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Reproductive strategies of flowering plants in Afromontane grasslands along an elevational gradient on Mount Cameroon

Reprodukční strategie kvetoucích rostlin v afromontánních trávnících podél elevačního gradientu na Kamerunské hoře

Diploma thesis

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Prohlášení

Prohlašuji, že jsem práci zpracoval samostatně a že jsem uvedl všechny použité zdroje a literaturu.

V Praze, 25.4.2021

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Dominik Anýž

Abstract in English

Montane conditions represent a relatively unfavorable climate for most plants and their pollinators. Consequently, zoogamous plant species growing at high elevations are expected to be pollen limited. According to the “reproductive assurance” and “transmission advantage” hypotheses, such pollen-limited plants will adapt more toward self-pollination than plants in communities with more reliable pollination. To test such predictions, I studied reproduction strategies and pollen limitation of ten zoogamous plant species at three elevations (2,800 m a.s.l. 3,500 m a.s.l. and 4,000 m a.s.l.) in montane grasslands on Mount Cameroon, West/Central Africa. I compared seed sets produces by plants with four treatments in our extensive hand-pollination experiments: autogamy, geitonogamy, outcrossing, and natural control. One experimental species was found to be self-incompatible, six species were partially self-compatible, and one was completely self-compatible and predominantly selfing. In five of these plant species, I compared the reproduction strategies and pollen limitation among the elevations. I found that pollen limitation did rise in two species, we expect this to be due to the fact that the species were already pollen limited at 2,800 m a.s.l. Contrary to the two hypotheses, selfing did not rise with elevation in any of our experimental species at the intraspecific level. I believe it to be due to the fact that plants in our lowest elevation were not fully capable of unassisted self-pollination, which other studies have shown is necessary in species which exhibit rising selfing rates with elevations. It is however possible, that the studied species are more selfing than species in lower elevations on an interspecific level.

Key words: self-compatibility, pollen limitation, self-pollination, elevation, adaptation

Abstrakt v čestině

Vysokohorské oblasti představují relativně nepříznivé klimatické podmínky pro většinu druhů rostlin a jejich opylovačů. Proto se předpokládá, že u zoogamních rostlin se s rostoucí nadmořskou výškou bude zvyšovat i limitace opylení (tzv. *pollen limitation*). Hypotézy o "reprodukční jistotě" (*reproductive assurance*) a "výhodě přenosu" (*transmission advantage*) předpokládají, že u takových druhů či populací začnou převládat adaptace vedoucí k samoopylení. Předpoklady reprodukčních strategií a limitace opylení ověřuji v této studii u deseti zoogamních druhů rostlin ve třech nadmořských výškách (2 800 m n.m., 3 500 m n.m. a 4 000 m n.m.) v horských trávníkách Kamerunské hory. Porovnával jsem počet vyprodukovaných semen květů po čtyřech experimentálních zásazích: *autogamie, geitonogamie, cizoprašnost* a *přirozená kontrola*). Jeden druh nebyl self-kompatibilní (schopný se opylit vlastním pylem), šest bylo částečně self-kompatibilních a jeden druh byl plně self-kompatibilní. U pěti druhů jsem rozdíly v reprodukčních strategiích a v limitaci opylení porovnal mezi nadmořskými výškami. Míra limitace opylení se zvyšovala s nadmořskou výškou pouze u dvou druhů, což zdůvodňuji potenciálně vysokými hodnotami již v nejnižší nadmořské výšce (2 800 m n.m.). U samoopylení jsem nezaznamenal předpokládány výrazný nárůst s nadmořskou výškou. Zkoumané rostlinné druhy v nejnižší elevaci nevykazovaly výraznou schopnost samoopylení, která byla zaznamenána u druhů rostlin, u nich se zvýšená schopnost se stoupajícím gradientem prokázala. Je však možné, že zkoumané druhy jsou více schopné samoopylení na mezidruhové úrovni, než druhy v nižších polohách.

Klíčová slova: self-kompatibilita, limitace opylení, samo-opylení, elevace, adaptace

Contents

1. Introduction

Most angiosperms (~90% of diversity; Ollerton et al., 2011) rely on animal vectors for transfer of male gametes (pollen) onto the female reproductive organs (stigmas) of flowers to ensure efficient reproduction (pollination). These vectors are known as pollinators and are extremely important for the plant life cycle. Plants have been able to colonize most biomes on the planet, some plant species adapting to the harshest of conditions, even high arctic and alpine environments, where pollinators tend to be scarce (Arroyo et al., 1982; Blionis et al., 2001; McCall & Primack, 1992). When there are not enough pollinators to efficiently pollinate plant flowers, plants are often subjected to what is known as pollen limitation. Pollen limitation is defined as a reduction of reproductive success because of a shortage in pollen supply (Knight et al., 2005; Torres-Díaz et al., 2011). Plant species experiencing such situations have various ways of coping with it to ensure reproduction, especially in species that are short lived and under reproductive time pressure.

Plants are primarily self-incompatible, meaning that they are only able to be pollinated by pollen genetically different from their own, which is called outcrossing (or xenogamy) (Ferrer & Good-Avila, 2007; Wright et al., 2013). However, many plant species have become secondarily self-compatible (or at least partially self-compatible) meaning that they become capable of reproducing using the pollen from a genetically identical individual (Ferrer & Good-Avila, 2007; Takebayashi & Morrell, 2001). When plants become self-compatible, they are usually able to produce seeds by self-pollination (or selfing). Dafni et al., (1995) defines two categories of selfing; facilitated selfing, where a pollen vector is necessary, and autonomous selfing, which does not require a vector. We can further distinguish facilitated selfing in plants by whether their ovule is able to be pollinated from pollen within one flower (autogamous selfing) or having to rely on pollen from a different flower of the same plant (geitonogamous selfing)(Dafni et al., 1995). However, the terminology of various types of selfing has been used inconsistently in the past throughout pollination studies. For this thesis, we use the terminology found in Arroyo et al., (2006), where autogamous and autonomous selfing are viewed as synonymous, i.e. autogamous selfing refers to self-pollination within one flower, regardless of involvement of a pollen vector. Some plants have also been known to completely avoid the need for ovule fertilization altogether by becoming apomictic, i.e. producing a genetically identical copy of the female plant from an unfertilized ovule (Schinkel et al., 2016).

There has been some debate on self-compatibility in the past years, mostly focusing on the criticism that self-compatibility has for long been viewed as a qualitative trait, treating plants as either self-compatible or self-incompatible (Razanajatovo et al., 2016). However, many plant species are at least partially self-compatible, meaning they are capable of reproducing with genetically identical pollen, although the seed quality and/or quantity could be lesser than if reproducing with outcrossing pollen (Ferrer & Good-Avila, 2007). It has been proven that the transition to self-compatibility (or at least partial self-compatibility) is among the most common plant adaptations (Barrett, 2002; Takebayashi & Morrell, 2001). Although the debate is still ongoing, self-compatibility of plants is starting to be viewed more as a gradient between the two strategies (Razanajatovo et al., 2016).

Although selfing can provide plants the ability to reproduce when pollen from conspecific individuals is scarce, it has often been referred to as an “evolutionary dead end” (Schemske & Lande, 1985; Takebayashi & Morrell, 2001). The reason for this is that lack of genetic diversity (among other things) could potentially lead to inbreeding depression in a population, in other words the accumulation of negative mutations, which may reduce fitness of a species (Barrett et al., 2014; Park et al., 2017). Plants utilize various adaptations to avoid selfing, from spatial and temporal separation of reproductive organs within a flower (herkogamy and dichogamy, respectively)(Goodwillie et al., 2005) to separating the two sexes within plant individuals completely (dioecy) (Henry et al., 2018; Willmer, 2011). Plants that have colonized environments inhospitable for pollinators utilize various additional adaptations to minimizing selfing.

