Should I sex or should I go?

Expansion of species niche through autopolyploidy

Ingrid Vesterdal Tjessem

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Centre for Ecological and Evolutionary Synthesis
Department of Biosciences
Faculty of Mathematics and Natural Sciences
UNIVERSITY OF OSLO

Department of Arctic Biology
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Abstract

Polyploidy is widespread in the plant kingdom and can lead to phenotypic alterations and novel variation, which can be advantageous in certain ecological, spatial and climatic settings, for instance, by promoting shifts in reproductive strategy. Ecological and reproductive competition from parental diploid species is a major barrier to polyploid establishment (minority cytotype exclusion principle). To survive over time, newly formed polyploids can adapt to a somewhat different niche (niche shift hypothesis) than the one occupied by their progenitor. However, our knowledge on the establishment and niche differentiation in natural populations of autopolyploids is still scarce.

Saxifraga oppositifolia (Saxifragaceae) is an autopolyploid, arctic-alpine plant with diploids, triploids and tetraploids growing in a wide range of habitat types. Reproductive strategy and output have in former studies been related to the two main growth forms (cushions and trailing plants), but have not been tested for the effect of ploidy levels. With this thesis, I investigated whether autopolyploidization in *S. oppositifolia* created a shift in reproductive strategy, from sexual reproduction to vegetative propagation with shoot fragments, and if so, whether this has led to niche expansion for the species as a whole.

Fieldwork was carried out in Svalbard in 2020 using five transects covering three habitat types (ridge, slope, riverbed) where *S. oppositifolia* is growing. Data on sexual reproduction was obtained from 720 plants by recording number of flowers and capsules with seeds, counting seeds per capsule, and conducting a germination experiment. Data on asexual reproduction was obtained through a rooting ability experiment including cuttings from plants of different ploidy levels and from different habitat types. The resulting data was analyzed through explorative analyses with AICc and likelihood-ratio chi squared tests.

Sexual reproduction dominated in diploids, which had a much higher reproductive output in terms of flower, capsule and seed production compared to triploids and tetraploids. Triploids and tetraploids were, on the other hand, more efficient in vegetative propagation. Sexual reproduction in diploids was promoted in ridges, whereas triploids and tetraploids were found in more moist and less disturbed riverbeds, which seem to be optimal for vegetative propagation. I have found that ploidy levels affect sexual and asexual reproduction, supporting that autopolyploidy has led to a shift in reproductive strategy allowing a niche expansion and polyploid colonization in less disturbed and more moist areas.

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«In natural science the principles of truth ought to be confirmed by observation.»

Carl Linnaeus – Philosophia Botanica, 1751.

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1 INTRODUCTION

Polyploidy, the process leading to more than two complete sets of chromosomes, is widespread in the plant kingdom and has contributed to speciation and the development of many floral novelties during the major diversification of angiosperms (Soltis & Soltis, 1999; Chanderbali et al., 2016; Soltis & Soltis, 2016). Polyploidy is in fact so frequent in many plant groups, that formation of polyploids is often considered the rule rather than the exception (Soltis et al., 1993; Soltis & Soltis, 1999).

Polyploids are generally classified as two main types: autopolyploids, with multiple chromosome sets derived from a single species, and allopolyploids, which have a hybrid origin followed by a polyploidization event. Allopolyploids are often morphologically different from their diploid progenitors (Spoelhof et al., 2017). This has led to a higher degree of taxonomic recognition of allopolyploids, whereas autopolyploids, which often resemble their progenitor, have been considered less common and less evolutionary significant (Spoelhof et al., 2017). Compared to allopolyploids, autopolyploid linages are therefore understudied and regarded as variation within the same species-complex (mixed-ploidy species), rather than separate systems. Accordingly, autopolyploids are easily overlooked, and our knowledge on the evolutionary consequences of autopolyploidy is particularly scarce (Soltis et al., 2007; Parisod et al., 2010; Soltis et al., 2014).

As an evolutionary mechanism, polyploidy is known to be particularly important in stressful environments, where specialized adaptations to extreme surroundings can be necessary for species survival (Van de Peer et al., 2021). Several studies have investigated how polyploidization can contribute to rapid change in gene expression, which leads to phenotypic alteration and novel variation (Ramsey & Schemske, 2002; Adams & Wendel, 2005; Chen, 2007). This can be particularly advantageous in certain ecological, spatial and climatic settings (te Beest et al., 2012; Rice et al., 2019). Climate, and especially temperature, seems to influence and predict the distribution of polyploid linages, both allopolyploids and autopolyploids (Rice et al., 2019). For instance, autotetraploids of *Dendranthema nankingense* (Asteraceae) showed enhanced tolerance to cold stress, but also drought and salt stress, due to morphological and physiological alterations (Liu et al., 2011). Godfree et al. (2017) looked at the perennial grass *Themeda triandra* (Poaceae), and found that total output of viable seeds in drought- and heat-stressed tetraploids was much higher compared with

diploids. Cheng et al. (2020) found evidence for a successful autopolyploidy-driven invasion of *Solidago canadensis* (Asteraceae) from temperate areas to pan-tropical regions, which exemplify how invasive plants may owe their success to polyploidization (te Beest et al., 2012; Cheng et al., 2020).

The area north of the Arctic Circle (hereinafter the Arctic) can be considered an extreme and stressful environment for organisms to survive in, as it is characterized by strong seasonality, extreme temperatures, and severe weather events. New, favourable novelties provided through polyploidy might increase the chance of survival, for instance increased mutational robustness which can buffer small populations and low genetic diversity, or altered gene expressions which may increase stress tolerance (Van de Peer et al., 2021). It is therefore not surprising that the frequency of polyploidy has been shown to increase with latitude (Brochmann et al., 2004; Rice et al., 2019). Polyploid linages have the potential to immediately acquire a range of new traits enabling expansion into new environments or adjustments to changed conditions (te Beest et al., 2012). Polyploidization may thus play an important role during periods of larger climatic changes and seems for instance to have been important during re-colonization of the glaciated regions after the last ice age (Brochmann et al., 2004; Brochmann & Brysting, 2008).

Although the formation of polyploids is rather frequent in stressful environments, most polyploids are ephemeral and short-lived (Arrigo & Barker, 2012). To be evolutionary successful, polyploids must locally establish and propagate once formed, but formation and establishment are still poorly understood aspects of polyploid evolution (Spoelhof et al., 2017). Ecological and reproductive competition from parental diploid species is a major barrier to polyploid establishment and can be explained through the minority cytotype exclusion (MCE) principle (Levin, 1975; Husband, 2000). The MCE principle stresses that the reproductive success of a species with several ploidy levels, also known as cytotypes, is frequency dependent. It predicts that the minority ploidy level in a certain population of mixed ploidies will become excluded from the population within a few generations. This is because the minority cytotype will suffer from a greater portion of ineffective pollination events, as there are fewer mates that can secure successful pollination, and thereby fewer individuals, which can maintain the minority cytotype population. However, there is an exception that proves the rule. Levin (1975) pointed out one way to avoid the disadvantages from MCE: by adapting to other ecological conditions than that of the majority cytotype,

polyploids can avoid a competition that is already lost. However, to release the potential for new adaptations and expansion, newly formed polyploids (neopolyploids) must establish efficient reproductive barriers towards their progenitors to avoid back-crossing.

Neopolyploids are often reproductively challenged through chromosomal paring problems (Grant, 1981; Ramsey & Schemske, 2002) or incompatibility in the endosperm (Köhler et al., 2010), which usually leads to reduced fertility or nonviable progeny. Several studies have shown that neopolyploids have better odds for establishment, if they can reproduce asexually through clones or by self-reproduction (Otto & Whitton, 2000; Ramsey & Schemske, 2002; Baack, 2005; Buggs & Pannell, 2006; Soltis et al., 2010). For instance, autopolyploidy has shown to immediately increase investment in asexual instead of sexual reproduction in *Chamerion angustifolium* (Onagraceae), and thus increase establishment of neopolyploids (Van Drunen & Husband, 2018). Furthermore, Wakui and Kudo (2021) found that tetraploids of *Vaccinium vitis-idaea* (Ericaceae) have differentiated from their self-incompatible, diploid progenitor by developing self-compatibility and increasing vegetative growth. This could also explain the ecotypic differentiation, which has led to tetraploids mainly being distributed in low-elevation populations, whereas diploids are dominating in alpine populations.

Change of reproductive strategy is just the first step towards successful establishment. The second step is to successfully survive in the long term. To survive over time and to avoid competition, one solution is adapting to a somewhat different niche than the one occupied by the parental species. The competitive exclusion principle, also referred to as Gause's law (Gause, 1934), addresses how two species cannot coexist if both occupy the exact same niche. The primary solution to both MCE and Gause's law is habitat segregation through niche divergence, as suggested by the niche shift hypothesis (NSH) (Husband, 2000; Levin, 2004). Karunarathne et al. (2018) looked into how reproductive shifts and ecological niche divergence may foster various ploidy levels. They found that tetraploid populations of the grass *Paspalum intermedium* (Poaceae) were maintained by successfully expanding the species' ecological preferences together with a shift from sexual reproduction to apomixis, a type of asexual reproduction. Diploids, on the other hand, continued to dominate in the core distribution area of the species, and did not compete with tetraploids for resources. As previous studies (Levin, 1983; Husband et al., 2013), the study of *P. intermedium* thus supports the NSH and represents an example of a polyploid range expansion into wider or more extreme ranges than the diploid progenitors.

Asexual reproduction, for example through ramets, may be beneficial for local population growth through rapid, short distance spread. However, asexual reproduction reduces genetic diversity and increases the risk of diseases and other effects caused by low genetic diversity (Yang & Kim, 2016). Sexual reproduction, on the other hand, promotes genetically diverse offspring through recombination, mutation, and gene flow. Many plants utilize both reproductive modes, sexual and asexual, which can facilitate adaptation to temporally or spatially variable environments. Zhang and Zhang (2007) hypothesized that plants living in habitats with fluctuating environmental conditions and strong competition allocate most resources towards sexual reproduction, whereas clonal reproduction is dominating in stable habitats, although this balance may vary among species depending on environmental conditions.

As reproduction and niche shifts are key in the establishment of neopolyploids, these factors are of particular interest when searching for a good model system. The Arctic is an excellent region for polyploidy research, mainly because of the high number of polyploid species already present (Brochmann et al., 2004), but also because many species may be dependent on rapid evolution for surviving the enhanced climatic changes (Franks et al., 2007; Lustenhouwer et al., 2018). The arctic environment includes habitat heterogeneity that are strongly linked to reproductive traits (Crawford, 2008). In arctic environments, the interplay between climatic factors and topography causes a heterogeneous distribution of snow, creating a strong habitat gradient over relative short distances (Billings & Mooney, 1968; Cooper et al., 2011). An important habitat gradient is from dry and windblown ridges with little or no snow cover, to sloping heaths and hollows with a more stable snow cover. Traits related to reproduction are found to vary along this gradient: early flowering species, which typically are found closer to the ridge, have breeding systems favouring out-crossing. On the other hand, late flowering species, which often are found in areas with deeper snow cover and later melt out, are frequently selfing or reproducing asexually (Yang & Kim, 2016).

Saxifraga oppositifolia (Saxifragaceae) is an arctic-alpine species with two main ploidy levels resulting from autopolyploidy: diploids (2n = 26) and tetraploids (2n = 52), but also triploids (2n = 39) do occur (Flovik, 1940; Elven et al., 2011; Müller et al., 2012; Eidesen et al., 2013). *Saxifraga oppositifolia* mainly reproduces sexually but is also known to reproduce asexually through vegetative propagation with trailing branches or ramets (Kume et al., 1999). It is

protogynous and a predominant outcrosser, and is one of the earliest flowering species in its habitat, and as an entomophilous species, it is especially important for pollinating insects early in the growth season. Crossing experiments have shown that it is pollen limited particularly late in the growth season and that seed set is strongly reduced in self-pollinated flowers (Kevan, 1972; Stenström & Molau, 1992; Gugerli, 1998).

Saxifraga oppositifolia has high level of phenotypic variation (Figure 1.1), shown for instance as different growth forms varying from trailing plants to cushions, and with intermediate growth forms in-between (Brysting et al., 1996). Different growth forms have been related to different reproductive strategies, with trailing plants having more efficient vegetative propagation compared to cushion formed plants (Kume et al., 1999). Different growth forms have also partly been related to ploidy levels, with cushions mainly being diploid, while trailing plants can be of all three ploidy levels (Eidesen et al., 2013). Finally, growth form has been related to habitat type, with cushions dominating on exposed ridges, while trailing plants are more common in moist riverbeds (Crawford et al., 1993; Crawford et al., 1995; Eidesen et al., 2013). Eidesen et al. (2013) additionally found that tetraploids seem to have shifted towards a narrower pH range, as they are commonly found in more alkaline habitats compared to diploids, which have a lower pH optimum. Generally, *S. oppositifolia* is considered a weak competitor, yet tetraploids have been recorded as slightly better competitors than diploids (Eidesen et al., 2013).

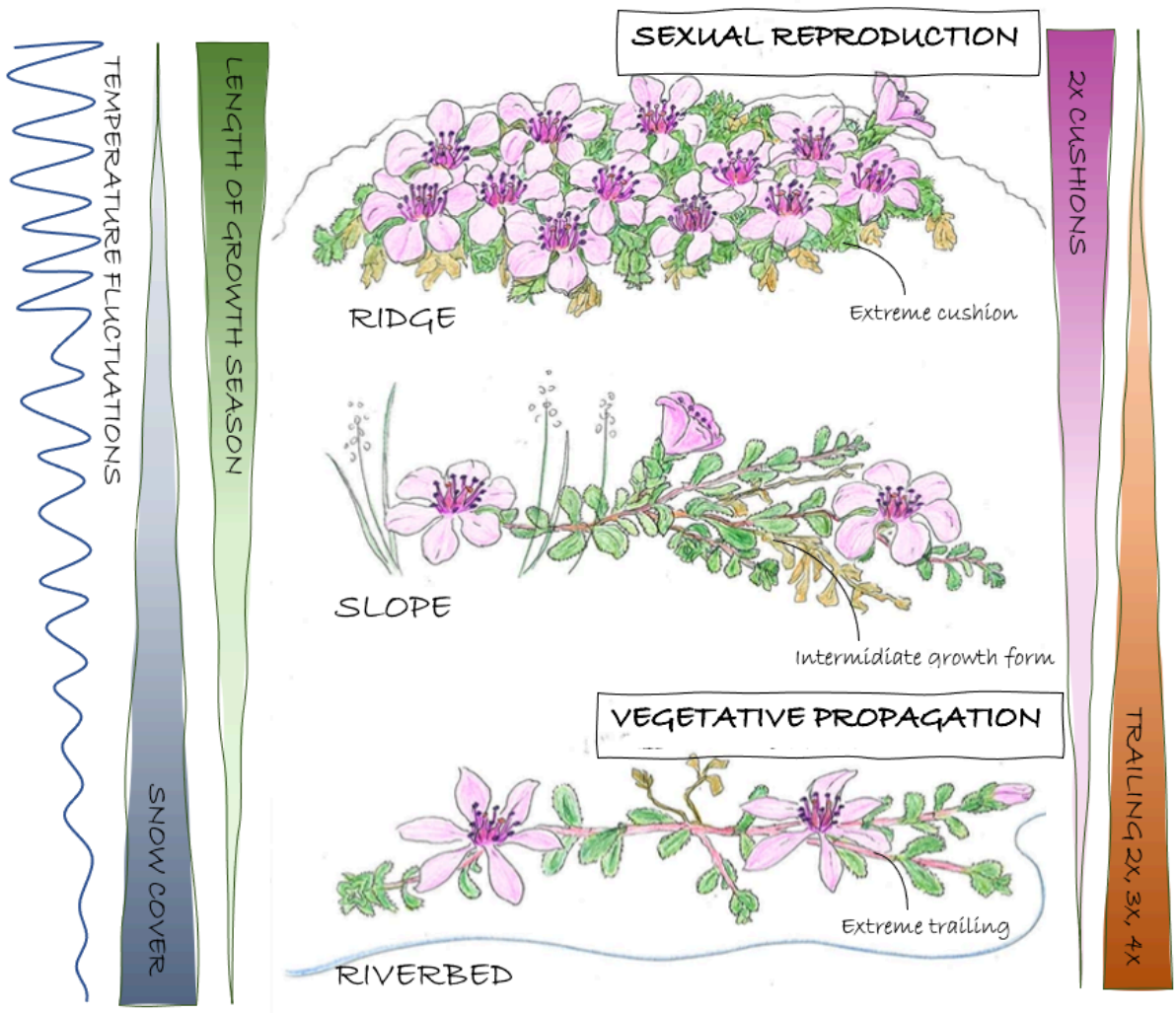

Figure 1.1. Abiotic factors impacting phenotypic variation in the arctic-alpine *Saxifraga oppositifolia*, with different growth forms inhabiting a range of habitat types, from extreme cushions dominating in exposed ridges to trailing plants being more common in sheltered riverbeds. Cushions are mainly diploid while trailing plants can be both diploids, tetraploids and triploids. Ridges are characterized by early snowmelt and long growth season, whereas riverbeds melt out later and have shorter growth season. Slopes have a higher vegetation cover compared to the other habitat types, and with higher biomass production, the soil typically contains higher levels of soil organic matter (SOM). Ridges are more weather exposed, with more fluctuating temperatures than riverbeds.

