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Influence of environmental and intrinsic factors on the flowering success and petal morphology of *Pulsatilla patens* and the hybrid *Pulsatilla patens* × *vernalis* in Finland

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Abstract
Combatting the biodiversity crisis through management and conservation requires specific information on the basic biology of species in decline. The Eastern pasqueflower, *Pulsatilla patens*, has declined drastically in Europe and in Finland is currently confined to a few locations. The aim of this study was to assess which factors influence fitness-related traits of *P. patens* and a hybrid species *P. patens* × *vernalis.* To this end we recorded morphological and environmental variables over a three-year period on individually marked *P. patens* and hybrid individuals in eight locations in southern Finland. The number of flowers decreased significantly each year for *P. patens* while for the hybrid there was first an increase and then decrease. Hybrids produced on average more flowers than *P. patens* individuals and the share of flowering individuals was higher in hybrids than in pure pasqueflowers. In general, for both *P. patens* and the hybrid, larger plant diameter, cooler and drier conditions and greater canopy openness positively affected number of flowers and flowering probability, although some species-specific differences were evident with a preference for greater canopy density for *P. patens* than the hybrid. Although our results support current conservation practices of creating more open environments to benefit flowering of *P. patens*, there also appears to be a limit to the amount of direct sunlight *P. patens* will tolerate, thus warranting caution. At present, the hybrid does not appear to be of immediate concern to the endangered *P. patens*, although continued monitoring of the populations is recommended.

Keywords Conservation of plant species · Management · Canopy density · Threatened species · Structural equation modelling

Introduction

Biodiversity loss and climate change are serious challenges for the planet and humans, and they should be tackled together. Land use change, demand for natural resources and phenomena caused by climate change in particular influence already rare and threatened species (Hansen et al. 2001; Diaz et al. 2019, Sala et al. 2000) and habitat types. We can expect that biodiversity loss will progressively increase during this century caused by a higher demand for natural resources. In fact, not only the rarest plant species, but also the more common ones are in danger (Hansen et al. 2001; Lienert 2004). Indeed, there are many documented examples of previously common species that are now listed as threatened with extinction due to a drastic loss of populations, which has resulted in the rapid change in conservation status from least concern to threatened (Parmesan 2006; Czerepko et al. 2014; Kricsfalusy 2016). The most important factor threatening plant species is reduction of habitats and deterioration of their quality (Hilton-Taylor 2000).

A good illustration of a previously more abundant plant undergoing drastic reduction in numbers is *Pulsatilla patens.* The species' European range is highly fragmented, and the size of the populations has dramatically reduced in the past 80 years. The species has become threatened with extinction

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in the boundaries of the distribution range (Uotila 1969; Jutila 2009, 2011; Szczecińska et al. 2016; Juśkiewicz-Swaczyna & Grzybowski 2013). *Pulsatilla patens* is particularly well suited for a case study to investigate the broader phenomenon of population declines. First, it can be used as an indicator of warm, well-lit and sloping boreal and temperate forest sites, which have several threatened species and second, it has a circumpolar distribution in boreal and grassland habitats in North America and Eurasia, allowing for research to be carried out on stable and declining populations across much of the northern hemisphere.

Due to its rapid decline, *P. patens* has been placed on the IUCN Red List of Threatened Species and in Finland *P. patens* has been listed as "endangered" in the Red Book of Species (Hyvärinen et al. 2019). There are 150 sites (Kalliovirta et al. 2006) and an estimated average of 2000 individuals of *P. patens* left in Finland (Jutila 2019). This species is protected by the Bern Convention online Appendix 1 (Council of Europe 1979) and the European Commission's Habitats Directive Annex II (92/43/EEC) and IV.