High montane habitats are an example of a setting unfavorable for pollinators (Egawa & Itino, 2020; Lefebvre et al., 2018). The higher a mountain is (of course relative to the latitude), the more inhospitable the conditions become, with low temperatures and strong winds being only some of the factors limiting pollinator abundance, diversity and in turn, activity (Arroyo et al., 1985; Totland, 1997). The decrease in pollinators results in pollen limitation of the plant species which occur in these conditions (Arroyo et al., 1985; Jiang & Xie, 2020; Knight et al., 2005). The “increased pollination probability hypothesis” provides one solution to this problem. It states that flower showiness and longevity should increase with the rising elevation, in order to attract as many pollinators for as long as possible under the sub-optimal scenarios (Bingham & Orthner, 1998). In theory, the increasing longevity would provide more time for pollinators to locate and pollinate the flower. Torres-Díaz et al., (2011) conducted a study in the high Andes of central Chile and observed *Chaetanthera renifolia* (Asteraceae) had an increased receptiveness of its stigmas when it was not pollinated, compared to experimental flowers of the same species which had been pollinated. Trunschke & Stöcklin, (2017) described an increase in longevity in several species between two elevations in the Central Swiss Alps, located 1,000 m a.sl. apart. Out of the six experimental species observed, increased flower longevity was detected in three species: *Euphrasia montana* (Orobanchaceae), *Primula farinose* (Primulaceae) and *Trifolium alpinum* (Fabaceae). In both mentioned studies, the experimental plants were shown to rely on pollinators and did not have tendencies to selfing, while also not suffering from pollen limitation.

Decrease in vegetative growth is another common trend in species in high elevations (Hautier et al., 2009; Olejniczak et al., 2018; Yaqoob & Nawchoo, 2017). Studies have also shown that maximum reproductive output (i.e. the maximum quantity of seeds a flower is capable of producing) can decrease with rising elevations and inhospitable conditions (Straka & Starzomski, 2015; Wenk & Falster, 2015; Yaqoob & Nawchoo, 2017). The "energy-constraint hypothesis" suggests that fluctuating environmental conditions at high elevations may reduce photosynthetic rates, the energy for seed development and seed provisioning (Herbert G. Baker, 1972; Guo et al., 2010). It has been documented that for some plant species in higher elevations, it is more advantageous to stop reproducing sexually and only disperse clonally (Hautier et al., 2009; Milla et al., 2009). There is a fine line between cases in which these changes are considered adaptations and when they become inevitable reactions to the harsh conditions of the environment. This however depends on individual species and their ability to adapt toward inhospitable conditions (Fischer et al., 2013; Halbritter et al., 2018).

Certain plant species have however been known to buffer some of the hitherto mentioned negative effects by becoming selfing (de Vos et al., 2012; Zhang & Li, 2008). It is believed that ~15% of plants are predominantly selfing (>80% instance of selfing) and many more are capable of reproducing through a combination of selfing and outcrossing (Barrett, 2002; Vogler & Kalisz, 2001). The reason for this is that for some plants, the risks of selfing outweigh the disadvantages of not reproducing at all in conditions unfavorable to pollinators, and may therefore even provide an advantage (Mattila et al., 2020). Baker's law is a theory stating that selfing species may be more readily adapted towards colonizing new habitats due to not having to rely on pollinators (Baker, 1955; Cheptou, 2012; Lowry & Lester, 2006; Randle et al., 2009). This theory, which was first observed on several species from the family Plumbaginaceae (Baker, 1948) has become one of the fundamental ideas of plant reproduction.

The argument for this theory was based around the idea that selfing species would be best equipped to colonize islands, that was however disproven by the reality that many island species are non-selfing or even strictly outcrossing (Cheptou, 2012). Although there is still debate around Baker's law, the idea that selfing facilitates plants when pollination conditions are sub-optimal has been explored in several other hypotheses.

The "autogamy reproductive assurance hypothesis" states that when the mates or pollinators are scarce, plants will evolve toward selfing in order to compensate for the insufficient pollen transfer (Arroyo et al., 2006). This might happen precisely in habitats where the environmental productivity is quite low, and the area cannot support many organisms, such as alpine conditions. This hypothesis argues that the main selective advantage of selfing lies in the assured seed set, meaning that the plant will produce at least some seeds instead of none at all (Schoen et al., 1996). The "automatic selection hypothesis" (also called the "transmission advantage hypothesis") provides a view on why plants might choose selfing over outcrossing on the genetic level. It argues, that selfing genes (i.e. genes that promote reproduction through selfing) have a 3:2 transmission advantage over outcrossing genes (i.e. genes that only allow outcrossing pollination) (Fisher, 1941; Stone et al., 2014). Although studies in the past tried to identify which of these hypotheses is more accurate, more recent studies have started viewing them as co-operative processes in the transition from outcrossing to selfing (Barrett et al., 2014; Busch & Delph, 2012; Cheptou, 2012).

High elevation mountains, which tend to have inhospitable conditions for pollinators, have served as a proxy to test these hypotheses. Some studies, which have been cited as proof of these hypotheses tested only one alpine elevation, in which they found a large amount of selfing species compared to lower elevations, but of different species. Berry & Calvo, (2016) compared selfing instances from fourteen plant species in a lower elevation with four different species in a higher elevation and discovered the four higher species to be more selfing than their lower counterparts. Medan et al., (2002) conducted a similar study and found higher selfing rates in species located in higher elevations in the Mendoza Province, Argentina. Biella et al., (2021) proved that a high elevated *Linaria* (Plantaginaceae) species is self-compatible, unlike its sister species in the lowlands and Ling et al., (2017) found *Prunella vulgaris* (Lamiaceae) to be selfing in alpine environments. Although these types of studies do provide examples of higher selfing in higher elevations on an interspecific level, they do not provide answers about the change along elevation within a species.

When studies assessed breeding systems along an elevational gradient on an intraspecific level, the results become more complicated. Some studies did find higher instances of selfing in higher elevation positioned species. Seguí et al., (2018) proved *Viola maculate* (Violaceae) to become more reliant on cleistogamy (a form of selfing, which occurs in an unopened flower) with rising elevation (1,600 m a.sl.- 2,500 m a.s.l. gradient), and Etcheverry et al., (2008) found *Vigna caracalla* (Fabaceae) to be predominately selfing in its highest elevation due to pollen limitation. More studies have been appearing that do not find the rising elevation to be a driving factor of increasing self-pollination in species, which are not already self-compatible. Gugerli, (1998) found no difference in selfing instances in *Saxifraga oppositofolia* (Boraginaceae) over a 500 m gradient, Young et al., (2002) came to same results in self-incompatible *Rutidosis leiolepis* (Asteraceae) over a 400 m gradient. Arroyo et al., (2006) did find selfing in all elevations over a 1 000 m gradient in Chilean Andes, it was however on a fully self-compatible, predominantly selfing species *Chaetanthera euphrasiodes* (Asteraceae). Wirth et al., (2010) proved that *Eritrichum nanum* (Boraginaceae) was more self-pollinated in lower, compared to higher elevations, where the cost of producing seeds was too high for the plant. More similar studies have come out in recent years (Black et al., 2019; Dai et al., 2017). However, in all of these mentioned studies, when the plant was found to have increased selfing in higher elevations compared to lower ones, the plant was already self-compatible in the lower elevations, but was not subjected to such intense pollen limitation and in turn did not have to resort to selfing. This would imply that the adaptation to self-compatibility would have had to happen before the plants colonized the higher elevations and only then started utilizing selfing more (Arroyo et al., 2006)

To my knowledge, only one recent study has tested the change in breeding systems along an elevational gradient in Africa. Black et al., (2019) found no pollen limitation and no instances of selfing in self-incompatible *Cineraria erodioides* (Asteraceae) along an elevational gradient of 500 m a.s.l. In this study, I examined the breeding systems of several plant species in montane grasslands along an elevational gradient of the Central/West Africa's highest peak, Mount Cameroon. Mount Cameroon is a volcanic mountain located in the Gulf of Guinea, starting at the sea level and extending inland for about 25 kilometers until reaching its highest point, 4,095 m a.s.l. Being host to some 2,300 species of plants (~100 of those endemic)(Cheek et al., 1996), Mount Cameroon National Park was established in 2009. The area has two main seasons, a rainy season from June to October and a dry season from November to May. The peak is surrounded by a tropical forest, but at around 2,200 m a.s.l. the forest is replaced by natural montane grasslands. The grasslands are prone to burning, either natural wildfires resulting from the lack of water in the dry season or by the National Park officials as a precautionary measure against wildfires getting out of hand.