Although *S. oppositifolia* has been studied for many decades, it is not until recently that a link between flower production and ploidy was suggested, as more flowers were observed in diploids than in triploids and tetraploids (P. B. Eidesen et al., unpublished data). More flowers are observed also in diploids with trailing growth form, suggesting that it might not only be an effect of growth form. If higher flower production is connected to ploidy level rather than growth form and habitat type, how do tetraploids and triploids compensate for the lower flower production? One solution is to produce more seeds per capsule compared to diploids.

Another solution could be to produce seeds of higher quality and hence have in higher germination percentage compared to diploids. A third solution could be higher investment in asexual reproduction, through vegetative propagation, with the consequence of reducing genetic diversity in the long term. However, it is yet to find out how sexual reproduction and vegetative propagation might vary with ploidy level.

A model system using *S. oppositifolia* to bridge the gaps of knowledge concerning the long-term evolutionary success of autopolyploids was established in Svalbard in 2018. It consists of five transects spanning the snow distribution gradient, and thus representing three habitat types: from exposed ridges and more sheltered and sloping heats (hereinafter referred to as slopes) to moist riverbeds. By using this system, I will investigate whether autopolyploidization in *S. oppositifolia* has created a shift in reproductive strategy, and if so, whether this has led to a niche expansion for the species complex as a whole.

More specifically, I will collect data on reproductive strategy and output to estimate the effect of ploidy level as well as other potential sources to variation in reproductive traits. I aim to address the following objectives:

OBJECTIVE I: Sexual reproduction

I will investigate whether ploidy or other potential sources of variation (growth form, habitat type, transect, plot no., flower production, total no. of seeds and seed weight (g)) best explain variation in sexual reproduction in *S. oppositifolia* by recording capsule and seed production and performing a seed germination experiment.

OBJECTIVE II: Vegetative propagation

I will investigate whether ploidy or other potential sources of variation (soil type, habitat type, plot no.) best explain variation in vegetative propagation in *S. oppositifolia* by recording rooting ability and leaf production in a rooting experiment of field collected cuttings.

2 MATERIAL AND METHODS

2.1 STUDY AREA

From June to August 2020, fieldwork was conducted in Spitsbergen, which is the largest of the islands in the Archipelago of Svalbard. The study area is situated around Longyearbyen, which is located innermost in Adventfjorden, in the western part of Spitsbergen (Figure 2.1). Adventfjorden is influenced by the West Spitsbergen Current, which contributes to a warmer and more Atlantic climate compared to the northern and eastern part of Spitsbergen. Based on vegetation composition and summer temperatures, Adventdalen is included in the Arctic bioclimatic subzone C, and commonly characterized by an open patchy vegetation with 5-50% cover of vascular plants (Walker et al., 2005).

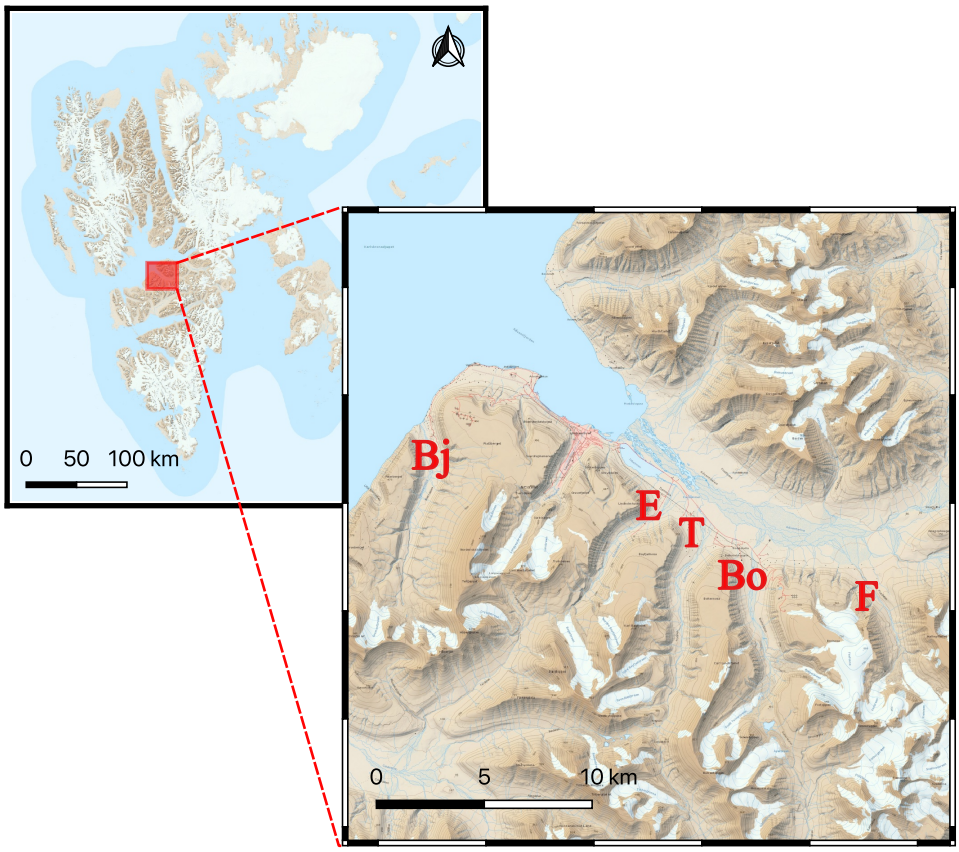

Figure 2.1. Map of the Archipelago of Svalbard (74–81ºN,10–35ºE) showing the location of the study area (insert), with the five valleys in which fieldwork was conducted in June-August 2020. Bj – Bjørndalen, E – Endalen, T – Todalen, Bo – Bolterdalen, F – Foxdalen.

2.2 FIELD DESIGN

Data was collected from five successive valleys representing a gradient from coast to inland (Figure 2.1). The field design was established by Pernille Bronken Eidesen in 2018 and 2019 as part of a project owned by The University Centre in Svalbard (UNIS), and included five transects, one in each valley. Each transect included three plots, which represent three habitat types where *S. oppositifolia* is commonly growing in Svalbard: ridge, slopes and riverbeds, and thus representing a gradient of snow distribution.

Windblown ridges (Figure 2.2) are reshaped year by year due to periglacial disturbances, which include freeze and thaw processes and solifluction, making ridges unstable the whole year-around. With little or no snow cover, ridges, are dominated by unsorted grain-sized till, and left exposed to wind abrasion and more extreme temperature fluctuations compared to slopes and riverbeds (Appendix A).

Figure 2.2. Fieldwork in August 2020 where capsules were registered and collected from *Saxifraga oppositifolia* plants found in plot 13 (ridge) in Foxdalen.

Slopes (Figure 2.3) are regarded as semi-dry heaths and have higher vegetation cover compared to ridges and riverbeds. This habitat type is typically sheltered from harsh weather but is influenced by interspecific competition from other herbs, grasses, mosses, and lichens. With higher biomass production, soil in slopes is slightly more acidic compared to the more alkaline and mineral rich soil in ridges and riverbeds.

Figure 2.3. Fieldwork in July 2020 where flower phenology and capsules were registered from *Saxifraga oppositifolia* plants found in plot 9 (slope) in Bolterdalen.

Riverbeds (Figure 2.4) are more inconsistent and can either be characterized by being drier and more stabilized with some vegetation cover and biological crust, or consist of more unstable sediment types varying from clay and silt to pebbles and larger gravel (Appendix B), and hence have less or no vegetation cover. The latter type is particularly unstable during the growth season as it occasionally is flooded with melt water influencing the habitat with fine moraine and till.

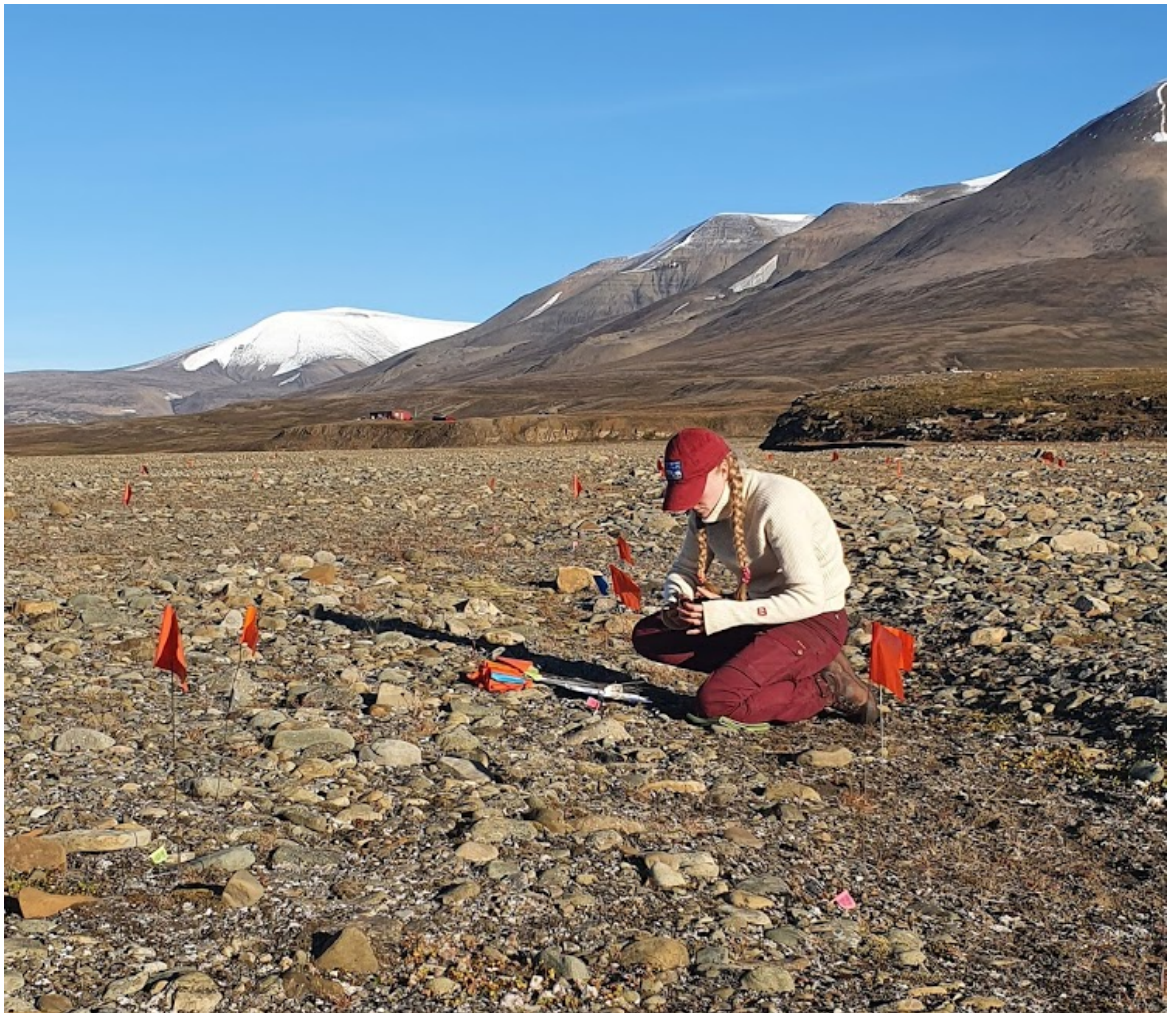

Figure 2.4. Fieldwork in August 2020 where capsules were registered and collected from *Saxifraga oppositifolia* plants found in plot 11 (riverbed) in Foxdalen.

Each plot contained 48 randomly marked plants, for which GPS coordinates (UTMs) were also registered in case the ID-marking would get lost. Ploidy levels of the plants were already determined by flow cytometry analyses in 2018. Ploidy levels were found to be distributed in a gradient with diploids clearly dominating in ridges, while triploids and tetraploids were more common in slopes and especially riverbeds (Table 2.1).

Table 2.1. Distribution of *Saxifraga oppositifolia* ploidy levels (diploids, triploids, tetraploids) in all 15 plots (plot no. 2-16) for the plants that were used for data collection in 2020. The table includes transect name/valley, location of each transects (UTM coordinates) and habitat type.

	BJØRNDALEN	**ENDALEN**	**TODALEN**	**BOLTERDALEN**	**FOXDALEN**
	33 x 0507436 (E) 8682526 (N)	33 x 0518259 (E) 8680004 (N)	33 x 0520011 (E) 8678638 (N)	33 x 0522529 (E) 8675971 (N)	33 x 0526870 (E) 8677263 (N)
RIDGE	*Plot no. 16*	*Plot no. 4*	*Plot no. 7*	*Plot no. 10*	*Plot no. 13*
Diploids	47	39	41	46	38
Triploids	0	1	0	0	8
Tetraploids	0	3	2	1	1
SLOPE	*Plot no. 15*	*Plot no. 3*	*Plot no. 6*	*Plot no. 9*	*Plot no. 12*
Diploids	47	38	15	5	11
Triploids	0	1	23	11	35
Tetraploids	0	7	7	32	0
RIVERBED	*Plot no. 14*	*Plot no. 2*	*Plot no. 5*	*Plot no. 8*	*Plot no. 11*
Diploids	12	3	9	47	26
Triploids	3	14	12	1	9
Tetraploids	33	30	24	0	13

2.3 DATA COLLECTION

2.3.1 SEXUAL REPRODUCTION

Flower, capsule, and seed production

Flower phenology was registered twice during the field season 2020. All plots were visited for registration during June, and a second registration during July. Number of flowers were originally recorded into different flower phenology categories, but due to phenological variation between transects, i.e., potential sampling bias, all flower phenology categories were merged into one, hereinafter referred to as *Flower production*.

Capsules and seeds were recorded and collected during fieldwork in August 2020. Total number of observed capsules was registered to estimate a capsule:plant ratio, hereinafter referred to as *Capsule production*, and up to 10 capsules were collected from each individual plant (an upper limit of 10 capsules was set to minimize the negative impact on the plants). Only closed capsules were collected to ensure that seed count corresponded to the actual number of produced seeds and that seeds had not already been dispersed. The collected capsules were stored in paper bags in a cold room (4 degrees) until capsules were dissected and seeds counted by hand. Based on the seed count and number of collected capsules, a seed:capsule ratio was estimated, hereinafter referred to as *Seed production.*

Seed germination

To check germination rate, a seed germination experiment was set up (Figure 2.5). To cover variation in seed quality, only individuals with at least 25 seeds were initially included, which resulted in 51 diploid and four tetraploid individuals. As so few tetraploids reached the limit of 25 seeds, two additional tetraploids were included (with 22 and 16 seeds). Both these plants were found in riverbeds. Triploids were not represented in the experiment due to the very low sexual reproductive output (< 1 seed per capsule on average).

Eppendorf tubes were filled with 25 seeds per tube, marked and weighed. The seeds were sterilized in a BSC A2 safety cabinet. First, 1000 µl 70% EtOH solution was added to the Eppendorf tubes, and after 5 min incubation the solution was removed. Then, 1000 µl bleach (20% commercial Klorix, 0.1% Tween20 and 80% dd H_2O) was added, followed by centrifugation. After 5 min the bleach solution was pipetted out. As a last treatment, wash

buffer (0.0009% Tween20 and 99.9999% dd H_2O) was pipetted into the Eppendorf tubes and then removed, and 1 ml 0.1% agar was added. Agar and seeds were distributed into a Petri dish by rotating the dish. The Petri dish were sealed with parafilm and placed in a growth cabinet at 4°C and dark for a three-week stratification period.