The destruction of habitats due to sand excavation, building and picking and transplanting to gardens were the most important threats to *P. patens* ssp. *patens* during earlier times. Currently these factors are expected to play less of a role, because this species has been protected by the Nature Conservation Act since 1952 in Finland. Currently, canopy closure and eutrophication of forests, scarcity of light in the field layer and a thick moss layer are more important reasons for vulnerability (Nieminen & Ahola 2017). In north-eastern Europe *P. patens* ssp. *patens* inhabits dry, pine-dominated heath forests, especially esker forests (Uotila 1996; Pilt and Kukk 2002). The structure of these forests has changed due to intensive forestry, which not only decrease old-growth forests, but also reduce natural, open or semi-open phases of forest succession by excluding fire and cattle grazing and by speeding up forest closure by planting trees and fertilizing (Vanha-Majamaa et al. 2007). These changes have increased interspecific competition for light and nutrients in the field layer causing the decline of *P. patens*. Forest fires prevented forest closure and thereby improved the light conditions in the herbaceous layer that enhanced *Pulsatilla patens*' germination (Kalamees et al. 2005) and reduced competition with other plant species by removing the ground vegetation and moss layer (Uotila 1996). Livestock grazing created patches for germination and left the forest structure open. Furthermore, protoanemonins in *P. patens* caused grazers to leave this plant intact. Currently forest fertilization leads to increased soil nutrient concentrations, soil acidity and competition by other plant species such as grasses, mosses and herbs (Röder & Kiehl 2006).

In Finland, the eastern limit of *P. patens* co-occurs with the western limit of *P. vernalis*. The global distribution area of *P. vernalis* is more restricted than that of *P. patens*. These species differ somewhat in morphology and in ecological needs (Grzyl et al. 2014; Sandström et al. 2017), but their plastid genome polymorphism patterns revealed close relationships (Ronikier et al. 2008). Consequently, a hybrid *P. patens* × *vernalis*, can be found in those areas where *P. patens* and *P. vernalis* co-occur and hybridize (Uotila 1980; Jutila 2009, 2011). There are eleven known populations of the hybrid in Finland. Also hybrid is protected in the Finnish Nature Conservation Act.

An important issue in conservation biology is the task of evaluating the causes of species' endangerment and based on that, finding appropriate measures to save them. To do this, it is necessary to consider the species' ontology in relation to ecology and to identify the life history stages most critical to population growth or extinction (Iriondo et al. 2003). The sexual reproductive stage is considered amongst the most sensitive phases of the plants' life cycle. Indeed, flower and fruit production were found to be major factors affecting differences in population dynamics (Newell & Tramer 1978; Jacquemyn et al. 2010; Smith & Menges 2016). Some studies in arctic plants (Arft et al. 1999) have shown an increase and some studies in the Tibetan plateau (Liu et al. 2012) decrease of flower production due to winter warming. In Finland eastern pasqueflower, which can be regarded as continental plant species (Uotila 1996), rather seems to suffer from thinner snow cover and resulting dry spring conditions, which may prevent buds to develop into flowers as they dry up instead (personal observation, Heli Jutila).

The aim of this study was to find out which factors influence flowering and seed production of *Pulsatilla patens* and *P. patens* × *vernalis*. The work bases on a long-term project in the Hämeenlinna region to study *P. patens* and hybrid populations (Jutila 2011) and the effects of management. The data are more precise than used in many previous pasqueflowers studies since the individual marking allowed exact follow-up of individuals and in addition population size estimation.

Based on previous studies, it was hypothesized that forest canopy openness, and light availability would influence fitness-related traits of both *P. patens* and *P. patens* × *vernalis*. Individuals in sites with a high canopy openness were expected to have more flowers, larger petals, more and bigger seeds because they would have more energy available to invest in them.

Moreover, the species were expected to differ in fitness characteristics. *P. patens* was expected to have smaller petals than *P. patens* × *vernalis*, an observation that was made earlier by Uotila (1980) but has not yet been statistically tested. The seed mass was expected to be bigger in *P. patens* than in *P. patens* × *vernalis*, because hybrid seeds mostly remain thin and undeveloped.