We chose this location to test the mentioned hypotheses about plant breeding systems along elevation by hand-pollination experiments on ten plant species at three elevations in the grasslands above the timberline. We set three hypotheses to test: (1) reproductive output of individual plant species decreases towards the higher elevations; (2) populations at higher altitudes are more pollen limited; (3) autonomous self-pollination increases towards the higher elevations.

2. Materials and methods

2.1 Study area and plant species

The study was situated above the timberline on the western slope of Mount Cameroon, along the Guiness Trail from Buea to the summit. Originally, the study was meant to include four elevations (2,300 m a.s.l. 2,800 m a.s.l., 3,500 m a.s.l. and 4,000 m a.s.l., sampled over two years) to represent the complete elevational gradient of the montane grasslands. However, the outbreak of Covid-19 made it impossible. In 2019, following the plans, the study was performed at the two mid-elevations 2,800 m a.s.l. (surroundings of the Hut 2, 4°11'38"N, 9°11'51"E) and 3,500 m a.s.l. (surroundings of the Wevondi Cave, 4°12'14"N, 9°11'9"E). The epidemiological situation allowed to carry the study out at the highest elevation 4,000 m a.s.l. (surroundings of the Hut 3, 4°12'33"N, 9°10'48"E) in 2020, under the leadership of Ishmeal N. Kobe and his local assistants. The lowest elevation 2,300 m a.s.l. (surroundings of Mann's Spring; 4°8'40"N, 9°7'14"E) would have been right above the timberline and would have been the most species-rich site. The 2,800m elevation hosts species-rich grasslands, supplemented by abundant shrubs and even a few solitary trees. The 3,500m elevation was characterized by species-poorer grasslands on a steep windy slope with rare shrubs. The 4,000m elevation was already in the alpine zone with species-poor sparse montane vegetation growing mostly on old lava screes. In each elevation, we evenly set study plots, where we would carry out our hand-pollination experiments. Plots were chosen based on their accessibility, plant species abundance, and expected protection against potential fires.

In 2019, we identified all flowering zoogamous plant species within the studied elevations. We selected 10 plant species (Table 1) following these criteria:

i. Distribution in at least two of the studied elevations.
ii. Enough specimens at individual elevations.
iii. Suitability for hand pollination experiments, i.e. with large and robust flowers or inflorescences, with several flowers or inflorescences per plant specimen, and with easily accessible pollen and stigmas.

In the four studied Asteraceae species (Table 1) which have inflorescences, we assessed each inflorescence (hereinafter called flower) as one experimental unit, instead of each floret individually.

Table 1. *– List of the experimental plant species with the amounts of treatment replicates produced in each elevation. Treatments: A (Autogamy), G (Geitonogamy), O (Outcrossing), C (Control), n.f. (not flowering) - where there were not enough flowers for a certain treatment or when species did not appear in a given elevation.*

Family Plant species	**Total no. of experimental flowers**	**Total no. of mature seed sets**	**Matured seed sets for individual treatment per elevation (m a.s.l.)**											
			Elevation 2,800				**Elevation 3,500**				**Elevation 4,000**			
			A	**G**	**O**	**C**	**A**	**G**	**O**	**C**	**A**	**G**	**O**	**C**
Asteraceae														
Crepis hypochoeridea	225	223	13	10	12	10	7	5	10	15	38	34	23	35
Senecio purpureus	345	277	31	25	27	35	7	8	7	7	38	34	23	35
Lactuca inermis	127	94	14	n.f.	9	18	9	n.f.	10	11	10	n.f.	1	4
Senecio burtonii	192	86	19	17	15	21	3	5	1	6	n.f.	n.f.	n.f.	n.f.
Hypericaceae														
Hypericum revolutum	124	94	7	11	15	13	n.f.	n.f.	n.f.	n.f.	15	9	12	11
Geraniaceae														
Geranium arabicum	42	35	9	n.f.	10	18	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
Geranium mascatense	41	29	4	n.f.	7	21	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
Caryophyllaceae														
Silene biafrae	32	29	n.f.	n.f.	n.f.	n.f.	9	n.f.	10	6	n.f.	n.f.	n.f.	n.f.
Ranunculaceae														
Clematis simensis	85	77	18	19	19	22	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.	n.f.
Gentianaceae														
Swertia mannii	29	18	5	3	1	5	3	1	0	0	n.f.	n.f.	n.f.	n.f.

2.2 Pollination experiment

To reveal the plant species breeding system, we carried out four treatments (Fig. 1) on the chosen plant species and compared the quantity of seed sets (i.e. the number of seeds produced by individual experimental flowers) among different treatments and elevations. Flowers treated by *autogamy* were kept bagged to avoid transfer of pollen from another flower, flowers treated by *geitonogamy* were hand-pollinated with pollen from a different flower of the same individual, flowers treated with *outcrossing* were hand-pollinated with pollen from a different individual (at least 100 m far away, to increase the probability that it would be a genetically different individual; Fig 2B), and *control* flowers were left exposed for natural pollination.

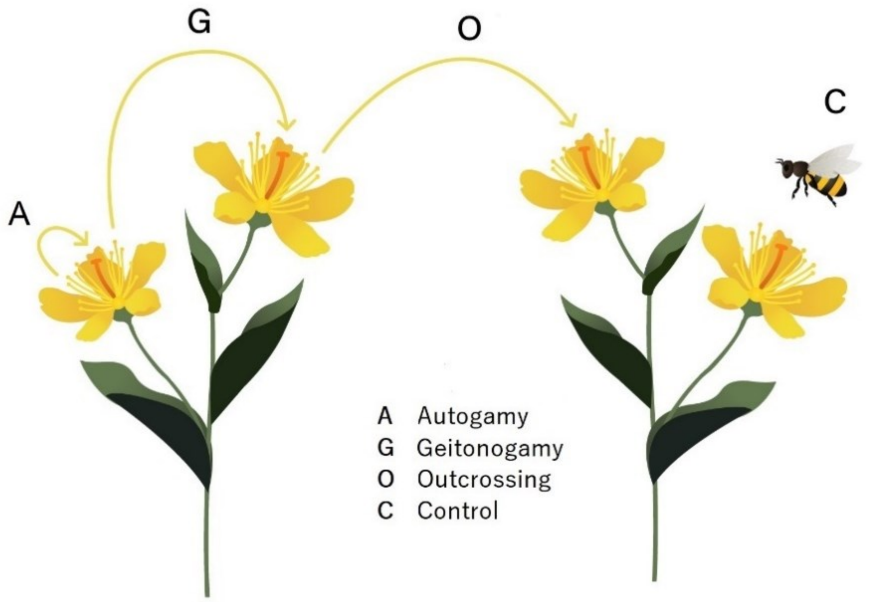


Figure 1. *The four experimental treatments illustrating pollen transfer (yellow arrows) on the studied plant species. Illustrator: Barbora Drozdová*

In each study plot, we identified the experimental plant species and covered several dozens of their unopened buds with nylon mesh bags (M. Bartoš et al., 2020) to avoid any pollination event prior to our own experiment (Fig 2A). The nylon bags were made in various sizes to fit the different plant species' flower sizes. The smallest bags (6x8 cm) were used to cover *Swertia mannii* and both *Geranium* species, the medium bags (8x10 cm) were used to cover both *Senecio* species, *Lactuca inermis*, *Silene biafrae*, and *Clematis simensis*, and the largest bags (10x15 cm) covered the buds of *Crepis hypochoeridea* and *Hypericum revolutum.* For each three experimental buds covered on a single plant we left one bud uncovered and labeled it with a cotton string to make sure the *control* flowers will be of the same age as the experimental flowers. We made sure to leave enough uncovered untreated flowers on each plant individual as a pollen source for the treatment *geitonogamy*. If, however, there were even more available buds, we bagged them as well, to ensure having as many replicates per plant as possible. For each plant species, we aimed to test no less than 40 replicates (10 for each treatment) in each elevation. The total amount of the experimental plants in each elevation can be found in Table 1. Each experimental plant individual was assigned a unique number. We observed flowering of each experimental plant species, especially how long it takes flower buds to open, how long the anthers produce (potentially viable) pollen, and how long the flower stigmas are receptive (by observation of their drying though a magnifying glass). Based on this, we concluded the best time to carry out our hand pollination experiments.