After the stratification period, the plates were placed in a growth chamber with light conditions 24 hours a day and a temperature set on 16°C the first week, 18°C the second week, and for the rest of the period raised up to 20°C. During the second week, 500 µl dd H_2O was added to each plate under sterile conditions to avoid the small seedlings to dry out. After seven weeks in the growth chamber, a germination percentage was calculated.

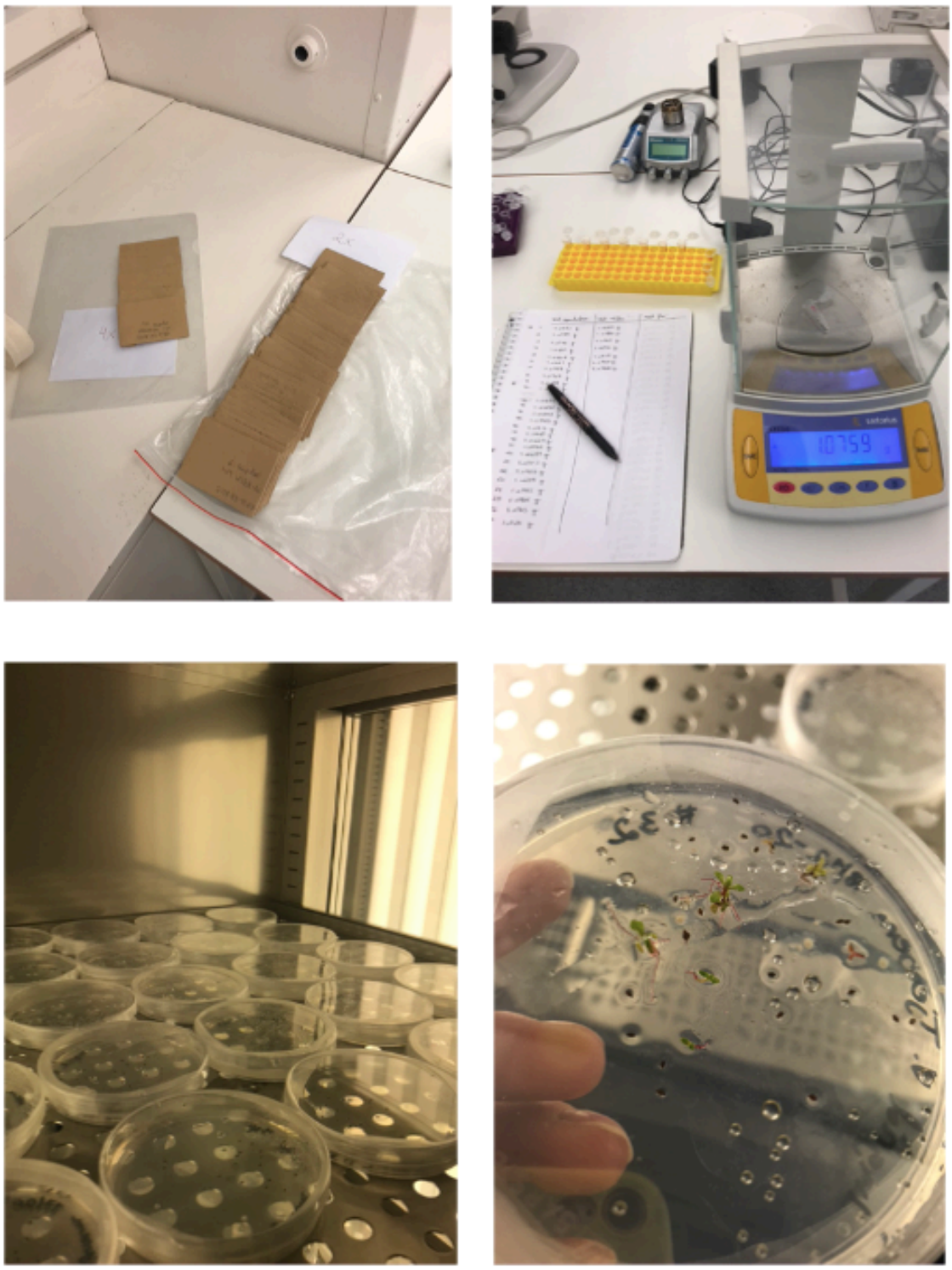

Figure 2.5. Seed germination experiment with seeds from *Saxifraga oppositifolia* collected in Svalbard during fieldwork in August 2020. The seeds were weighed, sterilized, and placed in agar for stratification for three weeks, before being exposed to 24 h daylight and increasing temperatures in a growth chamber for seven weeks.

2.3.2 VEGETATIVE PROPAGATION

Rooting ability and leaf production

To investigate differences in vegetative propagation, rooting ability in cuttings from plants with different ploidy levels and/or from different habitat types were tested in a rooting experiment (Figure 2.6). In the beginning of July (13th-17th of July 2020), two cuttings were collected from 150 plants, resulting in the total of 300 cuttings: 100 cuttings from each ploidy level (2x, 3x, 4x) covering three habitat types (ridge, slope, riverbed). Cuttings were only taken if a plant had two branches that measured at least 10 cm. The branches were stored in a refrigerator until the experiment started 22nd of July 2020.

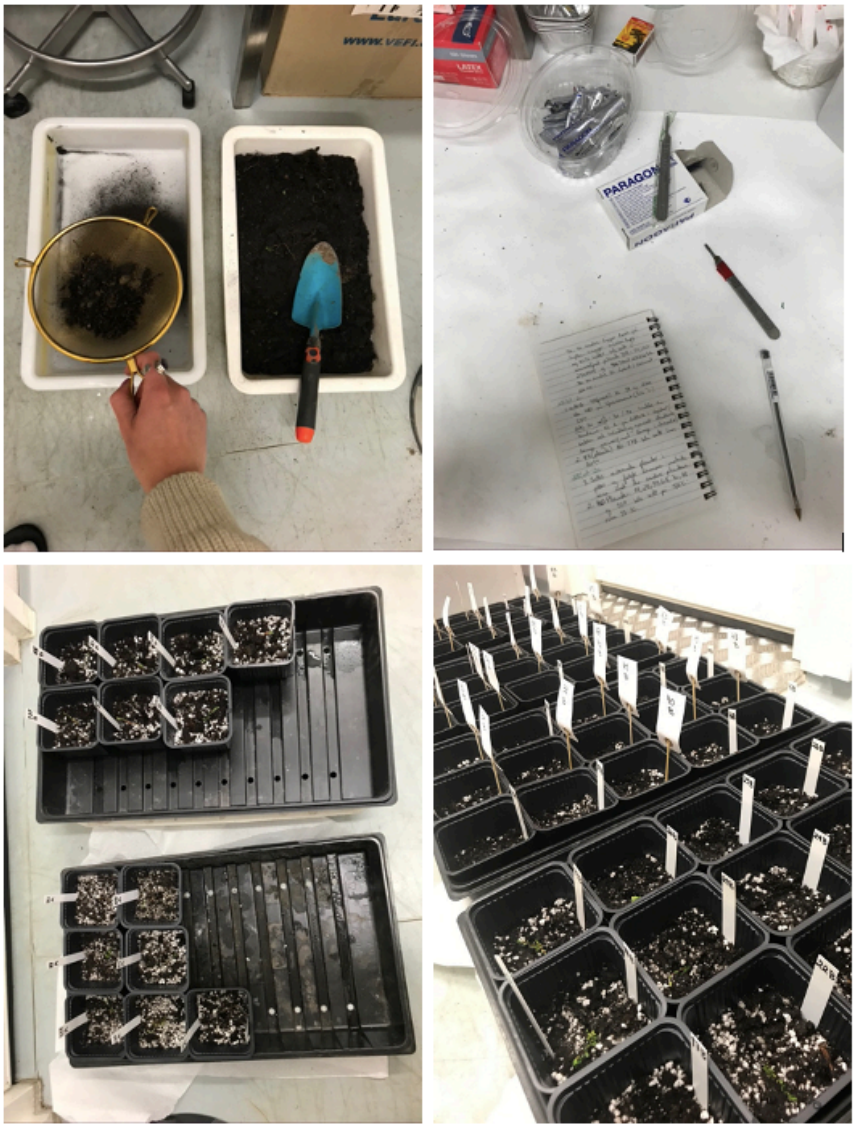

Figure 2.6. The rooting ability experiment was conducted with cuttings from *Saxifraga oppositifolia* collected during fieldwork in Svalbard. The cuttings were placed in pots filled with either soil type A, a mineral rich soil treatment, or soil type B, an organic soil treatment. Rooting ability was scored as five stages of root development in 300 cuttings, two cuttings per plant.

The experimental setup included two different soil treatments: soil A – a more alkaline and mineral rich soil, and soil B – a more acidic and organic soil. Both soil mixes were made by Cornell's Ratio with two parts peat moss and one part perlite (by volume) (Boodley & Sheldrake, 1972). Soil A contained 10 L perlite and 20 L peat moss mixed with sand (15.5 L peat moss and 4.5 L sieved sand), and soil B contained 10 L perlite and 20 L peat moss mixed with organic soil (18.4 L peat moss and 1.6 L sieved organic soil) (Figure 2.6).

In total 150 pots with organic soil mix (A) and 150 pots with mineral soil mix (B) were used in the experiment. All pots were watered with 200 ml and then well drained. Cuttings were shortened with a sterilized scalpel making a clean cut and a final branch length of 5 cm and put into separate pots: one branch in a pot with mineral soil (A) and the other branch from the same plant in a pot with organic soil (B). In total, 50 cuttings from each ploidy level (2x, 3x, 4x) were kept in mineral soil (A) and 50 cuttings from each ploidy level were kept in organic soil (B).

The plants were given 1 dl additional water and placed in a 5-6°C growth room with daylight 24 hours a day. One week later the plants were moved to a 14-15°C growth room with the same light conditions. The plants were watered with 10 sprays from a spray bottle every second day. After nine weeks, 29th of September, all branches were evaluated for root growth, defined as rooting ability, and scored as stage 0 (no visible roots) to 4 (rooting with branching) (Table 2.2, Appendix C). Additionally, leaf production was recorded as a binary variable (present/absent). The presence of leaf production could vary from just one recently developed new leaf to a full rosette of new leaves disregarding difference in leaf production.

Table 2.2. The five categories (rooting stage 0-4) used in the rooting ability experiment to classify *Saxifraga oppositifolia* cuttings based on their rooting ability.

Rooting stage	Definition
0	No visible roots
1	Rooting, but only visible in a stereo microscope
2	Some indication of new roots
3	Rooting, but the new root/roots are small
4	Clearly rooting. Roots are branching

2.5 STATISTICAL ANALYSES

2.5.1 OVERVIEW

As the effect of ploidy level on reproductive strategy was the main focus of this thesis, the variable *Ploidy level* was addressed separately with hypothesis tests (Ha-1.1, HA-1.2, HA-1.3, HA-2.1 and HA-2.2, Table 2.3) to better avoid so called "data dredging", which can occur when looking at too many possible covariates and associations in the same analysis (Smith & Ebrahim, 2002).

Research questions addressing other potential sources to variation (Q1.1, Q1.2, Q1.3, Q2.1 and Q2.2, Table 2.3) were investigated with explorative analyses carried out by a model search for potential single or interacting covariates. The explorative analyses were meant to construct models, which to some extent would explain the variance in each response variable by using "nuisance variables". Nuisance variables, in this case other potential sources to variation (Table 2.3) than *Ploidy level*, are variables not directly relevant for the objectives, although they might correlate with the hypothesised independent variable, which in this thesis was *Ploidy level*. By using nuisance variables, less of the variance in the response remained to be tested and explained by the likelihood-ratio chi squared test, and thus increased the test power in the hypothesis tests.

The nuisance variable analyses, or explorative analyses, were carried out by using two stepwise model selection functions written by Trond Reitan (https://folk.universitetetioslo.no/trondr/R/regress_search.R). These functions, *regress.ic.dredge* and *regress.ic.search*, looked through various generalized linear mixed effect models (GLMM, CLMM) using the R packages "glmmTMB" (Brooks et al., 2017), "glmmADMB" (Fournier et al., 2012), "lme4" (Bates et al., 2014) and "ordinal" (Christensen, 2018) to find the best model based on an information criterion (IC). The first function, *regress.ic.dredge*, compared models by looking through all possible combinations and was used in analyses with less than six covariates (Rooting ability and Leaf production). The second function, *regress.ic.search*, was less time consuming as it stepwise added, removed or replaced covariates, instead of searching through all possible combinations, and was used in analyses with six or more covariates (Capsule production, Seed production and Seed germination).

Overdispersion was accounted for by comparing Poisson distribution regression models with negative binomial regression models, also known as the overdispersed Poisson. In a Poisson distribution the variance of the observations equals the mean, however, when recording biological data, the mean might be larger than the expected value and is thus overdispersed (Hinde & Demétrio, 1998). A mean larger than the expected value is considered irregular variation, and occurs when the expected value in single observations or samples vary from each other, i.e., a per-sample random effect (Harrison, 2014), hereinafter referred to as unexplained variation. Depending on the distribution of these per-sample random effects, either lognormal distribution or gamma distribution, the expected values from the distribution imply the Poisson regression model with an extra per-sample random factor (lognormal-Poisson) or the negative binomial regression model (Gamma-Poisson), respectively.

The package "glmmTMB" was used for Poisson regression in capsule production, "glmmADMB" was used for negative binomial regression in seed production, "lme4" was used for binomial regression in seed germination and leaf production and "ordinal" was used for ordinal logistic regression in rooting ability. The best models were those with the lowest IC score.

Second order Akaike Information Criterion (AICc) was used in the search algorithm to account for small sample sizes (Hurvich & Tsai, 1989). This information criterion is recommended when number of parameters (K) is large relative to sample size (n), or more specific when $n / K < 40$ (Burnham & Anderson, 2002). AICc suggests the model, which is best in the sense of minimizing bias and variance in the fitted model parameters, and AICc was preferred for exactly this reason: to minimize the variance in the nuisance variable analyses and thereby increase the test power in the hypothesis tests including *Ploidy level*. There was no need in using a more conservative information criterion, like the Bayesian Information Criterion (BIC), which converges towards the true model (Schwarz, 1978). The nuisance variable analyses did not aim for the true models, as the true models might include *Ploidy level*, which was hypothesis tested after the nuisance variable analyses. Furthermore, the AIC weights (w_i), or "the weight of evidence", for the top five models were calculated to account for model uncertainties (Appendix D). The AICc weight (w_i) can be interpreted as the probability for a model to be the best model (Burnham & Anderson, 2004).

After the explorative analyses, *Ploidy level* was included in the best model and tested in the likelihood-ratio chi squared tests. *Ploidy level* was included in the final model if it was significant with 95% confidence, i.e., with a p-value < 0.05, hereinafter referred to as significant. The final model coefficient estimates, along with their respective contribution to the model and the objectives, were evaluated and furthermore reviewed in the discussion. Variance contributions, given in percentages, were estimated as var(*Estimate**variable) for the fixed effects, while the variance contributions from random effects were obtained straight from the analyses.

Table 2.3 gives an overview of all the investigated objectives as well as including response variables of interest, statistical methods, and potential sources of variance, i.e., nuisance variables approached with an explorative analysis and *Ploidy level*, which served as independent variable in the likelihood-ratio chi squared tests. A second ploidy variable (*Diploids vs. Polyploids*) was also tested in the likelihood-ratio chi squared tests in case variation primarily was found to be between diploids and polyploids (tetraploids and triploids). Support was given to the explorative research questions, if at least one nuisance variable was included in the model. All statistical analyses and diagrams were performed and produced in RStudio version 1.3.1093 (R Core Team, 2020).

Table 2.3. Objectives including the explorative research questions (Q) and hypotheses (HA – alternative hypothesis) that were analyzed, with their respective response variables, regression types, model selection methods and parameters of interest, i.e., list of covariates, that was used to investigate variation in reproductive parameters in *Saxifraga oppositifolia*.

