in the rhizome section sprouting in the following year. During the growing season, belowground organs of *R. glacialis* store plenty of starch (Moser et al. 1977), which might be converted to low molecular carbohydrates in cold periods (Körner 2003). However resource allocation within clonal plants may be complex because of internal reallocation processes and, in addition, may vary with environmental conditions (Bazzaz et al. 2000). Thus further detailed studies on the dynamics of allocation in *R. glacialis* are necessary to confirm the above-assumed pattern.

In *R. glacialis*, floral meristems are initiated 2–3 years before flowers become functional. As stated before, the transition from the vegetative to the reproductive apex occurs belowground either at the end of the growing season after aboveground structures have died back (3-year flower preformation) or at the beginning of the growing season during sprouting at the latest (2-year preformation). In both cases, floral primordia remain in an early state during the prefloration period and continue development parallel to anthesis and fruit development in the current season. A multi-season strategy has up to now only been reported for the alpine

species *Acomastylis rossii* (Meloche and Diggle 2001) and *Polygonum viviparum* (Diggle 1997), whose inflorescences require 3 and 4 years to progress from initiation to structural and functional maturity. In a recent study, a three-year strategy was found in the alpine-nival species *Geum reptans* and *Oxyria digyna* (Mauracher 2017). In the year of floral initiation, floral meristems complete development in an early developmental stage. During the second growing season flower bud preformation continues after anthesis in the current year and reaches an advanced stage at the end of the vegetation period. Finally, in the third year, anthesis starts 1–2 weeks (*Geum*) and 3 weeks (*Oxyria*) after snowmelt. All species mentioned here colonize, similar to *R. glacialis*, climatically and physically highly variable environments at higher elevations and latitudes. A multi-year strategy of flower preformation seems to be advantageous under these conditions as it allows an early regulation of reproductive resource allocation (Diggle 1997). Moreover, two or more flower cohorts in preparation at the same time offer the advantage of a permanent reserve pool of flower buds which might even prematurely develop further in the case the preceding flower cohort gets lost by e.g. frost spells, hail, rockfall or herbivory (snow voles and hares in particular). Since developmental processes are strictly coordinated, the missing signal of the current year's flowers could stimulate the next leaf and flower cohort to become functional (Huhta et al. 2009). An indication that preformed flower cohorts can mature precociously is provided by Prock and Körner (1996): in a cross-continental transplantation study, alpine genotypes of *R. glacialis* that had been transplanted to northern Scandinavia sprouted and flowered up to 3 times during the growing season under the extreme long-day conditions.

The date of snowmelt hardly affected the dynamics of flower preformation in *R. glacialis* which reflects a close link to the vegetative and reproductive aboveground development in the current season. As stated in the introduction, the lengths of reproductive phenophases vary only little at different sites at different times in the alpine-nival ecotone: in a single flower, the prefloration period lasts 2–3 weeks, anthesis 1 week, and seed development 4–5 weeks—in total 7–8 weeks (Wagner et al. 2010). Obviously, under the given temperature conditions at the lower distribution boundary, developmental speed cannot be accelerated any more. Similar observations made Gugger et al. (2015) in a reciprocal transplantation experiment with congeneric species pairs originating from mid and high elevation. At the lower and thus warmer site, phenophases were less advanced in high-elevation species than in mid-elevation species which led to the conclusion that high-elevation species are genetically constrained to their specific adaptations to a cold environment. *R. glacialis* undoubtedly is adapted to cold climatic conditions. Within the climatic gradient between the upper and the lower distribution

boundary, however, individuals respond quite plastic. In accordance with the date of snowmelt, there are considerable shifts in the timing and in the lengths of reproductive phenophases (Wagner et al. 2010). It can be assumed that the same applies to the phases of floral development belowground though there can be discrepancies in the coordination between aboveground and belowground processes. Such differences became apparent in the present study at the late melting site where due to the short growing season seeds did not mature in time and, together with all aboveground organs, prematurely died off. Belowground development, however, proceeded below the snow before plants passed into winter dormancy. This shows that the aboveground reproductive development is more time-limited than the belowground development which may continue as long as the ground is not frozen. Nevertheless, a premature leaf death negatively affected the size and number of fertile floral organs and the development status (most L-plant flowers entered winter in stage 4 while most E- and M-site plants had attained stage 5). Furthermore, the number of preformed flowers per inflorescence decreased the later the growing season started.

Subnivean development is widespread among perennial plants inhabiting cold regions or flowering in early spring (Forbis and Diggle 2001; Hamerlynck and Smith 1994; Kimball and Salisbury 1974; Körner et al. 2019). This phenomenon is particularly pronounced towards the end of winter when below the thinning snow cover vegetative and reproductive organs resume growth by absorbing long-wave radiation and heating up above zero. Cold adapted plant species may even grow at zero as shown for the alpine snow bed plant *Soldanella pusilla* (Körner et al. 2019). Flower buds are initiated in the previous growing season and remain dormant in an early developmental stage until mid-winter. Then the flowering shoots start to develop further at 0 °C below metre-thick snow in the dark and are ready for anthesis when they emerge from snow at the beginning of the growing season. The authors assume that an internal clock causes the timely beginning of development, rather than a day-length signal which would not work below a deep snowpack. *R. glacialis* did not develop further under deep winter snow. However, subnivean growth resumed under the thinning snow cover in spring and lasted the longer the later the melting date was. This explains why at snowmelt floral structures in late-thawing individuals were significantly larger than those of mid-thawing individuals though the reverse was true as winter began. During the prefloration period, in turn, flowers of mid-thawing individuals developed significantly larger fertile structures and had more carpels than those of late-thawing plants. It is conceivable that long-day conditions and maximum thermal input during the prefloration period in the first half of July promoted floral growth in M-site individuals, whereas the signal of decreasing

day-lengths in August limited growth of floral structures in L-site individuals.

In conclusion, plant architecture and developmental dynamics of *R. glacialis* are well adapted to life in the surroundings of glaciers at higher elevations and latitudes. The loose ramet clusters enable colonization of unstable scree which is typical for deglaciated areas. The preformed flower buds for the coming flowering season overwinter in an advanced state leading to anthesis soon after snowmelt. Subnivean development prolongs the period for flower preformation in case winter conditions set in too early and shortens the prefloration period when snowmelt is late. In the subnival zone, the duration of developmental stages is rather stable and largely independent of the date of snowmelt. The period from snowmelt to seed maturity of just 7–8 weeks is particularly short compared to other high-mountain species (Wagner et al. 2012). The staggered preformation of architectural units creates a permanent belowground reserve pool of reproductive units which ensure the survival of an individual in case aboveground tissues get lost due to herbivory or abiotic disorders. In addition, *R. glacialis* is ice tolerant during all reproductive stages which helps to survive sudden cold spells in summer and to tolerate night frosts whose frequency increases with elevation (Ladinig et al. 2013; Stegner et al. 2020). All these attributes are highly advantageous in the harsh and unpredictable high-mountain climate and contribute to the colonization success of *R. glacialis* in nival environments.

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Author contributions SM analysed the plant material, collected the data, and wrote parts of the manuscript. JW designed and supervised the study, carried out the field work, and wrote the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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