***Figure 2**. Various stages of the experiment on* Senecio purpureus*. (A) Bagged buds; (B) Outcrossing hand pollination;(C) Seed counting, the seeds in the upper left corner were counted as fully developed, whilst the rest were considered undeveloped.*

When a flower opened and we observed its stigma to be receptive, we unbagged it, performed one of the treatments, bagged it again, and labelled the mesh bag with a tag symbolizing the treatment. The hand pollination was performed by obtaining a conspecific flower/inflorescence and brushing the pollen of the obtained flower to a stigma(s) of the treated flower. The success of the pollen transfer was checked using a magnifying glass. After a flower had been treated, it was covered again with the mesh bag and left covered until the end of the experiment to prevent the loss of seeds, which the flowers would produce. *Control* flowers were bagged in the same way, after they had finished flowering. In the case of the Asteraceae species, in which the flowering period of an inflorescence took ~one week, hand pollination was repeated each day until all the individual florets in the inflorescence had flowered. Some plant species did not have enough flowers open at one time to carry out all treatments. In the case of *Lactuca inermis,* both *Geranium* species, and *Silene biafrae*, a maximum of two opened flowers were found per plant. In such cases, the available bagged flowers were then only treated with *autogamy* + *control* or *outcrossing* + *control*.

2.3 Quantification of the reproductive success

All bags from the experimental and control flowers were collected ~30-40 days after the experiments when the flowers were fruiting. Each mesh bag containing a fruit was cut off, placed inside a paper bag, and labeled with the plant code containing elevation, study plot number, plant species, plant number, and applied treatment. The bagged fruits were dried by silica gel.

Once the fruits had been transported to the Czech Republic, they were separated based on species. For each plant species, a chart was produced based on ca 10 bags (each containing one flower/inflorescence) to compare which seeds should be counted as fully developed and which should not. Most species' seeds were counted in a small paper tray using a stereomicroscope (Fig 2C). The seeds of *Hypericum revolutum* were produced in a pod made up of five individual chambers. Due to their small size, the *Hypericum* seeds were counted using a counting grid.

Of the ten species sampled, only eight were usable. For *Swertia mannii* and *Geranium mascatense*, only a few replicates were considered viable. Due to this, the species were not used in the analysis of breeding systems and pollen limitation. Of the remaining eight species, all produced a varied amount of "useless" experimental flowers. Most often, the fruits labeled "useless" were infested by an insect herbivore, in other cases the flower fell down from unknown reasons (i.e. flower abortions were unrecognizable from mechanical disturbances), or the experiment obviously failed (such as a lost tag, or development of another flower inside the bag). "Useless" fruits were not counted in the data analysis. In the case of *Hypericum revolutum*, at least one chamber was labeled as "useless" in the majority cases. In turn, the number of seeds produced per flower was counted as the total number of seeds divided by the amount of uninfected pods. In the case of *Crepis hypochoeridea*, many of the bags collected were labeled as "useless" (39.5 %). However, the ability of the species to produce seeds even when it had a pest led us to use said specimens. The amounts of used bags by elevation and treatment for each experimental species is summarized in Table 1.

2.4 Statistical analysis

For the five species, from which we had treatments from in at least two elevations (*Senecio purpureus, Senecio burtonii, Lactuca inermis, Crepis hypochoeridea, Hypericum revolutum*) we analyzed the changes in seed sets throughout elevations. For the whole statistical analysis of our results we used R version 1.3.959 (R Core Team, 2020).

To answer the main questions, we used the amounts of individual flower seeds sets and compared them with each other. Maximum reproductive output of individual plant species between elevations was tested by comparing the *outcrossing* seed sets throughout elevations for individual plants. *Outcrossing* seed sets were regarded as the highest number of seeds a flower can produce under pollen saturation from a genetically different individual. To uncover the severity of pollen limitation in different elevations, we used the seed sets of *outcrossing* (the maximum) and *control* (the realized amount) treatments. By comparing the treatments, we analyzed the proportion of seeds which the *control* flower is capable of producing under the same condition in a given elevation. To assess the ability of a flower to become self-pollinated, we compared the seed sets of *outcrossing* treatment with both *autogamy* and *geitonogamy*. Whenever comparing any treatment with *outcrossing*, we primarily used the values from the same plant individual. When there was more than one *outcrossing* replicate per individual, we used their average. In the case where there were no outcrossing treatments for a given plant, we used the average seed set from the outcrossing treatment of the plant species in each elevation.

To analyze whether pollen limitation (PL) rises with elevation on Mount Cameroon, we calculated a pollen limitation index for each *control* (C) treatment. We used an index developed by Larson & Barrett (2000):

$$PL = 1 - (S_C / S_O)$$

where S_C is the quantity of seeds produced per C treatment and S_O is the quantity of seeds produced by the *outcrossing* (O) treatment. PL ranges from 0 to 1, 0 meaning no pollen limitation and 1 meaning full pollen limitation. For the few cases of $PL<0$, we considered $PL=0$. The negative value means that the control produced more seed than the outcrossing treatment, which would mean no pollen limitation anyway. To answer the question of whether plants tend to reproduce more by self-pollination in higher elevations we used two indexes from our hand-pollination experiments. The first index was of treatments *autogamy* (A) against

O: (A/O), by which we analyzed the ability of a flower to reproduce without any additional pollen transfer. The second index was of *geitonogamy* (G) against O: (G/O), used to analyze self-compatibility of a species along with the reproductive output when pollen saturated from a genetically identical individual.

To identify self-compatibility among species, we used the distributions found in Matallana et al., (2010); G/O < 0.2 was considered self-incompatible, 0.7 > G/O > 0.2 was considered partially self-compatible and G/O > 0.7 was considered self-compatible. In the cases of plant species on which we did not carry out the treatment *geitonogamy*, we used A/O to assess self-compatibility.

Due to the high amount of zero values and integers which the individual species ratios produced, the criteria for using a parametric test were not met in any of the analyses. We therefore performed non-parametric Kruskal-Wallis tests to assess the relationship of these numbers on elevation. We analyzed the dependent variable "index" as numeric and tested its' relation to the variable "elevation", which we treated as an ordinal categorical factor. First, we assessed the p-value of the overall dataset for each plant, analyzing the values based on elevation. If inter-elevational differences were significant (p-value <0.05) for the plant species flowering at three elevations, we performed a Nemenyi post-hoc test to reveal differences among elevations in the form of their individual p-values. We set distance to "Chisquare" in order to break ties between individual values in the Nemenyi post-hoc tests.

3. Results

Overall, the hand pollination experiments were carried out with 1,242 individual flowers from the ten experimental plant species. Out of the eight species with sufficient data, one species was found to be self-compatible (*Crepis hypochoeridea),* six species were found to be partially self-compatible (*Senecio purpureus, Lactuca inermis, Hypericum revolutum, Geranium arabicum, Silene biafrae, Clematis simensis)* and one species was found to be self-incompatible (*Senecio burtonii*) (Fig. 3). Altogether, only five of the experimental species flowered at multiple studied elevations (Table 2). Three species (*Crepis hypochoeridea, Senecio purpureus and Lactuca inermis)* were treated at three elevations, whilst the remaining two species (*Hypericum revolutum and Senecio burtonii)* were treated at two elevations only.