Research questions and hypotheses	Response variable	Regression type	Model selection method	Potential sources of variance
OBJECTIVE I Sexual reproduction				
Capsule production				
Q 1.1 Is capsule production affected by any nuisance variables?	Capsules per plant	Poisson regression	Explorative analysis, AICc	"Nuisance variables" *Growth form, Habitat, Transect, Plot no.*
HA-1.1 Ploidy level has an effect on capsule production (HA)	Capsules per plant	Poisson regression	Likelihood-ratio chi squared test	*Ploidy level*
Seed production				
Q 1.2 Is seed production affected by any nuisance variables?	Seeds per capsule	Negative binomial regression	Explorative analysis, AICc	"Nuisance variables" *Flower production, Growth form, Habitat, Transect, Plot no.*
HA-1.2 Ploidy level has an effect on seed production (HA)	Seeds per capsule	Negative binomial regression	Likelihood-ratio chi squared test	*Ploidy level*
Seed germination				
Q 1.3 Is germination affected by any nuisance variables?	Germinated seeds vs not germinated seeds	Binomial regression (logistic)	Explorative analysis, AICc	"Nuisance variables" *No. of seeds, Seed weight (g), Growth form, Habitat type, Transect, Plot no.*
HA-1.3 Ploidy level has an effect on germination (HA)	Germinated seeds vs not germinated seeds	Binomial regression (logistic)	Likelihood-ratio chi squared test	*Ploidy level*
OBJECTIVE II Vegetative propagation				
Rooting ability				
Q 2.1 Is rooting ability affected by any nuisance variables?	Rooting category	Ordinal regression (logistic)	Explorative analysis, AICc	"Nuisance variables" *Soil type, Habitat type, Plot no.*
HA-2.1 Ploidy level has an effect on rooting ability (HA)	Rooting category	Ordinal regression (logistic)	Likelihood-ratio chi squared test	*Ploidy level*
Leaf production				
Q 2.2 Is leaf production affected by any nuisance variables?	Leaf production	Binomial regression (logistic)	Explorative analysis, AICc	"Nuisance variables" *Soil type, Habitat type, Plot no.*
HA-2.2 Ploidy level has an effect on leaf production (HA)	Leaf production	Binomial regression (logistic)	Likelihood-ratio chi squared test	*Ploidy level*

2.5.2 SEXUAL REPRODUCTION

Flower and capsule production

Data on flower and capsule production were summarized for each ploidy level through descriptive statistics including a tabulated description of mean flower and capsule production and box plots of capsule production per plant and estimated capsule:flower ratio.

To test if capsule production was explained by ploidy level or nuisance variables, I used Poisson regression defined by the command *glmer* from the R package "lme4" (Bates et al., 2014) with *Capsules* serving as the response variable (Table 2.4). The nuisance variables that were used in the explorative analysis to address Q 1.1 "Is capsule production affected by any nuisance variables?" (Table 2.3), are listed in Table 2.4.

Table 2.4. Overview of variables used for analysing capsule production in *Saxifraga oppositifolia* plants, including variable name, type of variable, and categories if the variable is categorical or minimum to maximum value if numeric.

	Variable	Categories / Min. – max.
Response variable	*Capsules*	1 – 204
Hypothesis tests		
	Ploidy level	*2x, 3x, 4x*
	Diploids vs Polyploids	*Diploids* (2x), *Polyploids* (3x + 4x)
Nuisance variables		
Fixed effect		
	Growth form	*0-100*
	Habitat type	*Riverbed, Slope, Ridge*
	Transect	*Bjørndalen, Endalen, Todalen, Bolterdalen, Foxdalen*
Random effect		
	Plot number	*2 – 16*
	Unexplained variation	*ID*

The Poisson regression models the log of the expected counts, in this case capsule counts, as a function of the predictor variables, where the regression coefficients can be interpreted as the difference between the log of expected counts. For a one unit change in the predictor variable, the exp(regression coefficient) will have a multiplicative change on the expected response, given that the other predictor variables in the model are held constant. Regarding categorical variables, the expected response will also change with the exp(regression coefficient) when comparing the control category, or the intercept, to a specific category in a predictor variable.

Seed production

Seed production was estimated for all plants that were recorded with capsules during the fieldwork. Data on seed production was first summarized through descriptive statistics including a tabulated description of total seed production for each ploidy level and box plots of seed production according to ploidy level and habitat type.

To assess, which variables best explained seed:capsule ratio, I used negative binomial regression to define the model. For the explorative analysis I used the "glmmADMB" package (Fournier et al., 2012) while I used the package "MASS" (Venables & Ripley, 2002) with the command *glm.nb* for the hypothesis test. Number of seeds served as dependent variable and number of capsules as an offset variable to avoid loss of information in the data (Reitan & Nielsen, 2016). The nuisance variables that were used in the explorative analysis to address Q 1.2 "Is seed production affected by any nuisance variables?" (Table 2.3) are listed in Table 2.5.

To begin with, it was reasonable to assume a Poisson-distribution for the dependent count variable, number of seeds (*No. of seeds*) (Table 2.5). However, due to potential dependent or correlated incidents regarding the failure or success in seed development, there seemed to be evidence of overdispersion in the data. To be certain, a Poisson-based regression model with overdispersion was compared to the negative binomial regression model by AICc. The negative binomial regression model had the lowest AICc and was therefore the preferred alternative instead of a Poisson-based regression model with overdispersion (Osgood, 2017).

Table 2.5. Overview of variables used for analysing seeds per capsules in *Saxifraga oppositifolia* plants, including variable name, type of variable, and categories if the variable is categorical or minimum to maximum value if numeric.

	Variable	Categories / Min. – max.
Response variables		
	No. of seeds	0 – 338
	No. of capsules	1 – 11
Hypothesis tests		
	Ploidy level	*2x, 3x, 4x*
	Diploids vs Polyploids	*Diploids* (2x), *Polyploids* (3x + 4x)
Nuisance variables		
Fixed effects		
	Flower production	0 – 208
	Growth form	0 – 100
	Habitat type	*Riverbed, Slope, Ridge*
	Transect	*Bjørndalen, Endalen, Todalen, Bolterdalen, Foxdalen*
Random effects		
	Plot number	*2 – 16*
	Unexplained variation	*ID*

As with Poisson regression, the negative binomial regression models the log of the expected counts, in this case seeds per capsule, as a function of the independent variables, -or predictor variables. The coefficients for the negative binomial regression model can therefore be interpretated in the same way as with the Poisson: for a one unit change in the predictor variable, the exp(regression coefficient) will have a multiplicative change on the expected response, given that the other predictor variables in the model are held constant.

Seed germination

Data on seed germination were both summarized through descriptive statistics including a tabulated description of mean germination percentage and mean weight (g) of 25 seeds from each habitat type and box plots of germination percentage according to ploidy levels and habitat types.

To test if seed germination was explained by ploidy level or nuisance variables, I used binomial logistic regression defined by the command *glmer* from the R package "lme4" (Bates et al., 2014) with *Germinated seeds vs not germinated seeds* serving as the dependent variable. The variable *Germinated seeds* was treated as success as it included the seeds that had successfully germinated, whereas *Not germinated seeds* (estimated as *Seeds per plate - Germinated seeds*) was treated as failure. The nuisance variables that were used in the explorative analysis to address Q 1.3 "Is germination affected by any nuisance variables?" (Table 2.3) are listed in Table 2.6.

In logistic regression, odds are used as a measure for the probability to be in a certain category, divided on the probability not to be in that specific category (Morgan & Teachman, 1988), and this regression type models the expected change in log odds of having a certain outcome, per change in the predictor variables. Moreover, odds ratio is a measure connecting two categories by their odds value. The odds ratio is telling how much the odds value decrease or increase when going from the reference category to an alternative one.

Table 2.6. Overview of variables used for analysing the results from the germination experiment with *Saxifraga oppositifolia* seeds, including variable name, type of variable, and categories if the variable is categorical or minimum to maximum value if numeric.

	Variable	Categories / Min. – max.
Response variables		
	Germinated seeds	0 – 14
	Seeds per plate	16 – 25
	Not germinated seeds	11 – 25
Hypothesis tests		
	Ploidy level	*2x, 4x*
Nuisance variables		
Fixed effect		
	No. of seeds	16 – 338
	Seed weight (g)	0.0016 – 0.0057
	Growth form	0 – 100
	Habitat type	*Riverbed, Slope, Ridge*
	Transect	*Bjørndalen, Endalen, Todalen, Bolterdalen, Foxdalen*
Random effect		
	Plot number	*2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15*
	Unexplained variation	*ID*

2.5.3 VEGETATIVE PROPAGATION

Data on vegetative propagation, i.e., scoring of cuttings according to rooting stage categories (Table 2.2) and whether plants produced new leaves, were summarized through descriptive statistics including a tabulated description to ploidy levels and stacked bar plots of rooting ability and leaf production according to ploidy levels, habitat types and soil types.

The dataset on vegetative propagation consisted of only categorical variables (Table 2.7), making logistic regression a reasonable choice (Christensen, 2018).

Table 2.7. Overview of variables used for analysing the results from the rooting experiment with *Saxifraga oppositifolia* cuttings, including variable name, type of variable, and categories if the variable is categorical or minimum to maximum value if numeric.

	Variable	Categories / Min. – max.
Response variables		
	Rooting stage	*0, 1, 2, 3, 4* (Table 2.5)
	Leaf production	*Present, Absent*
Hypothesis tests		
	Ploidy level	*2x, 3x, 4x*
	Diploid vs Polyploid	*Diploids* (2x), *Polyploids* (3x + 4x)
Nuisance variables		
Fixed effects		
	Soil type	*A* (mineral soil), *B* (organic soil)
	Habitat type	*Riverbed, Slope, Ridge*
Random effects		
	Plot number	*2, 3, 4, 5, 6, 7, 8, 9,10, 12, 13, 15*
	Unexplained variation	*ID*

Three types of logistic regression were considered for handling the data: logistic regression, ordinal logistic regression, and nominal logistic regression. Logistic regression allows only two outcomes in the response variable, a binary response as in *Leaf production*, while both ordinal and nominal logistic regression accept more than two factors in the response variable as well as several predictor variables.

Rooting ability

I used ordinal logistic regression to address Q 2.1 "Is rooting ability affected by any nuisance variables?" (Table 2.3), as the categories in the dependent variable *Rooting stage* followed a certain order (Table 2.7). Ordinal logistic regression compares cumulative probabilities, rather than probabilities for discrete categories as in nominal logistic regression where all categories in every variable are compared, which thereby results in a more complex model (Greene & Hensher, 2010; Long, 2014). The key assumption in ordinal regression is that the effects from any of the explanatory variables are consistent or *proportional* across the different thresholds. Hence, this is usually termed the *assumption of proportional odds.* How this model concept worked with the rooting ability data is best described in three equations:

$$log(Pr(root=1)/Pr(root= 2|3|4))=\beta a+\beta 1(Ploidy) +\beta 2(Habitat) +\beta 3(Soil) +\beta 4(1|Plot)$$
$$log(Pr(root=1|2)/Pr(root= 3|4))=\beta b+\beta 1(Ploidy) +\beta 2(Habitat) +\beta 3(Soil) +\beta 4((1|Plot)$$
$$log(Pr(root=1|2|3)/Pr(root= 4))=\beta c+\beta 1(Ploidy) +\beta 2(Habitat) +\beta 3(Soil) +\beta 4(1|Plot)$$

The log likelihood for a root to be in a certain rooting stage was divided on the log likelihood for a root to *not* be in a that rooting stage, which equals the coefficient for a threshold between rooting categories ($\beta a, \beta b$ *or* βc) plus coefficients for fixed effects *Ploidy level* ($\beta 1$), *Habitat type* ($\beta 2$), *Soil type* ($\beta 3$), and the random factor *Plot no.* ($\beta 4$).

The ordinal logistic regression model was defined by the *clmm* command loaded from the R package "Ordinal" (Christensen, 2018) and compared whether odds were below versus above any point on the rooting stage response scale, i.e., cumulative odds ratio.

To test if the assumption of proportional odds was held, a goodness-of-fit test was run for the final model which best explained rooting ability. However, this final model was defined by the *clmm* command, and thus included a random factor, which was not accepted in the

likelihood ratio test of model terms in nominal formulae, as it was designed for Cumulative Link Models (CLM) only. Therefore, the final model was redefined for the test of nominal effects by using the *clm* command where the random factor instead was defined as a fixed factor. The test was run by using the command *nominal_test* also loaded from the R package "Ordinal" (Christensen, 2018). No significant p-values were found for the independent variables, i.e., no evidence was found of non-proportional odds.

Leaf production

The variable *Leaf production* was used to examine variance related to production of new leaves. Contrary to *Rooting stage*, *Leaf production* is a binary response where *Present* served as success as it meant that a plant had successfully grown new leaves, while *Absent* served as failure as no new leaves were recorded. Leaf production was therefore analysed with binomial logistic regression defined by the *glmer* command loaded from the "lme4" R package (Bates et al., 2014). The nuisance variables that were used in the explorative analysis to address Q 2.2 "Is leaf production affected by any nuisance variables?" (Table 2.3) are listed in Table 2.7.

3 RESULTS

3.1 SEXUAL REPRODUCTION

Flower and capsule production

Out of 720 marked plants, 25 were not found during fieldwork due to flooded plots or loss of the plant in other ways. Flower and capsule production were estimated for 695 plants (424 diploids, 118 triploids and 153 tetraploids). Even though both number of capsule per plant and capsule:flower overlapped between ploidy levels, diploid plants had overall higher reproductive output in terms of flowers and capsules compared to triploids and tetraploids (Table 3.1, Figure 3.1).

Table 3.1. Sexual reproductive output of *Saxifraga oppositifolia* in Svalbard registered as mean flower production (flowers per plant) and mean capsules production (capsules per plant) for each ploidy level (2x, 3x and 4x).

Ploidy level	n	Flower production	Capsule production
2x	424	13.6	1.3
3x	118	4.6	0.4
4x	153	3	0.3

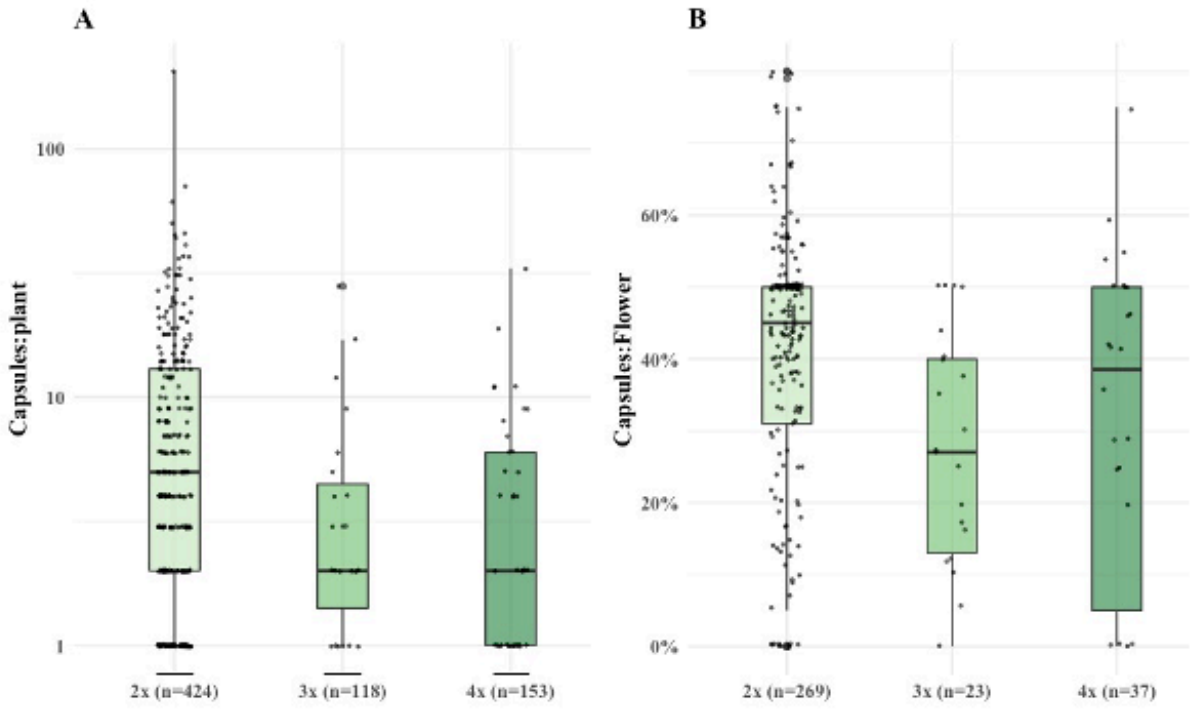

Figure 3.1. Boxplots showing sexual reproductive output of *Saxifraga oppositifolia* in Svalbard as number of capsules per plant (A) and capsule:flower ratio (percentage) (B) in diploid (2x), tetraploid (3x) and tetraploid (4x) plants. Boxplots include the median (horizontal line), the 1st and the 3rd quartile (lower and upper box limits, respectively), with whiskers extending from the boxes to the minimum (lower whisker) and the maximum quartile (upper whisker).

Research question Q 1.1 "Is capsule production affected by any nuisance variables?" was supported by the explorative analysis, as habitat, transect and growth form were included in the final model, although growth form had close to zero effect (Table 3.2, Appendix D: Table D.1.1). Plants from slopes had lower capsule production compared with plants from ridges and riverbeds. Regarding the transects, Endalen, Todalen and Bolterdalen had a higher capsule production, than what was found in Bjørndalen (intercept) (Table 3.2.).

Hypothesis HA-1.1 "Ploidy level has an effect on number of capsules per plant" was supported, as the variable *Diploids vs. Polyploids* had a significant effect according to the likelihood-ratio chi squared test (Appendix D: Table D.2.1). According to the final model, polyploids had lower capsule production compared with diploids (intercept) (Table 3.2.), and of all the fixed effects, ploidy level (*Diploids vs. Polyploids*) explained most of the variation in the model (Table 3.3.).

Table 3.2. The final GLMM of capsule production in *Saxifraga oppositifolia* in Svalbard. Intercept corresponds to *Diploids* (for *Diploids vs. Polyploids*), *Ridge* (for *Habitat*) and *Bjørndalen* (for *Transect*). Confidence limits (95%) were estimates as *Estimate* +/- 1.96 x *SE*.

				95% confidence limits	
	Fixed effects	**Estimate**	SE	Lower	Upper
	Intercept	- 0.23	0.33	- 0.87	0.42
Diploids vs. Polyploids	*Polyploids*	- 2.17	0.30	- 2.77	- 1.57
	Growth form	0.01	0.01	- 0.0001	0.02
Habitat	*Riverbed*	0.67	0.55	- 0.40	1.74
	Slope	- 2.11	0.60	- 3.29	- 0.94
Transect	*Endalen*	1.38	0.42	0.57	2.20
	Todalen	1.84	0.41	1.04	2.65
	Bolterdalen	1.92	0.40	1.15	2.70
	Foxdalen	0.13	0.43	- 0.70	0.97
Interactions	*Riverbed:Growth form*	0.0001	0.01	- 0.01	0.01
	Slope:Growth form	0.01	0.01	- 0.01	0.02
	Riverbed:Endalen	- 1.30	0.64	- 2.55	- 0.04
	Slope:Endalen	1.00	0.64	- 0.25	2.25
	Riverbed:Todalen	- 1.18	0.62	- 2.40	0.03
	Slope:Todalen	0.11	0.69	- 1.24	1.46
	Riverbed:Bolterdalen	- 2.68	0.63	- 3.92	- 1.44
	Slope:Bolterdalen	- 0.35	0.73	- 1.77	1.08
	Riverbed:Foxdalen	0.18	0.63	- 1.06	1.41
	Slope:Foxdalen	2.42	0.69	1.07	3.76

Table 3.3. Variance contribution (%) from covariates in the final model (GLMM, Table 3.2.) explaining capsule production in *Saxifraga oppositifolia* in Svalbard.

	Covariate	**Variance contribution**
Fixed effects	*Diploids vs Polyploids*	22.58%
	Habitat:Growth form + Habitat:Transect	19.44%
Random effect	Unexplained variation	57.97%

Seed production

To estimate a comparable seed:capsule ratio, only closed capsules were collected for this part of the thesis. Seed production was estimated for 163 plants (136 diploids, 13 triploids and 14 tetraploids). Altogether, 32% of the 424 diploids, 11% of the 118 triploids and 9% of the 153 tetraploids were registered with closed capsules. Diploids had a much higher seed production compared to triploids and tetraploids (Table 3.4, Figure 3.2). Seed production had overlapping medians for diploids all habitat types, where especially slopes had a large spread in the data (Figure 3.2.B). Riverbeds included an outlier, with more than 30 seeds per capsule.

Table 3.4. Sexual reproductive output of *Saxifraga oppositifolia* in Svalbard registered as total number of capsules and seeds per ploidy level.

Ploidy level	**n**	No. collected capsules	No. counted seeds
2x	136	500	5145
3x	13	46	7
4x	14	46	297

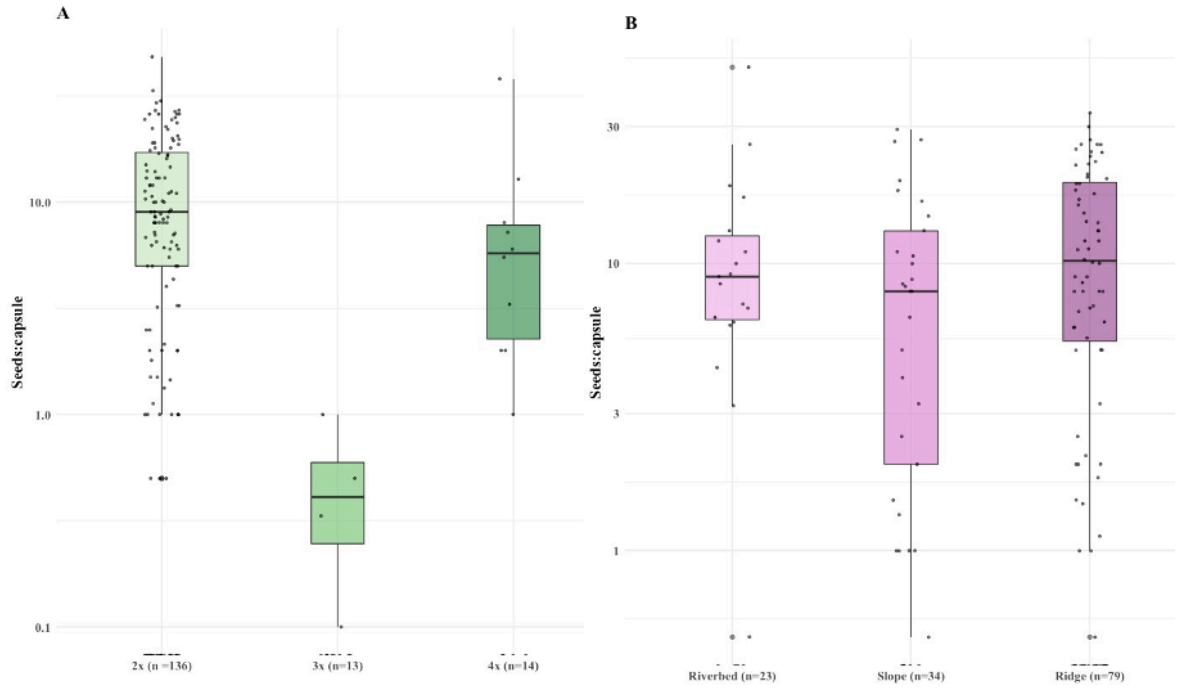

Figure 3.2. Boxplots showing sexual reproductive output of *Saxifraga oppositifolia* in Svalbard as number of seeds per capsule in diploid (2x), tetraploid (3x) and tetraploid (4x) plants (A) and habitat types (B, only diploid plants). Boxplots include boxes which marks the median (horizontal line), the 1st and the 3rd quartile (lower and upper box limits, respectively), with whiskers extending from the boxes to the minimum (lower whisker) and the maximum quartile (upper whisker).

Research question Q 1.2 "Is seed production affected by any nuisance variables?" was supported by the explorative analysis, although the only nuisance variables that were included were an interaction with *Growth form* and *Plot* as random factor (Table 3.5, Appendix D: Table D.1.2).

According to the final model, hypothesis HA-1.2 **"**Ploidy level has an effect on seed production" was supported as *Ploidy level* had a significant effect from the likelihod-ratio chi squared test (Appendix D: Table D.2.2). As none of the other nuisance variables were included with a fixed effect, *Ploidy level* was the only variable included in Table 3.5. Triploids and tetraploids had a lower seed production than diploids (Table 3.5.).

Table 3.5. The final GLM of seeds per capsule in *Saxifraga oppositifolia* in Svalbard. Intercept corresponds to *Diploids*. Confidence limits (95%) were estimated as *Estimate* +/- 1.96 x *SE*.

				95% confidence limits	
	Fixed effects	**Estimate**	SE	Lower	Upper
	Intercept	2.25	0.11	2.03	2.47
Ploidy level	*Triploids*	- 4.07	0.55	-5.15	-2.99
	Tetraploids	- 0.43	0.36	-1.14	0.28

Seed germination

Out of 57 petri dishes with sterilized seeds, 46 contained at least one germinated seed. Germination was observed in seeds from all three habitat types (riverbed, slope, ridge), as well as in both diploid and tetraploid plants, although tetraploids only were represented by six individuals (Table 3.6, Figure 3.3). The mean seed weight of 25 seeds for all habitat types was almost the equal (Table 3.6). Seed germination had overlapping medians for diploids and tetraploids, and diploids had a large spread in the data (Figure 3.3).

Table 3.6. Germination of seeds collected from *Saxifraga oppositifolia* in three habitat types (riverbed, slope, ridge) in Svalbard, shown as total number of seeds, number of germinated seeds, and germination percentage (no. of germinated seeds/no. of seeds *100), as well as mean weight of 25 seeds.

Habitat type	**n (plants)**	**n (seeds)**	**Germination %**	**Mean weight (g) of 25 seeds**
Riverbed	14	338	8%	0.0035
Slope	11	275	13.5%	0.0033
Ridge	32	800	19.6%	0.0033

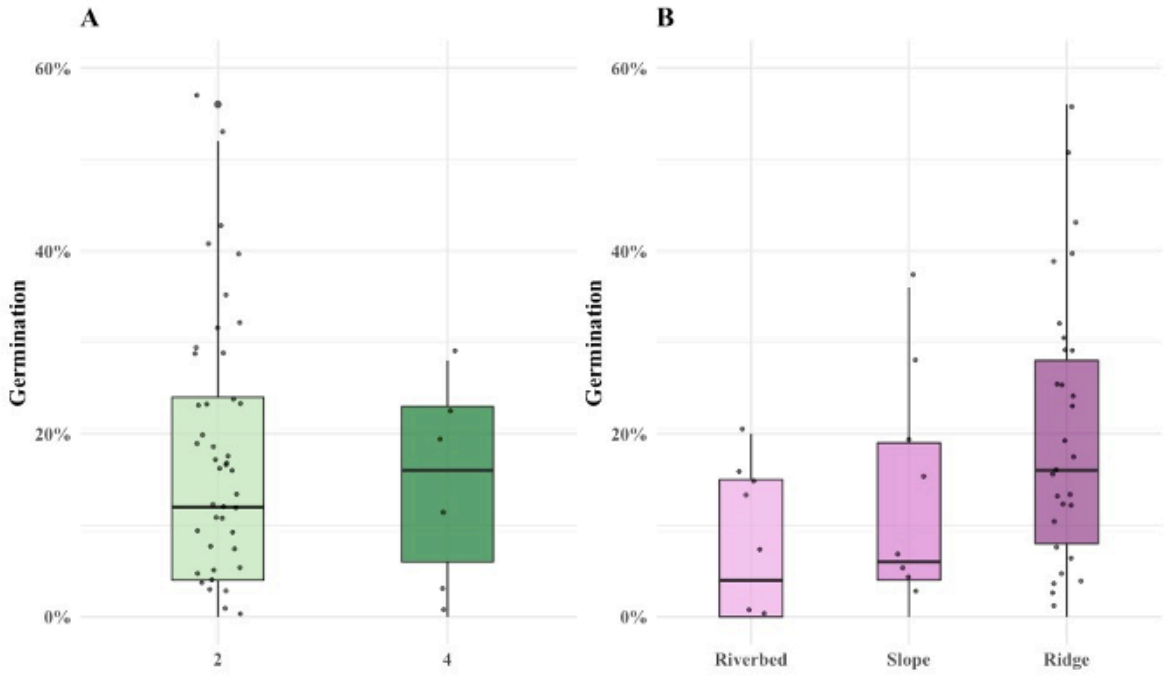

Figure 3.3. Boxplots showing germination percentage of *Saxifraga oppositifolia* seeds from diploid and tetraploid plants (A) and between different habitat types (B, only diploid plants). Boxplots include boxes which mark the median (horizontal line) and the 1st and the 3rd quartile (lower and upper box limits, respectively), with whiskers extending from the boxes to the minimum (lower whisker) and the maximum quartile (upper whisker). Mean plots include the mean represented by a dot and the 95% confidence intervals marked with whiskers.

Research question Q 1.3 "Is germination affected by any nuisance variables?" was supported by the explorative analysis, as the variables *Habitat, Transect* and *Growth form* were included in the final model explaining variation in germination percentage (Table 3.7, Appendix D: Table D.1.3). Hypothesis HA-1.3 "Ploidy level has an effect on germination" was not supported, as *Ploidy level* was not significant according to the likelihood-ratio chi squared test (Table 3.7, Appendix D: Table D.2.3).

Both slope and riverbed had lower germination compared to ridge. However, slope had a positive upper confidence limit, and thus might just as well have had a small positive effect on germination percentage. Endalen, Todalen, Bolterdalen and Foxdalen all had higher germination than Bjørndalen as they all had a multiplicative positive change in the odds of germination, i.e., an odd ratio larger than 1 which means that it is much more likely that seeds from the continental valleys germinated compared those produced by plants in the most coastal valley, Bjørndalen (Table 3.7). The transects also explained twice as much of the

variation as different habitats, nevertheless, half of the variation in the model was unexplained (Table 3.8).

Table 3.7. The final GLMM of results from germination experiment of seeds from *Saxifraga oppositifolia* in Svalbard. Intercept corresponds to *Ridge* (for *Habitat*) and *Bjørndalen* (for *Transect*). The odds ratio (OR) was estimated as exp(*Estimate*) and confidence limits (95%) as *Estimate* +/- 1.96 x *SE*.

					95% confidence limits	
	Fixed effects	**Estimate**	**OR**	SE	Lower	Upper
	Intercept	-4.05	0.02	1.21	- 6.42	- 1.68
Habitat	*Slope*	-0.06	0.94	0.50	- 1.03	0.91
	Riverbed	-1.01	0.36	0.37	- 1.73	- 0.28
Transect	*Endalen*	3.19	24.24	1.20	0.85	5.52
	Todalen	2.56	12.87	1.26	0.15	4.96
	Bolterdalen	2.79	16.25	1.23	0.40	5.18
	Foxdalen	3.20	24.53	1.26	0.72	5.68
	Growth form	-0.01	0.99	0.01	- 0.02	-0.0001

Table 3.8. Variance contribution (%) of covariates in the model (Table 3.7.) explaining germination of seeds collected from *Saxifraga oppositifolia* plants in Svalbard.

	Covariate	**Variance contribution**
Fixed effects	*Habitat type*	16.01%
	Transect	29.99%
	Growth form	6.74%
Random effect	Unexplained variation	47.26%

3.2 VEGETATIVE PROPAGATION

Out of 300 cuttings collected during field work, 265 cuttings (91 diploids, 83 triploids and 91 tetraploids) were scored for rooting ability and leaf production; the remaining were considered dead before or after the experiment started or not evaluated for other reasons (Table 3.9, Appendix C).

Table 3.9. Rooting ability of *Saxifraga oppositifolia* cuttings collected during fieldwork in Svalbard, shown as number of cuttings for each ploidy level classified into five rooting categories and number of cuttings with leaf production after nine weeks (Table 2.5, Appendix C).

Ploidy level	**n**	**Rooting stages**					**Leaf production**
		0	**1**	**2**	**3**	**4**	
2x	91	5	50	24	8	4	52
3x	83	1	25	29	14	14	76
4x	91	1	28	29	10	23	82

Rooting ability

Rooting ability were improved in triploids, tetraploids and in cuttings from slopes and especially riverbeds, compared to diploids and ridges, respectively (Figure 3.4 A and B). Mineral soil (soil A) also improved rooting, compared to organic soil (soil B), although the difference seems to be small (Figure 3.4 C).