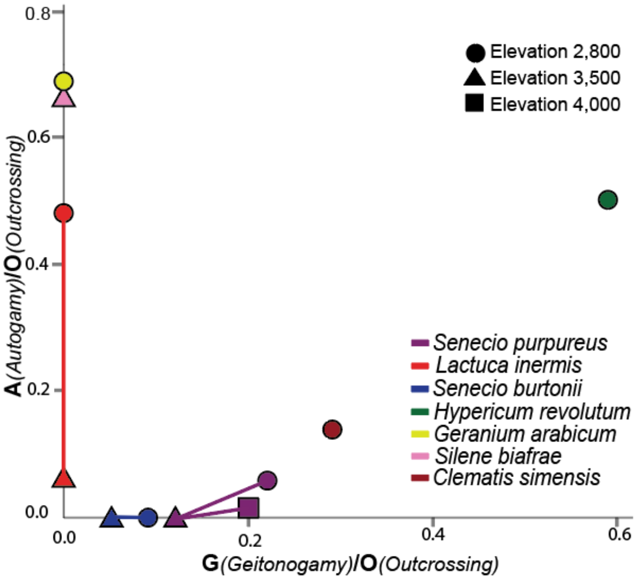


***Figure 3**. Scatterplot of A/O and G/O based on species by elevations.*

***Table 2.** Results of non-parametric Kruskal-Wallis tests analyzing differences in reproduction systems of individual plant species among elevations on Mount Cameroon. Indexes included are O (outcrossing), PL (pollen limitation), A/O (autogamy/outcrossing), G/O (geitonogamy/outcrossing)*

Species	O			PL			A/O			G/O		
	chi-square	df	P-value	chi-square	df	P-value	chi-square	df	P-value	chi-square	df	P-value
Crepis hypochoeridea	0.242	2	0.886	11.923	2	0.003**	13.915	2	< 0.001***	3.083	2	0.214
Senecio purpureus	28.338	2	< 0.001***	48.835	2	< 0.001***	6.778	2	0.034*	4.531	2	0.104
Lactuca inermis	5.26	2	0.072	5.354	1	0.021*	16.978	1	0.005**	*not analysed*		
Senecio burtonii	0.296	1	0.587	3.212	1	0.073	NA	1	1	0.597	1	0.440
Hypericum revolutum	21.13	1	< 0.001***	*not analysed*			*not analysed*			*not analysed*		

***Table 3**. Results of Nemenyi post-hoc tests comparing differences in reproduction systems among individual elevations; the post-hoc tests were performed only for the species flowering at three elevations (el)*

Species	O			PL			A/O		
	el 2,800, el 3,500	el 2,800, el 4,000	el 3,500, el 4,000	el 2,800, el 3,500	el 2,800, el 4,000	el 3,500, el 4,000	el 2,800, el 3,500	el 2,800, el 4,000	el 3,500, el 4,000
Crepis hypochoeridea	*not analysed*			0.849	0.133	0.007**	0.602	0.034*	0.006**
Senecio purpureus	0.863	< 0.001***	0.014*	0.054	< 0.001***	0.399	< 0.001***	< 0.001***	< 0.001***

3.1 *Crepis hypochoeridea*

Even though the self-compatible *Crepis hypochoeridea* did not have a significant difference in outcrossing ability between elevations (Kruskal-Wallis; O: H=0.242, df= 2, P=0.886) (Table 2, Fig. 4A), we found a significance difference in PL (K-W; PL: H=11.923, df= 2, P=0.003) (Table 2). The Nemenyi test in Table 3 revealed, that the significant outcome was between elevation 3,500 and elevation 4,000 (since *Crepis* produced less seeds in the former elevation) but did not differ much from elevation 2,800 (Fig. 4B). When fully saturated with genetically identical pollen, no significant difference was found between seed sets throughout elevation (K-W; G/O: H=3.083, df= 2, P=0.214), but there was a difference in the *autogamous* index treatments (K-W; A/O: H=13.915, df= 2, P=<0.001). Results of the post-hoc Nemenyi-test show significant variance of A/O between elevations 2,500 with 4,000 and 3,500 and 4,000 (Fig. 4C). This suggests that even though the self-compatibility did not decrease, *Crepis* was less capable of unassisted selfing in the elevation 4,000 than in elevations 2,500 and 3,500.

3.2 *Senecio purpureus*

The *outcrossing* values of partially self-compatible *Senecio purpureus* turned out to be significantly different among elevations (K-W: O; H=28.338, df= 2, P=<0.001) (Table 2) and the Nemenyi-test revealed, that the reproductive output of the species differed between the lower two elevations (2,800 and 3,500) and the highest elevation, 4,000 (Table 3, Fig 4A). Pollen limitation was also observed to be significant among elevations (K-W: PL; H=48.835, df= 2, P=<0.001) (Table 2). The Nemenyi-test showed us, that pollen limitation was not very high at elevation 2,800, but it did rise with increasing elevation (Table 3, Fig 4B). A/O of *Senecio* was found to be significant, but G/O was not (K-W; A/O: H=6.778, df= 2, P=0.034, G/O: H=4.531, df=2, P=0.104). From the Nemenyi-test for A/O we can interpret, that the differences were very significant between the outcomes of the individual indexes. However, when looking at Fig. 3 or Fig. 4C we can observe, that the values which were different were very small and *Senecio* was in fact quite incapable of *autogamous* selfing. Self-compatibility did not decrease with elevation, but *autogamous* selfing did (Fig. 4D).

3.3 *Lactuca inermis*

Partially self-compatible *Lactuca inermis* was sampled in three elevations, but only one replicate produced seeds in elevation 4,000 out of all the flowers treated. This replicate was treated with *autogamy* and because we did not have any seed-sets from replicates *outcrossing* or *control*, we were not able to produce PL and A/O indexes in elevation 4,000. When we analyzed the reproductive output of *Lactuca* based on all three elevations, the result was not significant (K-W; O: H=5.26, df= 2, P=0.072) (Table 2, Fig. 4A). The insignificance was because elevation 4,000 only had one replicate of *outcrossing* and it had a seed set of zero. Unfortunately, the second expedition in the year 2020 only produced one replicate of both *outcrossing* and *control* in the whole elevation 4,000 due to the overall low quantities of *Lactuca* in said elevation. The rest of the indexes were only calculated with elevations 2,800 and 3,500. A significant difference between elevations was found for both pollen limitation and autogamous selfing in *Lactuca* (K-W; PL: H=5.354, df= 1, P=0.021, A/O: H=16.978, df= 2, P=0.005) (Table 2). *Lactuca* became more pollen limited from elevation 2,800 to 3,500 (Fig. 4B) and produced less seeds in elevation 3,500 when treated with *autogamy* than in elevation (2,800) (Fig. 4C).