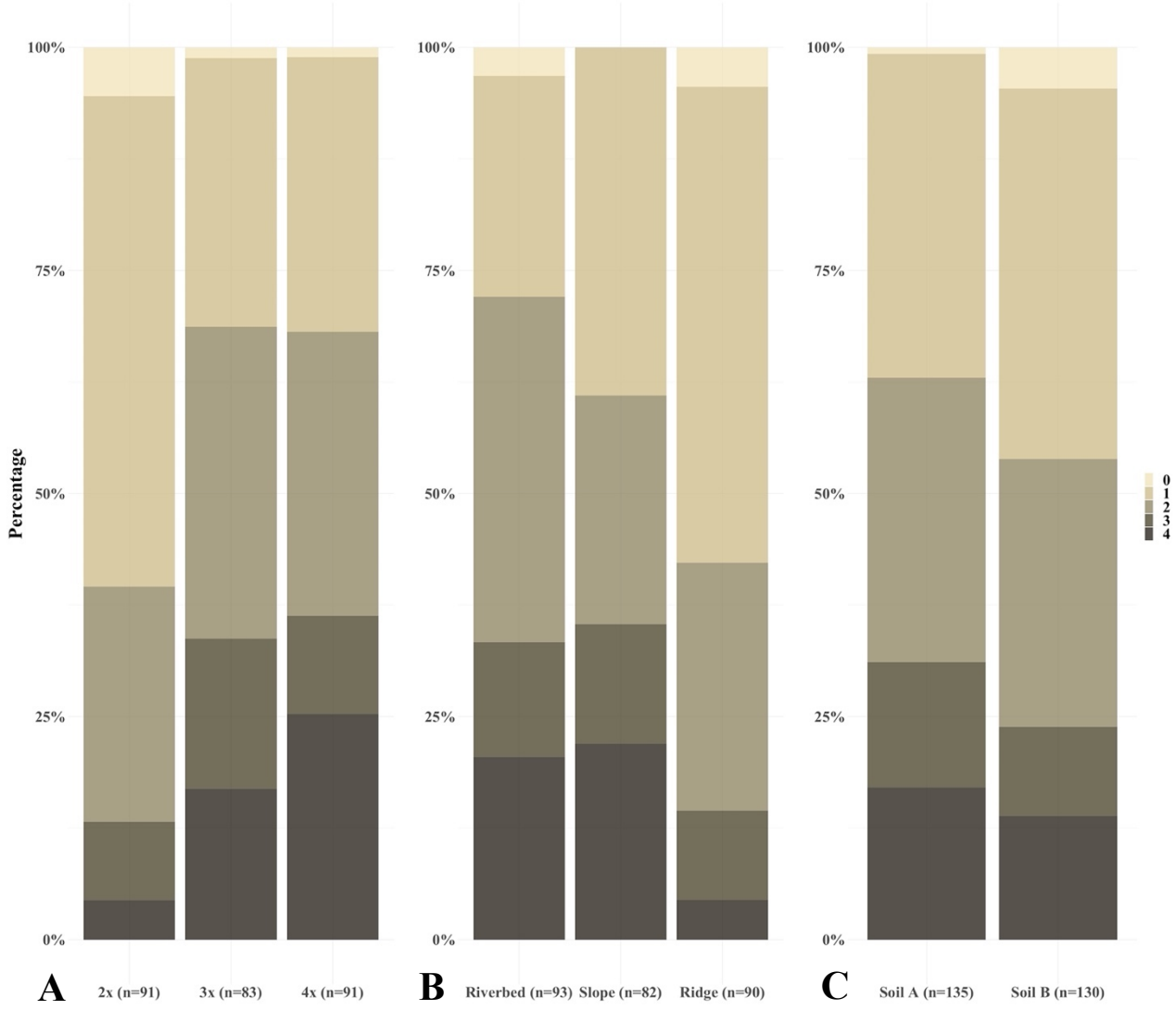

Figure 3.4. Stacked bar plots showing present percentage of *Saxifraga oppositifolia* cuttings classified in each of the five rooting stage categories (Figure 2.5) in diploid (2x), triploid (3x) and tetraploid (4x) plants (A), different habitat types (B), and soil types (C).

Research question Q 2.1 **"**Is rooting ability affected by any nuisance variables" and hypothesis HA-2.1 **"**Ploidy level has an effect on rooting ability" were both supported, as *Habitat type* and *Soil type* were included in the final model, and *Ploidy level* was regarded significant by the Likelihood-ratio chi squared test (Appendix E: Table E.1.4 and E.2.4). However, the variable *Diploids vs Polyploids*, was preferred over *Ploidy level* indicating that there was not that much difference in rooting ability between triploids and tetraploids (Table 3.10, Appendix D: D.2.4).

The coefficients for *Polyploids* (*Diploids vs Polyploids*), *Riverbed* (*Habitat type*) and *Slope* (*Habitat type*) were all positive, indicating that polyploids and plants from riverbeds and slopes were more efficient in vegetative propagation. According to the model, organic soil (*Soil B*) decreased the rooting ability (Table 3.10).

A complementing interpretation of this model is that the threshold *Diploids vs Polyploids = Polyploids* has been shifted a constant amount of 0.96 relative to the threshold when *Diploids vs Polyploids = Diploids*, meaning that the odds for rooting have shifted with a multiplicative factor of 2.61, when comparing polyploids with diploids. Similarly, the odds for increased rooting have shifted with a multiplicative factor of 1.54 and 1.90 when comparing cuttings from riverbed and slopes, respectively, with cuttings from ridges. The odds have also shifted with a multiplicative factor of 0.61 when comparing cuttings treated with organic soil with cuttings treated with mineral soil (Table 3.10, Table 3.11). *Ploidy level* and the random effect *Plot no.* explained most of the variation in the model (Table 3.12).

Table 3.10. The final CLMM of the results from rooting ability experiment of *Saxifraga oppositifolia* cuttings. Estimate gives the change in unit at each threshold value (Table 3.11) when going from reference value to a coefficient value. The reference values are *Diploids* (*Diploids vs. Polyploids*), *Ridge* (*Habitat*) and *Soli A* (*Soil*), respectively. The odds ratio (OR) was estimated as exp(*Estimate*) and confidence limits (95%) as *Estimate* +/- 1.96 x *SE*.

					95% confidence limits	
	Coefficients	**Estimate**	**OR**	**SE**	Lower	Upper
Diploids vs Polyploids	Polyploids	0.96	2.61	0.38	0.22	1.70
Habitat	Riverbed	0.43	1.54	0.52	- 0.59	1.44
	Slope	0.64	1.90	0.49	- 0.32	1.60
Soil type	Soil B	- 0.49	0.61	0.23	- 0.95	- 0.04

Table 3.11. Threshold estimates from the CLMM (Table 3.10) of results from the rooting ability experiment of *Saxifraga oppositifolia* cuttings. The thresholds estimates are based on the reference values for *Diploids* (*Diploids vs Polyploids*), *Ridge* (*Habitat type*) and *Soli A* (*Soil type*). Threshold estimates refer to the value on the y-axis between category 0 and 1 for threshold 0|1, and similar for 1|2, 2|3 and 3|4.

Threshold	**Estimate**	**SE**
0|1	-3.21	0.51
1|2	0.36	0.36
2|3	1.84	0.38
3|4	2.63	0.40

Table 3.12. Variance contribution (%) of covariates in the CLMM (Table 3.10) of rooting ability in *Saxifraga oppositifolia* cuttings.

	Covariate	**Variance contribution**
Fixed effects	*Ploidy level*	39.33 %
	Habitat type	13.05 %
	Soil type	11.44 %
Random effect	*Plot no.*	36.19 %

Leaf production

Triploids, tetraploids, slopes and riverbeds had a larger portion of cuttings with leaf production compared to diploids and ridges, respectively (Figure 3.5 A and B). Both soil types (A and B) seem to have an equal portion of cutting with leaf production, i.e., no visible difference in leaf production regarding soil treatment (Figure 3.5 C).

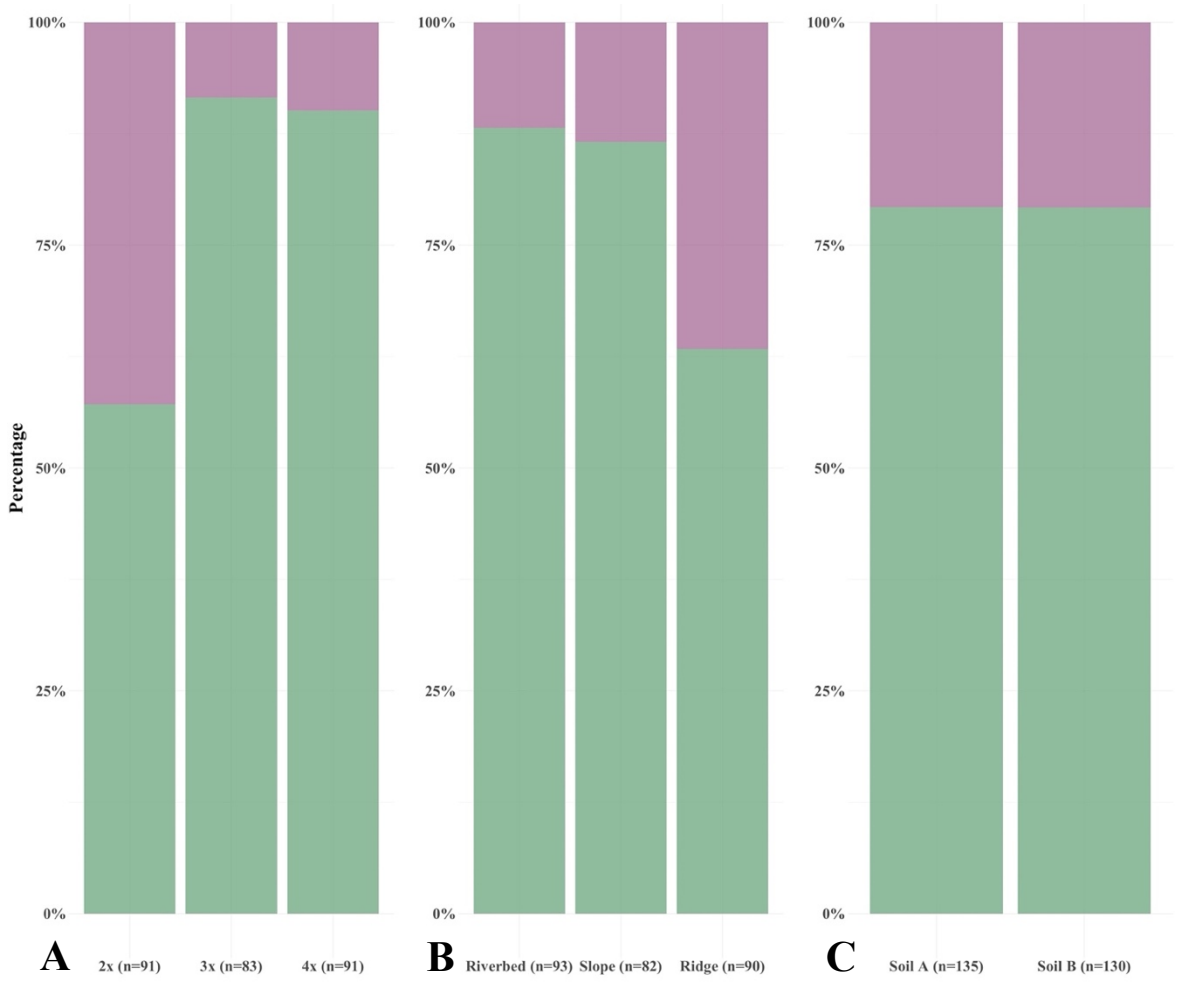

Figure 3.5. Stacked bar plots showing percentage of *Saxifraga oppositifolia* cuttings that produced new leaves (green) or not (pink) in diploid (2x), triploid (3x) and tetraploid (4x) plants (A), different habitat types (B), and soil types (C).

Q 2.2 **"**Is leaf production affected by any nuisance variables?" was supported according to the explorative analysis, although none of the nuisance variables were included in the final model except for *Plot* as a random effect (Table 3.13, Appendix D: Table D.1.5) and contributed with 12% of the variation in the model (Table 3.14).

Hypothesis HA-2.2 “Ploidy level has an effect on leaf production” was supported, as *Polyploids*, including both triploids and tetraploids, had a significant positive effect from the likelihood-ratio chi squared test (Appendix D: Table D.1.5 and D.2.5), indicating that it is more likely that polyploids produce new leaves. In fact, ploidy levels explained almost all (87.3 %) of the variation in leaf production (Table 3.14) and polyploids had a multiplicative positive effect on leaf production with an odds ratio at 6.66, meaning that the odds for leaf production increase with 6.66 if a plant is polyploid (Table 3.13).

Table 3.13. The final GLMM of leaf production in *Saxifraga oppositifolia* cuttings. The intercept corresponds to *Diploids*. The odds ratio (OR) was estimated as exp(*Estimate*) and confidence limits (95%) as *Estimate* +/- 1.96 x *SE*.

				95% confidence limits	
Fixed effects	**Estimate**	**OR**	**SE**	Lower	Upper
Intercept	0.40	1.50	0.31	- 0.21	1.01
Polyploids	1.90	6.66	0.41	1.10	2.70

Table 3.14. Variance contribution (%) of covariates in the final model (Table 3.14) of leaf production in *Saxifraga oppositifolia* cuttings collected during fieldwork in Svalbard in July 2020.

	Covariate	**Variance contribution**
Fixed effect	*Ploidy levels*	87.30 %
Random effect	*Plot no.*	12.70 %

4 DISCUSSION

In this thesis I investigate how ploidy level, or other potential sources of variation, affect reproductive strategy and output in *S. oppositifolia*. The results show that plants of different ploidy levels differ in sexual reproductive investment and output, i.e., flower, capsule and seed production, as well as in vegetative propagation, i.e., rooting ability and leaf production in cuttings. The results, thus, support autopolyploidy as a driver for phenotypic variation related to reproductive strategy and output in *S. oppositifolia* in Svalbard.

Variation related to the snow distribution gradient, and hence length of the growth season, has previously been used to explain why *S. oppositifolia* plants in ridges are observed with higher sexual reproductive investment. Former studies have, thus, suggested that growth form (Kume et al., 1999) or various abiotic factors (Opała-Owczarek et al., 2018) are responsible for the observed variation in reproductive strategy. Habitat heterogeneity can to some extent explain variation in reproductive output in *S. oppositifolia*, as also found in this thesis (Table 3.2, Table 3.7 and Table 3.10), but it cannot fully explain the uneven distribution of ploidy levels between habitat types, with diploids totally dominating on ridges, whereas triploids and tetraploids are more common in slopes and riverbeds (Table 2.1). The additional focus in this thesis on the connection between ploidy level and reproductive strategy can provide the missing link. The results support a shift in reproductive strategy towards more efficient vegetative propagation in triploids and tetraploids.

Sexually reproducing diploid plants are dominating on ridges

Sexually reproducing diploids, which dominates on ridges (Table 2.1), seem to benefit from the long growth season by producing more seeds compared to triploids and tetraploids (Table 3.5). In general, sexual reproduction seems to be common in plants growing in dry habitats (Herben et al., 2015) and especially under high light quantity conditions (Yang & Kim, 2016), which fits well with the characterization of the ridge habitat in Svalbard. Furthermore, higher reproductive output will generally be selected for when adult or juvenile mortality is high (Herben et al., 2015). The higher seed production in *S. oppositifolia* diploids on ridges might be a response to high mortality in germinating seedlings due to higher disturbance, like soil movement, in ridges.

Additionally, seeds from diploid *S. oppositifolia* growing on ridges may be of better quality (i.e., viability), resulting in higher germination compared to seeds produced by plants in slopes and riverbeds (Table 3.7). However, ridges can be a challenging environment for a seedling to emerge. Seedlings are extremely fragile and vulnerable to stress and disturbance compared to clonal ramets of the same species (Lei, 2010). Müller et al. (2011) found for instance that seed germination in various Arctic plant species, including *S. oppositifolia*, was much higher under optimal conditions in a growth chamber, compared to outdoor germination under natural conditions. The seed germination percentage for plants growing on ridges in the present study may, thus, have been overestimated compared to the situation under natural conditions, as in Müller et al. (2011). Additionally, Müller et al. (2011) recorded a rise in germination in *S. oppositifolia* seeds grown in field conditions from approximately 2% to 20% the year after the experiment started, which might suggest that germination percentage from this study could have been higher if the experiment had run for a longer time.
Further, the seed germination data may be impacted by variation in phenology across transects resulted in collection of seeds at different development stages. Seeds collected in Bjørndalen may for instance have been less mature than seeds collected from other transects.