3.4 *Senecio burtonii*

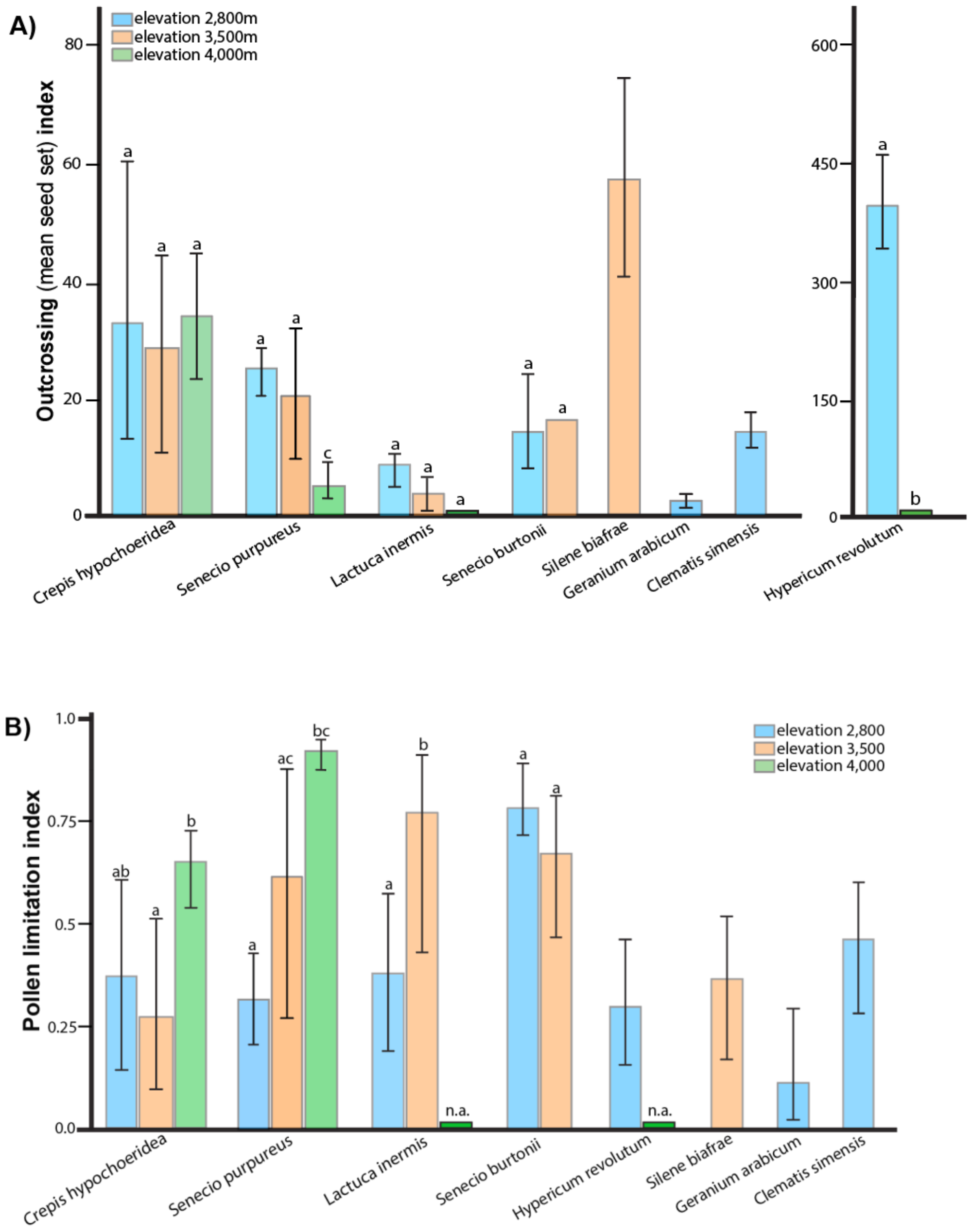
Self-incompatible *Senecio burtonii* was sampled in two elevations and was the most infested species, with 52.2% of all seeds resulting in being labeled “useless”. Because of this, only one replicate of *outcrossing* was used for elevation 3,500; it produced 17 seeds, which was used to measure all the subsequent indexes. The reproductive output of *Senecio* did not differ between the two elevations (K-W; O: H=0.296, df= 1, P=0.587) (Table 2, Fig. 4A). Pollen limitation looked as if it was lower in elevation 3,500 that elevation 2,800 (Fig. 4B), but the difference between the values was insignificant (K-W; PL: H=3.212, df= 1, P=0.073). G/O was insignificant for this species (K-W; G/O: H=0.597, df= 1, P=0.440), and in A/O there was no difference whatsoever (Table 3), as *Senecio* was unable to produce any seeds treated by *autogamy* in either elevation (Fig. 4C).

3.5 *Hypericum revolutum*

Partially self-compatible *Hypericum revolutum* was sampled in two elevations, however it did not produce seeds in elevation 4,000. Intuitively, the significance in reproductive output is evident (K-W; O: H=23.13, df=1, P=<0.001) (Table 2, Fig. 4A), however we were not able to produce results for PL, A/O and G/O. *Hypericum* did not produce seeds in elevation 4,000.

3.6 Species sampled in only one elevation

Unfortunately, we have three viable species sampled in only one elevation, since the second year of fieldwork did not go as originally planned. *Geranium arabicum* and *Silene biafrae*, which were both sampled in only one elevation, very capable of producing seeds by treatment *autogamy* in the elevation 2 800 and 3 500, respectively (Fig. 4C). Both species were found to be partially self-compatible, but seeing as the compatibility was measured from A/O, it is possible that the species are fully self-compatible. *Clematis simensis*, which was sampled in only elevation 2 800 was observed to have a twice as high G/O as A/O and was capable of selfing (Figs. 3, 4CD)



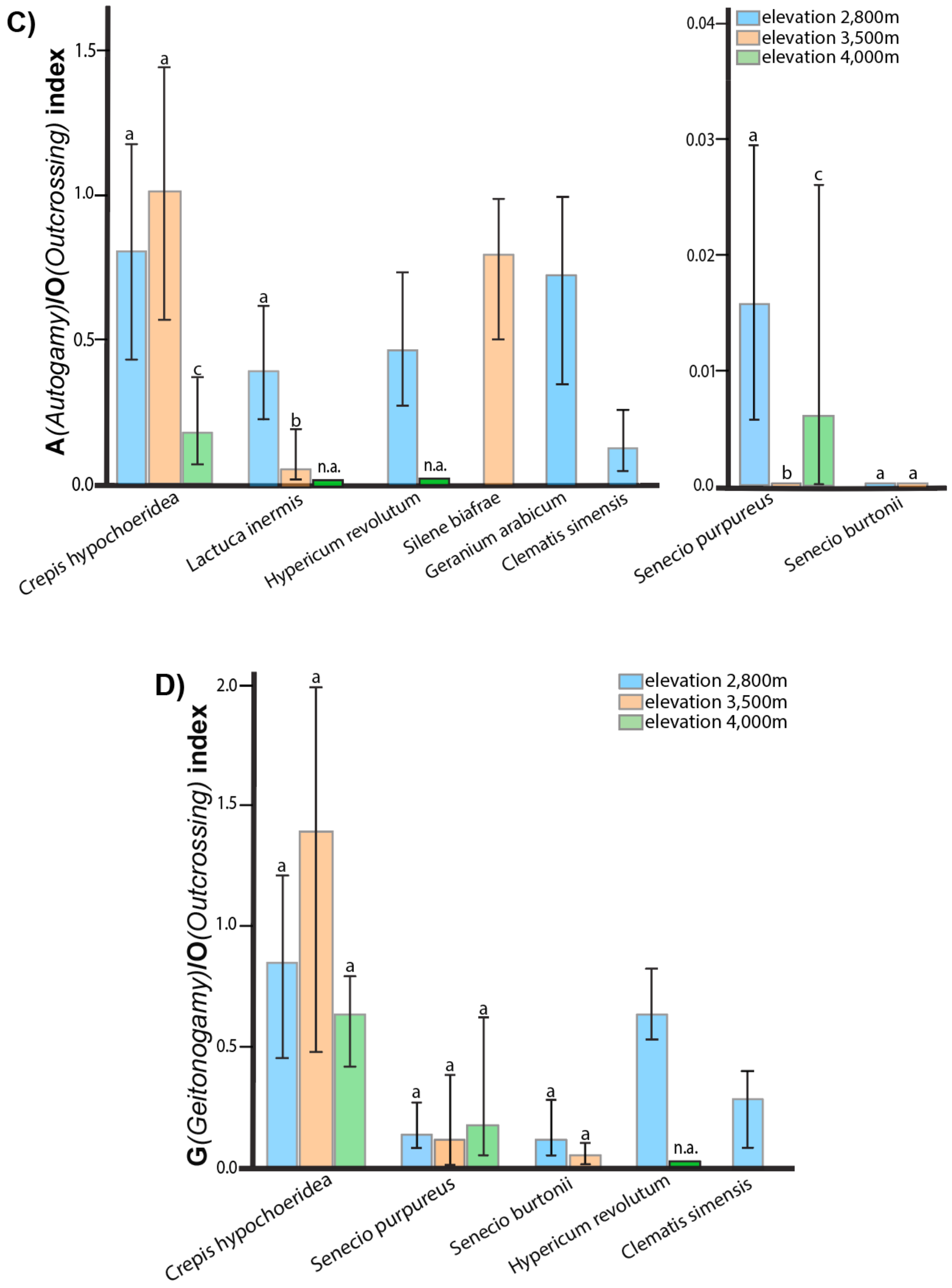


***Figure 4**. Outcomes of studied indexes on experimental plants species based on elevation: top of each bar indicates mean value, error bars are 95% confidence intervals. Individual letters symbolize significance results of Nemenyi post-hoc tests.*

4. Discussion

4. 1 Breeding systems of individual experimental species

Although plants are primarily self-incompatible, the shift from self-incompatibility to self-compatibility is one of the most common adaptations in the plant kingdom (Barrett, 2002; Vogler & Kalisz, 2001). Of our experimental species, I was only able to find previous breeding system studies carried out on *Geranium mascatense* (Geraniaceae), the rest have not been analyzed until now.