Capsule production might, however, be negatively affected by fluctuating temperatures in ridges. Plants growing on ridges were, thus, recorded with more flowers, but fewer capsules compared to riverbeds (Table 3.2). As one of the earliest flowering species in Svalbard, *S. oppositifolia* is frequently exposed to fluctuating temperatures and potentially freezing events. This is especially true for plants in ridges, which are the very first plants to flower. Compared to riverbeds temperatures on ridges are more fluctuating (Appendix A), and might have a negative impact on plant development, especially the complex processes involved in sexual reproduction (Zinn et al., 2010). It has been shown that both increased exposures to freezing temperature and altered temperatures during flowering may reduce flower abundancy in Arctic plants (Wheeler et al., 2015). In fact, early flowering arctic-alpine species have been shown to have relatively low reproductive success (fruits:flower ratio x seed:ovule ratio), although seeds had a high chance of maturing. Contrary, late flowering species had high seed output per individual, but lower chance of seed maturity (Molau, 1993; Körner, 2021). This also fits with the results on seed germination in *S. oppositifolia*, as seeds from ridges, or early flowering plants, had higher germination percentage than those from late flowering plants in riverbeds.

More vegetative propagation in tetraploids and triploids

Vegetative propagation was clearly more efficient in triploid and tetraploid compared to diploid *S. oppositifolia* plants. Additionally, vegetative propagation was higher in plants from slopes and riverbeds, compared with plants from ridges (Table 3.10 and 3.13). The more efficient vegetative propagation of triploids and tetraploids are probably beneficial for establishment in areas with shorter growing season. It has been argued that plants in growth-limiting conditions will allocate more biomass to clonal propagation and less to sexual reproduction (Yang & Kim, 2016). Furthermore, it has been hypothesized that smaller plants will evolve towards clonal reproduction because they fail to reproduce sexually under the competition, either conspecific or heterospecific, from taller neighbours (Aarssen, 2008). *Saxifraga oppositifolia* is considered a weak competitor, and with its relatively low growth, it can easily be overgrown or shaded by taller growing species, which has been shown to have a negative impact on sexual reproductive output (Stenström et al., 1997). Heterospecific competition has also been shown to increase competition for the already scarce number of pollinators in Svalbard, which might negatively impact diploid plants that strongly depend on insect pollination for sexual reproduction. *Dryas octopetala*, which is a common species in the typical slope habitat, has for instance been shown to negatively impact pollinator activity on other Arctic plant species (Tiusanen et al., 2020). This may contribute to explain the low capsule production of *S. oppositifolia* plants in slopes, disregarding ploidy level (Table 3.2), but also why diploids are less abundant in slopes compared to ridges.

Polyploidisation may trigger the development of vegetative reproductive modes that were not present in the diploid progenitors. It may also just enhance already existing vegetative propagation by increasing the number and subsequent differentiation of meristems (Müntzing, 1936; Husband et al., 2013). As *S. oppositifolia* diploids are capable of some vegetative propagation, it is likely that this reproductive mode has been enhanced in triploids and tetraploids. The change in reproductive mode following polyploidisation is generally discussed as an advantage for successful establishment and reduced risk of immediate backcrossing with their diploid progenitor (Otto & Whitton, 2000; Ramsey & Schemske, 2002).

Vegetative propagation in polyploids might also be favoured as a response to reduced fertility (Herben et al., 2017). This fits well with the lower flower, capsule and seed production in triploid and tetraploid plants, than diploids of *S. oppositifolia* (Table 3.1, Table 3.2 and Table

3.5). Although seed and capsule production was reduced in tetraploids, germination percentage did not vary between diploid and tetraploid seeds (Table 3.7). It seems like those few seeds that are produced by tetraploids are of sufficient quality and able to germinate just as well as diploid seeds. The ability of efficient vegetative propagation seems rather to have given triploids and tetraploids an advantage in habitats where diploids are less common, especially in riverbeds. On the other hand, vegetative propagation is known to reduce genetic diversity (Yang & Kim, 2016), which could then be a potential challenge for long term survival of tetraploid *S. oppositifolia*. However, Müller et al. (2012) investigated genetic diversity in *S. oppositifolia* by using amplified fragment length polymorphisms (ALFPs) and found no reduced genetic variation in tetraploid *S. oppositifolia* plants compared to diploids, suggesting that tetraploids do maintain genetic diversity despite higher investment in vegetative propagation. Gabrielsen and Brochmann (1998) studied genetic diversity at different spatial scales in *Saxifraga cernua*, which mainly reproduces clonally via bulbils, and found that even occasional sexual reproduction in exceptionally good years can maintain the genetic diversity in an Arctic clonal plant.

Triploids are usually reproductively challenged through chromosomal paring problems because of their odd number of chromosomes (Ramsey & Schemske, 1998). Triploid *S. oppositifolia* plants had a seed production close to zero (Figure 3.2) and seem to rely on vegetative propagation only. Whether these triploids are ephemeral hybrids recurrently produced between tetraploids and diploids, represent a triploid bridge as part of ongoing autopolyploidization (Ramsey & Schemske, 1998), or constitute an established lineage relying on vegetative propagation is currently unknown. Although triploids seem to strongly overlap with tetraploids when it comes to habitat preferences, the ability of tetraploids to reproduce sexually might create a slightly different niche compared to triploids.

More vegetative propagation in riverbeds

Riverbeds seem to be the optimal habitat type for vegetative propagation (Table 3.10). In the rooting experiment, the mineral rich soil (soil A) was supposed to represent the alkaline rich soils in wet riverbed habitats, whereas the organic soil (soil B) contained sieved soil with coal ash representing the carbon-rich soil found in slopes. Both riverbed and mineral soil were positive associated with bigger and more branching roots (Table 3.10). In line with these findings, it has been shown that when soil with low nutrient resources is supplied with carbon, for instance peat soil supplied with coal ash, it can reduce the biomass of plant roots (Bottner

et al., 1988). Furthermore, it has been shown that flooded environments can induce adventitious rooting (Visser et al., 1996), and that adventitious rooting in general happens more easily in wet soil (Herben et al., 2015).

Ye et al. (2014) found that woody clonality is frequent in wet or climatically stable environments, while herbaceous clonality is more frequent in cold, dry, or climatically instable environments. The ridges where *S. oppositifolia* grows are usually considered climatically instable because of movement of the substrates and fluctuating temperatures. Riverbeds, on the other hand, can also be considered challenging owing to occasionally flooding by large amounts of melt water, and plants must withstand both sudden increase in water stress, and mechanical stress from water movement across distances (Ostler et al., 1982). From another perspective, movement of melt water may actually contribute to dispersal of vegetative shoots, and hence be beneficial in vegetative propagation, which often only promotes local population growth through rapid spread around the mother-plant, but it is not a specialization for long-distance dispersal (Yang & Kim, 2016). The riverbeds in the present study vary from plot to plot, and not all are considered unstable or experiencing water stress to the same extent. Some of the riverbeds (Bjørndalen, Todalen and Bolterdalen) are found in open areas and might occasionally be flooded, resulting in regular deposition of fine sediments, while others are more stabilized with dry, less sorted glacial till and higher vegetation cover (Appendix B).

Slopes seem to be a less optimal habitat for both sexual reproduction and vegetative propagation in *S. oppositifolia*. On the other hand, it represents an intermediate habitat type where all ploidy levels co-exist and where both reproductive modes can be utilized although not optimized.

Autopolyploidy has created a shift in reproductive strategy and niche expansion

Taken together, autopolyploidization in *S. oppositifolia* has most likely facilitated both a shift in reproductive strategy towards more efficient vegetative reproduction and expansion of the species niche into riverbeds, by enabling tetraploids to thrive in environments less favourable for the sexually reproducing diploids. A similar example was seen for *Vaccinium vitis-idaea*, where tetraploids have ecotypically differentiated from their diploid progenitor and shifted from sexual reproduction to asexual reproduction (Wakui & Kudo, 2021). The divergence of the species niche of *S. oppositifolia* might be the beginning of a sympatric speciation process,

which may with time result in isolation and full reproductive barriers between diploids and tetraploids.

With the rapid climatic changes reported from Svalbard, and in the Arctic in general (ACIA, 2004), increased plasticity or rapid evolution are necessary for species to escape or adapt to the new conditions. The Arctic flora includes many specialist taxa, which live near the edge of physiological limits and may be genetically constrained to specific adaptions and thus less plastic in response to changing environments (Gugger et al., 2015). Arctic plant species that lack adaptive capacity may become the evolutionary losers with enhanced climatic changes (Somero, 2010). Range-restricted species, particularly arctic-alpine species, have already shown severe range contractions and have been some of the first species to go extinct due to recent climate change (Parmesan, 2006). In a scenario of rapid climate change, increase of a species' ecological preferences might be necessary to secure species survival. Polyploidization as an evolutionary mechanism that can promote niche expansion, as suggested for *S. oppositifolia* in this thesis, might play an important role for this to happen.

Future studies

In this thesis, I analysed and discussed data on sexual reproduction and vegetative propagation collected during only one growth season. Future studies should investigate variation in reproductive strategy and output over several growth seasons to account for variation between years, which most likely affects for instance phenology, pollinator availability and seed development, and thus also sexual reproductive output.

According to the statistical analyses, capsule production and rooting ability were affected not only by ploidy level, but for instance also by habitat type and soil type as well as variation not explained by the variables included in this thesis (a per-sample random effect) (Table 3.2 and Table 3.10). A great deal of unexplained variation is common in biological data, as natural systems include variation which often cannot be categorized, and it may reflect some of the variation for the specific growth season of 2020.

In line with the discussed heterospecific competition that may negatively impact pollinator availability in slopes and thus reduce capsule production, pollinator availability in different habitat types could be tested by using insect traps, to assess if pollination availability is correlated with and part of the habitat-effect on capsule production.

As this thesis focused on how reproductive strategy and output is related to ploidy level, another aspect that could be investigated is whether autopolyploidization could have resulted in other phenotypic traits, not captured by this thesis, which may promote niche expansion. Těšitelová et al. (2013) found for instance that polyploidy was associated with a shift in mycorrhizal symbionts, which may facilitate polyploid establishment and thus promote niche expansion within the *Gymnadenia conopsea* group (Orchidaceae). *Saxifraga oppositifolia* is associated with at least two fungal groups, that is Glomeromycota and Ascomycota (Oehl & Körner, 2014; Botnen et al., 2020), but if such fungal associations vary with ploidy level is yet to find out.

Furthermore, it would be interesting to do crossing experiment measure ploidy levels of the germinated *S. oppositifolia* offspring to see if any of the seedlings have another ploidy level than the mother plant. This could provide valuable knowledge about the origin and formation of autopolyploids. Triploids had a sexual reproductive output close to zero when it comes to seed set. The few seeds produced by triploid plants were not included in the seed germination experiment, but it would still have been interesting to see whether they would germinate and if so the ploidy level of the seedlings. Despite low seed set, triploid plants might still contribute to pollen flow. Triploid plants may potentially produce haploid, diploid or triploid gametes, which may contribute to the formation of higher level polyploids (Ramsey & Schemske, 1998).

5. CONCLUSION

This thesis is the first study to provide support for autopolyploidy as a driver for phenotypic variation related to reproductive strategy and output in *S. oppositifolia*. Autopolyploidy seems to have enabled a shift in reproductive strategy towards more efficient vegetative propagation in triploids and tetraploids. Although *S. oppositifolia* diploids are capable of some vegetative propagation, vegetative propagation has been enhanced in triploids and tetraploids and is beneficial for establishment in areas with shorter growth season. This ability of efficient vegetative propagation has given triploids and tetraploids an advantage in habitats where diploids are less common, especially in riverbeds. Sexually reproducing diploids are dominating on ridges and seem to benefit from the long growth season by producing more seeds than triploids and tetraploids. Although sexual reproductive output was reduced in tetraploids, germination did not vary between diploid and tetraploid seeds, and it seems like tetraploids seeds are of sufficient quality and able to germinate just as well as diploid seeds which might create a slightly different niche compared to triploids. This niche expansion for the species as a whole, might be the beginning of a sympatric speciation process. As the Arctic currently is experiencing some of the most severe rapid climatic changes on earth, Arctic species must either adapt to or go extinct and an increase of a species' ecological preferences through autopolyploidy might be one solution to secure species survival in the long run.

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APPENDICES

APPENDIX A

Temperature logger data from the transect in Endalen

The logger data is borrowed from Bronken Eidesen, P. (2021). Soil temperature and moisture recorded as part of a model-system for study of autopolyploidy in *Saxifraga oppositifolia*, Adventdalen, Svalbard [30.10.21]. Norstore. https://doi.org/10.11582/2021.00030

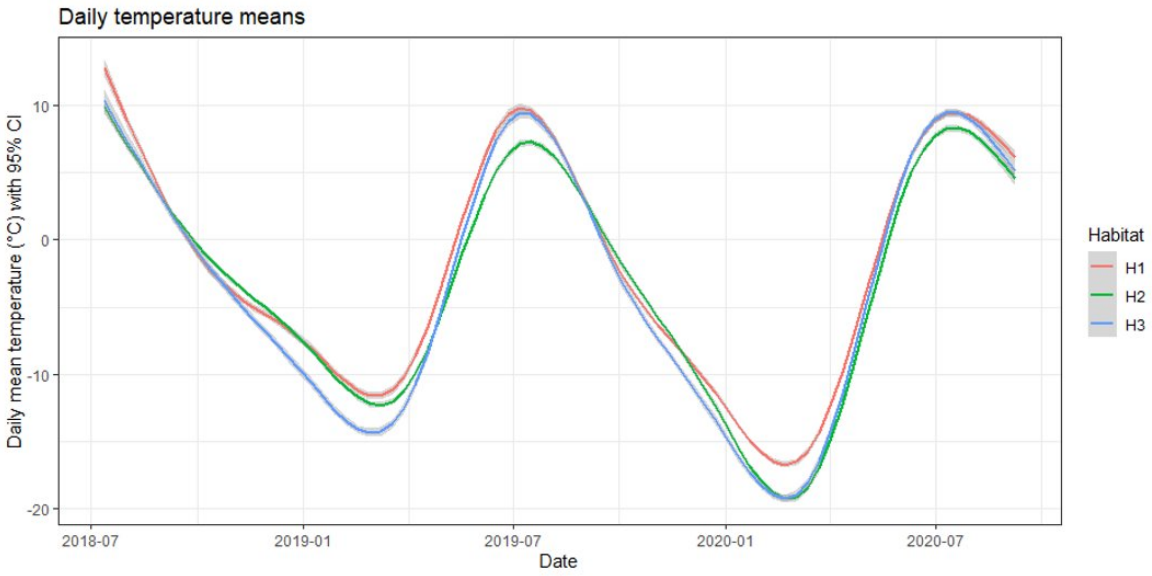

Figure A.1. Daily mean temperatures from 12th of July 2018 to 4th of September 2020 in the transect in Endalen. Riverbed (plot 2) = H1 (red), slope (plot 3) = H2 (green) and ridge (plot 4) = H3 (blue).

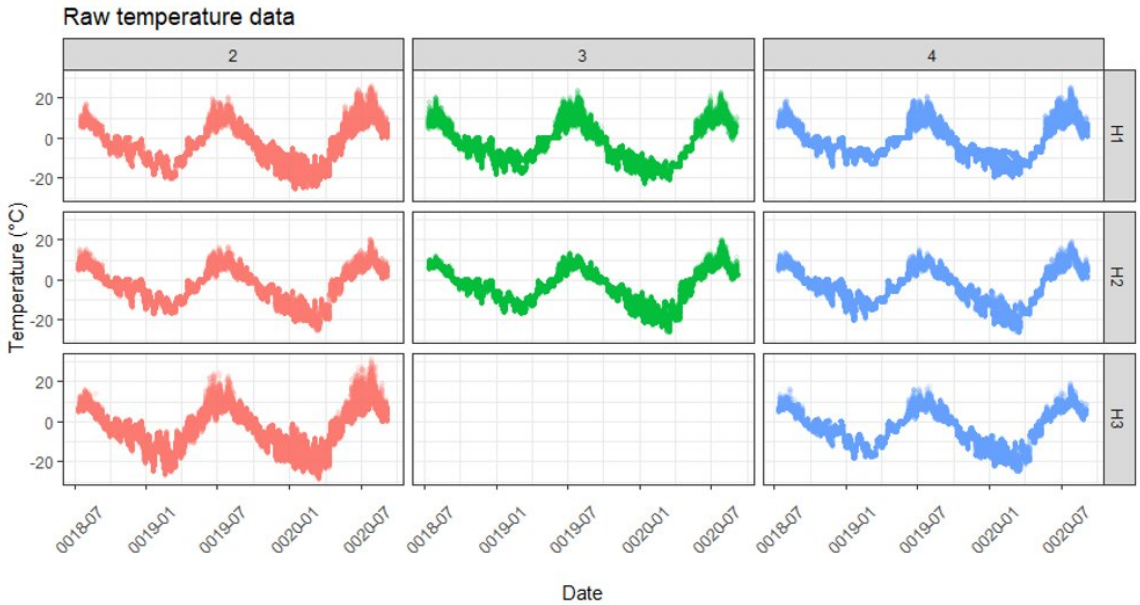

Figure A.2. Daily mean temperatures from 12th of July 2018 to 4th of September 2020 in the transect in Endalen. Riverbed (plot 2) = H1 (red), slope (plot 3) = H2 (green) and ridge (plot 4) = H3 (blue).