The family Asteraceae can be found worldwide and has various breeding strategies, from completely selfing *Conyza canadensis* to self-incompatible *Erigeron annuus* (Hao et al., 2011). Ferrer & Good-Avila, (2007) produced a survey of studies measuring self-compatibility in plants, and found that of 571 Asteraceae taxa represented, 61% were self-incompatible, 10% were partially self-compatible and the remaining 27% were self-compatible. Of the self-compatible (or at least partially self-compatible) species, only few have been proven to be predominately selfing, for example *Tragopogon mirus* (Soltis et al., 1995), or *Stephanomeria malheurensis* (Brauner & Gottlieb, 1987). Most species studies are predominately outcrossing, even when they can be self-compatible (Ferrer & Good-Avila, 2007).

Crepis is a genus of Asteraceae with some 200 known species (Andersson & Shaw, 1994). Hughes & Babcock, (1950) found various outcomes when testing the compatibility of *Crepis foetida*, observing some individuals to be able to produce autogamous seeds but others none at all. Andersson, (1989) found *Crepis tectorum* to be self-compatible, but not predominantly selfing. Cheptou et al., (2002) predicted that another species, *Crepis sancta* would be predominately selfing based on the assessed evidence but found no reproductive assurance through selfing in a study focusing on outcrossing in three populations around Montpellier, France. Our results provide evidence that the African species *Crepis hypochoeridea* is fully self-compatible and utilizes selfing in all elevations.

Senecio is another genus of the family Asteraceae that is diversified worldwide. A study from 1985 tested the breeding systems of 32 Australian *Senecio* species and found 19 species to be self-incompatible and 13 to be self-compatible (Lawrence, 1985). Hiscock, (2000) analyzed one species in the genus, *Senecio quallidus* and found it to be self-incompatible. More recently, Ferrer & Good-Avila, (2007) found that out of 12 species studied, most were self-compatible or at least partially self-compatible. *Senecio purpureus* is distributed throughout the grasslands across Africa, whereas *Senecio burtonii* is a species primarily found in Cameroonian montane grasslands. We found *Senecio purpureus* to be partially self-compatible but presume that it does not utilize selfing. *Senecio burtonii* was observed to be self-incompatible and is thus presumed primarily outcrossing.

The last of our studied Asteraceae was from the genus *Lactuca.* The genus is often viewed as completely self-compatible and selfing, due to the cultivation of *Lactuca sativa* (Lindqvist, 1960). Zohary, (1991) found seven species of *Lactuca* to be predominately selfing in a study comparing them to *Lactuca sativa.* The survey study of 571 Asteraceae species by Ferrer & Good-Avila, (2007) analyzes 124 species of *Lactuca* and found the majority to be self-compatible or at least partially self-compatible. They did not however provide information on selfing qualities of the species. The African species we studied, *Lactuca inermis,* seemed to be partially self-compatible, being able to produce half as many seeds through *autogamy* as the *outcrossing* treatment in the lowest elevation, but did not produce many seeds by selfing in the higher elevations.

The family Hypericaceae has a nearly worldwide distribution, with self-fertilization being common, but not universal (Robson, 1977). *Hypericum perforatum* was found to be self-compatible and sometimes prone to reproduction through apomixy (Mártonfi et al., 1996). *Hypericum cumulicola* was observed to produce almost as many seeds through self-pollination as with cross-pollination (Evans et al., 2003). On the other hand, Abrahamson & Vander Kloet, (2014) found *Hypericum edisonianum* to be self-incompatible and reliant on outcrossing pollen in hand-pollination experiments. Our experimental species, *Hypericum revolutum* is a typical Afromontane species and has been studied in experiments focusing on its pollinator assemblages (Bartoš et al., 2015; Janeček et al., 2007), no studies have yet been carried out to assess its breeding system though. Our results indicate, that *Hypericum revolutum* was partially self-compatible and capable of producing similar quantities of seeds through both *autogamy* and *geitonogamy* in the elevation 2,800, it was not however able to produce any seeds in the highest elevation studied.

The family Geraniaceae is also spread across most of the world, although mostly inhabiting temperate and sub-tropical regions and not so often found in the tropics. Fiz et al., (2008) produced a detailed pollination experiment and found that of the experimental *Geranium* species, 15 % were predominantly selfing, whilst the rest were more prone to mixed mating. Of our two experimental species Fiz et al., (2008) had already studied *Geranium* *mascatense*, and found it to have a mixed mating system. We do not have proper data for this species to produce studied indexes, but we do know that no seeds were produced in the replicants treated with *autogamy*. Our other experimental species from Geraniaceae, *Geranium arabicum* has not appeared in any study analyzing its breeding system. We found it to be partially self-compatible based on A/O since it was impossible to produce *geitonogamous* treatments. It is possible that this species is fully self-compatible, as it produced a high quantity of seems when treated with *autogamy*.

One of our experimental species was from the genus *Silene* of the Caryophyllaceae family. Studies examining the breeding systems of *Silene* point out that although primarily viewed as an outcrossing genus, some experimental species tend to reproduce by selfing (Buide et al., 2015; Kephart et al., 1999). Kephart et al., (1999) found selfing in *Silene douglasii* and Davis & Delph, (2005) identified that *Silene noctiflora* self-pollinated itself even before opening its flower. Buide et al., (2015) carried out a pollination experiment on two co-flowering species (*Silene niceensis* and *Silene ramosissima*) in southern Spain and discovered that although both were self-compatible, only *Silene ramosissima* was predominantly selfing. Our data indicated that the Cameroonian endemic species *Silene biafrae* was partially self-compatible based on its A/O index. Similarly to *Geranium arabicum*, it is possible that this species is fully self-compatible, since it produced almost as many seeds when treated with *autogamy* as with *outcrossing* in 3,500 m a.s.l.

Clematis is a genus with ~350 known species of the family Ranunculaceae. Redmond & Stout, (2018) pointed out that self-pollination was not uncommon in *Clematis*, which they tested on *Clematis vitabla*, and found it to be partially self-compatible, but not predominantly selfing. N. Jiang et al., (2010) produced results of hand pollination experiments which concluded that of their three experimental species, all were self-compatible, two of which were adapted toward high selfing rates (*Clematis akebiodes* and *Clematis rehderiana*) and one was predominantly outcrossing (*Clematis chrysocoma*). Our results indicate that the African species *Clematis simensis* is partially self-compatible and capable of reproducing by selfing, when outcrossing pollen is limited.

Unfortunately, the data of our last experimental species, *Swertia mannii* from the family Gentianaceae was insufficient and so we could not make any deductions about its breeding system. We presume that the pollination experiment could have been carried out incorrectly on this species, since the mean *control* treatment seed set was twice as high as the *outcrossing* treatment seed set. However, we cannot say for sure since only one replicate of *outcrossing* was not labeled "useless" in elevation 2,800 and none was recovered from elevation 3,500. To my knowledge, no other study on the breeding system of *Swertia mannii* exists which we could compare to our data.

4. 2 Variations in reproductive output among elevations

Overall, we expected a decrease in reproductive output with increasing elevation in our experimental plant species. The "energy-constraint hypothesis" states, that species in unfavorable conditions are limited by obtainable energy which they could use to invest in reproductive output. (Abdusalam & Li, 2019; Knight et al., 2005). It is well documented that the harsh conditions in higher elevations may impact the reproductive success of plants, resulting in plants not being able to produce as many seeds as plants in the lower elevations (García-Camacho & Totland, 2009; Hautier et al., 2009; Körner, 2003; Stigter, 2019). Of our five experimental plant species, we found a significant decrease in the maximum reproductive output in three of them (Fig. 3A).