APPENDIX B

Variation in the riverbed habitat type

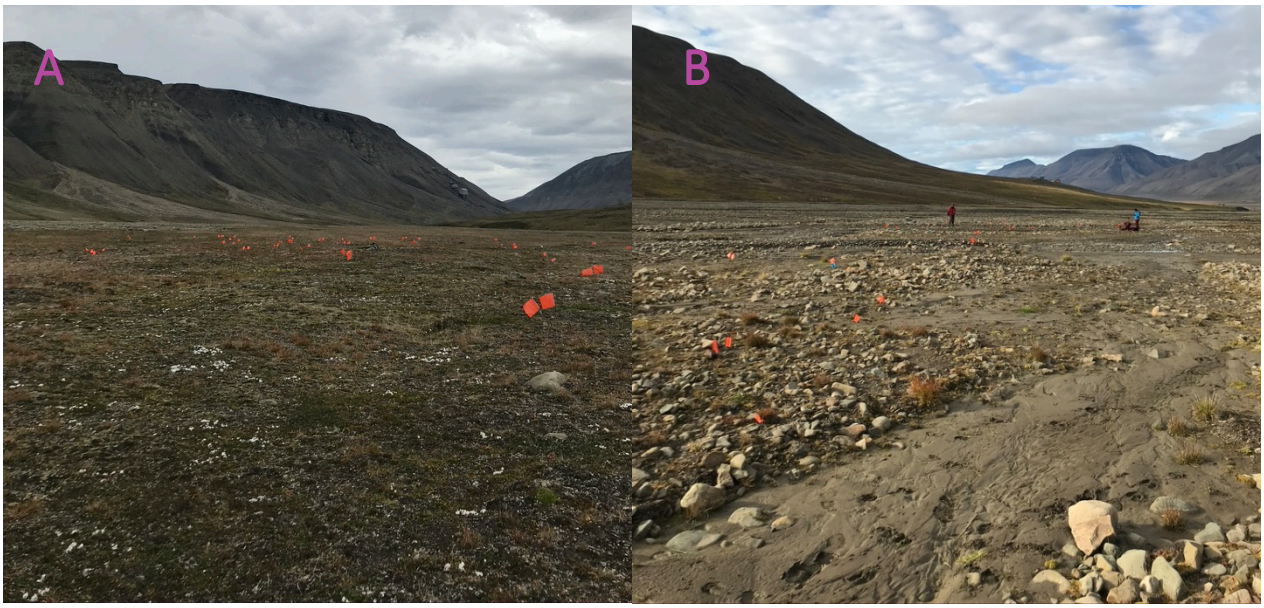

Figure B.1. Plot 2 – riverbed in Endalen (A), which is characterized by having vegetation cover and biological crust and being more stable and drier compared to plot 8 – the riverbed in Bolterdalen (B), which consists of more unstable sediment types varying from clay and silt to pebbles and larger gravel, and has almost no vegetation cover at all. This last riverbed type is occasionally flooded during the growth season, and hence results in regular deposition of fine and thin sediments.

APPENDIX C

Pictures of plants from each rooting stage

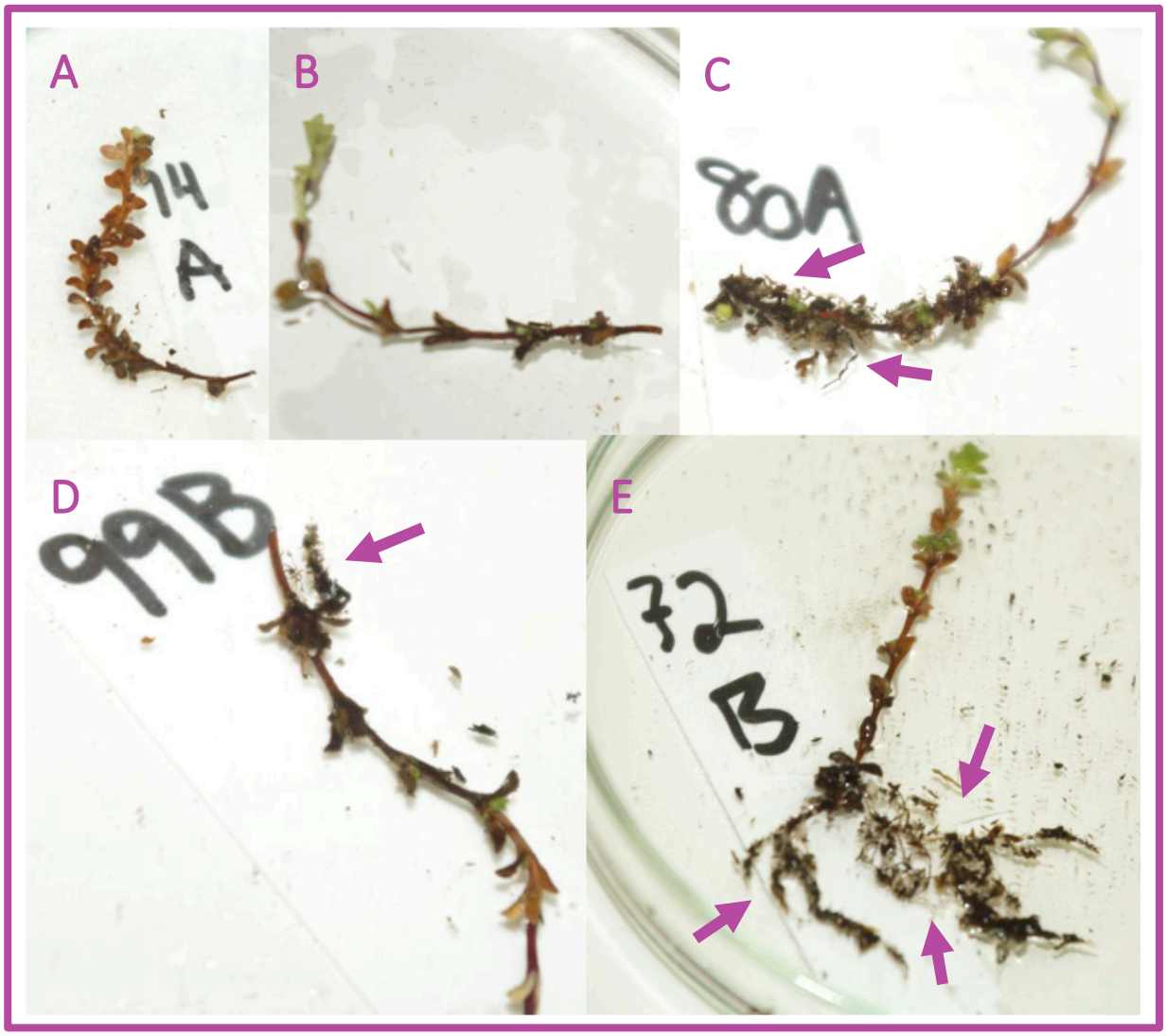

Figure B.1. Five cuttings from the rooting ability experiment representing rooting stage 0-4 (Table 2.5) with arrows pointing towards visible root initials/roots. Rooting stage 0 (A) had no visible roots, rooting stage 1 (B) rooted but only visible in a stereo microscope, rooting stage 2 (C) had indications of new roots, rooting stage 3 (D) had small roots, rooting stage 4 (E) clearly had branching roots.

Appendix D

D.1 Five best models from AICc model comparison procedures and AIC weights

AIC weights (w_i) can be interpreted as conditional probabilities for models and make it easier to interpretate the results of an AIC model comparison procedure (Wagenmakers & Farrell, 2004). The AIC weights were therefore used to evaluate the evidence for each of the five best models and to compare them thereafter. The weights were calculated from AIC values by the following formulae:

$$w_i = \frac{e^{-0.5AIC_i}}{\sum_{j=1}^{\#models} e^{-0.5AIC_j}} = \frac{e^{-0.5(AIC_i - AIC_{best})}}{\sum_{j=1}^{\#models} e^{-0.5(AIC_j - AIC_{best})}} = \frac{e^{-\Delta AIC_i/2}}{\sum_{j=1}^{\#models} e^{-\Delta AIC_j/2}}$$

Capsule production

Q 1.1 Is capsule production affected by any nuisance variables?

Table D.1.1. The five best models obtained from the explorative analysis, carried out by the function *regress.ic.search* which stepwise added, removed or replaced single or interacting covariates, instead of searching through all possible combinations. The tables includes both AICc and AIC-weight.

Model	*Habitat*	*Transect*	*Growth form*	(1| *Plot*)	(1| *ID*)	*Habitat*: *Transect*	*Growth form*: *Habitat*	*Growth form*: *Transect*	**AICc**	w_i
1	x	x	x		x	x	x		2860.63	0.44
2	x	x	x		x	x	x	x	2862.42	0.18
3	x	x	x	x	x	x	x		2862.70	0.16
4	x	x	x		x	x			2862.94	0.14
5	x	x	x		x	x		x	2864.15	0.08

Seed production

Q 1.2 Is seed production affected by any nuisance variables?

Table D.1.2. The five best models obtained from the explorative analysis, carried out by the function *regress.ic.search* which stepwise added, removed or replaced single or interacting covariates, instead of searching through all possible combinations. The tables includes both AICc and AIC-weight.

Model	*Habitat*	*Growth form*	(1|*ID*)	(*Growth form* | *Plot*)	*Habitat* * *Growth form*	**AICc**	w_i
1			x	x		1350.28	0.28
2	x		x			1350.70	0.22
3	x	x	x		x	1350.74	0.22
4			x			1351.35	0.16
5		x	x	x		1351.96	0.12

Seed germination

Q 1.3 Is germination affected by any nuisance variables?

Table D.1.3. The five best models obtained from the explorative analysis, carried out by the function *regress.ic.search* which stepwise added, removed or replaced single or interacting covariates, instead of searching through all possible combinations. The tables includes both AICc and AIC-weight.

Model	*Habitat*	*Transect*	*Growth form*	*Seed weight (g.)*	(1|*ID*)	*Habitat * Seed weight (g.)*	**AICc**	w_i
1	x	x	x		x		280.91	0.44
2	x	x			x		282.06	0.18
3	x		x		x		282.07	0.16
4	x				x		282.35	0.14
5	x			x	x	x	282.98	0.08

Rooting ability

Q 2.1 Is rooting ability affected by any nuisance variables?

Table D.1.4. The five best models obtained from the explorative analysis, carried out by the function *regress.ic.dredge* which looked through all possible combinations for potential single or interacting covariates. The tables includes both AICc and AIC-weight.

Model	*Habitat*	*Soil*	(1|*Plot*)	*Habitat*Soil*	**AICc**	w_i
1	x	x	x		710.99	0.39
2		x	x		711.50	0.30
3	x		x		713.09	0.14
4			x		713.51	0.11
5	x	x	x	x	714.56	0.07

Leaf production

Q 2.2 Is leaf production affected by any nuisance variables?

Table D.1.5. The five best models obtained from the explorative analysis, carried out by the function *regress.ic.dredge* which looked through all possible combinations for potential single or interacting covariates. The tables includes both AICc and AIC-weight.

Model	*Habitat*	*Soil*	(1|*Plot*)	(*Soil* | *Plot*)	**AICc**	w_i
1			x		249.13	0.45
2	x		x		250.40	0.24
3		x	x		251.18	0.16
4	x	x	x		252.47	0.09
5				x	253.23	0.06

D.2 Likelihood chi-square tests

For all hypotheses test the following null hypotheses (H0) and alternative hypotheses (HA) were used (HA is specified for each hypothesis test in Table 2.3: HA-1.1, HA-1.2, HA-1.3, HA-2.1 and HA-2.2):

H0: *Ploidy level* or *Diploids vs Polyploids* have no effect on the model

HA: *Ploidy level* or *Diploids vs Polyploids* have an effect on the model

SEXUAL REPRODUCTION

Capsule production

Table D.2.1 Results from the likelihood chi-square test of the best model from the explorative analysis (Table D.1.1) compared to the best model including the variable *Diploids vs. Polyploids* and the best model including *Ploidy level*. The first p-value refers to a comparison of model 1 and 2, and the second p-value of a comparison of model 2 and 3. According to the likelihood chi-square test, *Diploids vs. Polyploids* was significant ($p < 0.05$), and the null hypothesis was rejected.

Model		*P* value
1	*Capsules ~ Habitat + Transect + Growth form + (*1 | *ID) + Habitat:Location + Growth form:Habitat*	
2	*Capsules ~ Habitat* + *Location* + *Growth form* + (1 | *ID*) + *Habitat:Location* + *Growth form:Habitat* + *Diploids vs. Polyploids*	***
3	*Capsules ~ Habitat* + *Location* + *Growth form*+(1 | *ID*) + *Habitat*:*Location* + *Growth form*:*Habitat* + *Ploidy level*	
Significant codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Seed production

Table D.2.2 Results from the likelihood chi-square test of the best model from the explorative analysis (Table D.1.2) compared to the best model including the variable *Diploids vs. Polyploids* and the best model including *Ploidy level*. The first p-value refers to a comparison of model 1 and 2, and the second p-value of a comparison of model 2 and 3. According to the likelihood chi-square test, *Ploidy level* was significant ($p < 0.05$), and preferred over *Diploids vs. Polyploids,* and the null hypothesis was rejected.

Model		*P* value
1	*Seed production* ~ (*Growth form* | *Plot number*)	
2	*Seed production* ~ (*Growth form* | *Plot number*) + *Diploids vs. Polyploids*	**
3	*Seed production* ~ (*Growth form* | *Plot number*) + *Ploidy level*	***
Significant codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Seed germination

Table D.2.3 Results from the likelihood chi-square test of the best model from the explorative analysis (Table D.1.3) compared to the best model including the variable *Diploids vs. Polyploids* and the best model including *Ploidy level*. The p-value refers to a comparison of model 1 and 2. According to the likelihood chi-square test, *Ploidy level* was not significant ($p > 0.05$), and the null hypothesis "H0: *Ploidy level* have no effect on the model" was kept.

Model		*P* value
1	*Germination percentage* ~ *Habitat* + *Transect* + *Growth form* + (1 | *ID*)	
2	*Germination percentage* ~ *Habitat* + *Transect* + *Growth form* + (1 | *ID*) + *Ploidy level*	
Significant codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

VEGETATIVE PROPAGATION

Rooting ability

Table D.2.4 Results from the likelihood chi-square test of the best model from the explorative analysis (Table D.1.4) compared to the best model including the variable *Diploids vs. Polyploids* and the best model including *Ploidy level*. The first p-value refers to a comparison of model 1 and 2, and the second p-value of a comparison of model 2 and 3. According to the likelihood chi-square test, *Diploids vs. Polyploids* was significant ($p < 0.05$), and the null hypothesis was rejected.

Model		*P* value
1	*Seed production* ~ *Habitat* + *Soil* + (1 | *Plot*)	
2	*Seed production* ~ *Habitat* + *Soil* + (1 | *Plot*) + *Diploids vs. Polyploids*	*
3	*Seed production* ~ (*Growth form* | *Plot number*) + *Ploidy level*	
Significant codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Leaf production

Table D.2.5 Results from the likelihood chi-square test of the best model from the explorative analysis (Table D.1.5) compared to the best model including the variable *Diploids vs. Polyploids* and the best model including *Ploidy level*. The first p-value refers to a comparison of model 1 and 2, and the second p-value of a comparison of model 2 and 3. According to the likelihood chi-square test, *Diploids vs. Polyploids* was significant ($p < 0.05$), and the null hypothesis was rejected.

Model		*P* value
1	*Seed production* ~ (1 | *Plot*)	
2	*Seed production* ~ (1 | *Plot*) + *Diploids vs. Polyploids*	***
3	*Seed production* ~ (1 | *Plot*) + *Ploidy level*	
Significant codes	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	