The decrease in reproductive output of *Senecio purpureus* and *Lactuca inermis* was very similar to what was expected, with each rising population being able to produce less seeds than the previous. In *Lactuca* however, we only produced one replicant in the highest elevation, so the results might not indicate the proper outcome. The complete decrease of all flower seed sets in elevation 4,000 of *Hypericum revolutum* was not expected and could have two explanations. First, which I believe is the case, is that the inhospitable conditions made it nearly impossible for the plants to produce seeds due to the high energy demands, to which the species was not adapted. I do not have data to assess the viability of pollen, or the receptivity of stigmas in the elevation 4,000, which could be used to assess if the problem arose in just one part of the reproductive cycle or if the whole flower was sterile. I do however know that in the lower elevation, the species was quite capable of reproducing with selfing. From observation, it can be said that the *Hypericum* plants in elevation 2,800 were much larger and took on formations of big shrubs, whereas in the elevation 4,000 they were more bushes low to the ground, with less flowers blooming. These observations allow the prediction that the plant was resource limited, due to which the plants in the higher elevation might have been unable to produce seeds at all. Various studies have proven that if the conditions become too harsh for a plant, it might be forced to resort only to clonal reproduction, instead of sexual reproduction (Willmer, 2011; Young et al., 2002). It can also be possible that these plants reproduce sexually only once in a few years, or more specifically when the conditions are above average in the highest elevation, and the plant opts to invest into seeds. Unfortunately, our data does not provide this information.

A question might then arise about how the plants which inhabit these elevations got there in the first place, seeing as they are unable to reproduce regularly. The term "ecological trap" is used to describe scenarios, where organisms settle in sub-optimal habitats, which might have appeared to provide suitable conditions at first (Gardner et al., 2018; Robertson & Hutto, 2006). A simple answer to the question, how the plant species which do not reproduce in the highest elevation got there, is that the seeds from lower elevations were transported higher by wind. This could have been the case for some Asteraceae, since their seeds are adapted toward wind dispersal, with the crown consisting of a pappus of hairs. It is also possible, that the fruits of the flowers were eaten by birds, which transported the seeds into the higher elevations. The seeds were able to start germinating and growing when the conditions were exceptionally good, however due to the unfavorable conditions which inevitably arose, the plants were not able to reproduce sexually.

There is also a second explanation for low seed sets in elevation 4,000, it being that the flowers were harvested too early there. The fruits of flowers were harvested ~40 days after the last pollination experiment in the first year (2019), and ~30 days in the second year (2020). It is possible that the 30 days were not enough for the plants in the highest elevation to produce fruits, however the first replicates produced in 2020 had the same amount of time to mature as the last replicates produced in 2019 (40 days), since the experiment was carried out for ten days in the later year. It is however possible that for the two species, which produced low seed sets (*Lactuca inermis* and *Hypericum revolutum*), 40 days was not enough time. Both *Crepis hypochoeridea* and *Senecio purpureus* had set seed within the time limit provided.

4.3 Self-compatibility and pollen limitation

From published studies dealing with pollen limitation, we can imply that the highest levels of pollen limitation are found in species that are self-incompatible (Knight et al., 2005). Studies analyzing pollen limitation among elevations which did not find pollen to be limited were carried out on plant species, which were highly selfing and were not reliant on pollinators for pollen transfer in any elevation (Mary T.K. Arroyo et al., 2006; Gómez, 2002; Hargreaves et al., 2015). Of the studies which did observe rising pollen limitation with elevation, the vast majority reported it on plant species, which were self-incompatible or in some cases partially self-compatible (N. Jiang et al., 2010; Torres-Díaz et al., 2011; Totland & Sottocornola, 2001). With this in mind, we would expect to find highest levels of pollen limitation in *Senecio burtonii,* and also potentially in *Senecio purpureus* and *Lactuca inermis.*

We found pollen limitation to rise significantly in only two of our experimental studies (Fig. 3B). Partially self-compatible *Senecio purpureus* and *Lactuca inermis* suffered most from pollen limitation with rising elevation. Although pollen limitation did not rise among elevations in all species, it is important to keep in mind that our lowest elevation was already significantly high (2,800 m a.s.l.), thus the plant species in this elevation might have already been significantly pollen limited. I believe this is the case of self-incompatible *Senecio burtonii,* which did not show an increase in pollen limitation, probably because it was already severely pollen limited in elevation 2,800 (Fig. 3B). *Crepis hypochoeridea* was not pollen limited overall, which is presumed to be a result of its self-compatibility.

4.4 Selfing along elevation

Both the "reproductive assurance hypothesis" and the "transmission advantage hypothesis" predict, that selfing rates should rise with elevation in plants to make up for the decrease in pollination caused by the fluctuating environmental conditions (Busch & Delph, 2012; Schoen et al., 1996; Stone et al., 2014). If our results were to be in accordance with one of the two hypotheses, all the species would experience a raised amount of selfing in the higher elevations. It is true that studies which observed certain plant species in only one elevation found them to be able to reproduce with selfing (Biella et al., 2021; Ling et al., 2017). It is has also been observed, that selfing rates do tend to rise between elevations on an interspecific level (Berry & Calvo, 2016; Medan et al., 2002). However, from extensive research, increased selfing rates throughout elevations are mainly only found in species, which were already self-compatible in the lower elevations (Black et al., 2019; Dai et al., 2017; Etcheverry et al., 2008; Wirth et al., 2010; Young et al., 2002). Those species only utilized selfing more in the higher elevation, where the conditions for pollination were worse and selfing ensured reproduction. Considering what the studies mentioned above have examined, that selfing does not rise unless the species is already self-compatible, we could expect rise in four experimental species, which could potentially become more self-compatible.

Our results indicate that none of our experimental species became more selfing among elevations. Of the four species, it was not too surprising in the two *Senecio* species. Since *Senecio burtonii* was self-incompatible, a rise in selfing was not expected. Although *Senecio* *purpureus* was partially self-compatible, it was considered a primarily outcrossing species due to its' very low selfing capabilities. In accordance with studies quantifying breeding systems, both *Senecio* species would be the least expected to have selfing rise with elevation, since they were unable to properly utilize selfing in the lowest studied elevation. *Lactuca inermis* also showed a decrease in selfing rates, contrary to what would be expected from a species capable of producing half as many seeds through *autogamy* as *outcrossing* in the lowest elevation. It is possible, that *Lactuca* produced seeds by selfing only when the conditions were exceptionally good in the higher. In both elevation 3,500 and 4,000, only one *autogamous* treatment produced seeds in each elevation. I was surprised to find that the species, which was labeled as predominantly selfing, *Crepis hypochoeridea*, produced significantly less seeds when treated with *autogamy* in the highest elevation than in the two lower elevations. However, self-compatibility did not decrease among elevations *Crepis*.

Overall, although I did not find significant rise in selfing rates on an intraspecific level, it is possible that the experimental species have higher selfing rates on an interspecific level compared to lower elevations. Considering various published studies, it can be deemed improbable that our experimental species, which did not show significant selfing rates in the lowest elevation would be capable of self-pollination with rising elevation. To better understand the breeding systems of our experimental species, we must still carry out experiments on species in elevation 2,300. Unfortunately, until then, not much can be said about species with only one elevation sampled. In the future, I am looking forward to both increasing my knowledge of the species which we already have sampled in multiple elevations, along with learning about how species which we only have sampled in one elevation (such as *Clematis simensis* and *Geranium arabicum*) reproduce in the lowest elevation of the grasslands on Mount Cameroon.

5. Conclusion

We carried out experiments on 10 plant species on the grasslands above the timberline of Mount Cameroon to assess the differences between pollen limitation and breeding systems of these plants along an elevational gradient. We successfully identified previously unknown breeding systems of eight species, one which turned out to be self-incompatible, six which were partially self-compatible and one which was fully self-compatible. The maximum reproductive output of our experimental plant species located in multiple elevations either decreased (two species) or was statistically insignificant throughout elevations (three species). Contrary to predictions, our analysis does not reveal a significant increase in pollen limitation by elevation in all species, this could be however that the plants were already limited in our lowest elevation sampled. The results from our study are also contradictory to the "reproductive assurance hypothesis" and the "transmission advantage hypothesis", as none of the species became more adapted towards selfing with increasing elevation. In fact, even a predominantly selfing species produced less seeds when treated with *autogamy* in the highest elevation. However, taking into account that our lowest elevation was already very high (2 800 m.a.s.l.), we can assume that on an interspecific level, the plants which we studied might be more self-compatible than those located in lower elevations. We plan on sampling elevation 2 300 m.a.sl. in the upcoming year to assess how to better understand the whole reproductive system of montane plant species on the grasslands above the timberline of Mount Cameroon.

6. References

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