Material and methods

Pulsatilla patens and *P. patens* × *vernalis*

Pulsatilla patens (L.) Mill. is a long-lived perennial, tussock-forming, deeply rooted hemicryptophyte species with a vertical branching rhizome which can form several shoots (Röder & Kiehl 2006). *P. patens* is widely distributed in Eurasia and North America (Hultén and Fries 1986). It has recently been divided to several species and subspecies. In North America *P. nuttalliana* characterizes grazed, burned or mown prairies (Wildeman and Steeves 1982), which are among the most endangered ecosystems in North America. In Europe, *Pulsatilla patens* subsp. *patens* ranges from Germany, Hungary and Poland to the Baltic countries, and into Sweden, Finland, western Russia, Belarus and western-Ukraine. The eastern pasqueflower (*Pulsatilla patens*) is thought to be established during the Late Glacial Period (15 000–10 000 years B.P.; Jalas 1950) in those areas that were elevated and not covered with ice from the Baltic Sea. *P. patens* subsp. *multifida* is a subspecies distributed in East European Russia to Kamchatka and North China (powo.science.kew.org).

In Finland, *Pulsatilla patens* grows in dry heath or esker forests on fairly poor soils, and in particular, in *Vaccinium vitis-idaea -type, V. vitis-idaea—Fragaria vesca* -type and *V. vitis-idaea—Rubus saxatilis* -type and *Hypochoeris maculata—V. vitis-idaea* -type (Uotila 1969). The hybrid is found in quite similar sites (Uotila 1980). The well-illuminated habitat and cover of bare soil is a benefit for *P. patens* in germination, flowering and fruiting stages (Juśkievicz-Swaczyna & Grzybowski 2013).

In Finland, *P. patens* is one of the first plant species flowering in spring from the end of April to the beginning of May. The flower buds are formed already in autumn. *P. patens* flowers are bright dark purple and attract various insect groups with pollen as their main reward. In *Pulsatilla patens* × *vernalis* the flowers are lighter and somewhat dirty. Palmate, for winter decomposing leaves of *P. patens* differ from the wintering, opposite leaflets of the hybrid (see more distinctions in Uotila 1969, 1980).

Fruits of *P. patens* are one-seeded achenes. Seed production is fairly abundant (the average number of seeds per flower is 103 ± 24.9 according to Kalliovirta et al. 2006) and the seeds can germinate immediately. The seeds of *P. patens* disperse between the end of June and beginning of July (Uotila 1969; Kalamees et al. 2005; Jutila 2019). Dispersal abilities of seeds are poor and seedlings grow slowly: it can take several years before a seedling reaches the adult stage (Pilt & Kukk 2002; Kalamees et al. 2005; Jutila 2011). *P. patens* has no permanent seed bank (Pilt & Kukk 2002). It is likely that most of the seeds produced by *P. patens* × *vernalis* are ungerminable. However, some germination and introgression in the hybrid population is evident (Uotila 1980).

Knowledge of the life stages is crucial to characterize the demography of populations. According to the study of Kricsfalusy 2016, *P. patens* has four ontogenetic periods: latent (seed), pre-reproductive (seedling, juvenile, immature, and virgin), reproductive (generative), and post-reproductive (subsenile and senile). The pre-reproductive or vegetative stage lasts for at least three years, but most individuals remain in this state longer. In the generative or reproductive stage individuals flower. In the post-reproductive (post-generative) stage flowering ceases, root system degrades, individual senescences and dies.

Study area and field methods

This paper focuses on eight pasque flower populations across the Hämeenlinna vicinity and measures additional environmental conditions (Fig. 1). The populations were Ahvenisto, Hevospierettämänmäki, Kellotapuli, Kilparisti, Lakkola, Leutostentie, Ruskeanmullanharju and Tunturivuori. *P. patens* inhabits seven sites being absent only from Leutostentie. The hybrid *P. patens* × *vernalis* is found in five sites, Hevospierettämänmäki, Kellotapuli, Kilparisti, Lakkola and Leutostentie. These populations has been investigated in the Hämeenlinna region by Dr Heli Jutila for three decades. Indeed, she followed different populations across the region where she counted the number of individuals, the number of flowering individuals and the number of flowers. More detailed studies started in April 2017 by marking, numbering and counting the individuals in order to survey the fitness of several populations and individuals in them. In 2017, 79 *P. patens* and 38 *P. patens* × *vernalis* individuals were included in studies. In 2019 the same populations were studied.

Flowering and non-flowering *Pulsatilla patens* and hybrid individuals and their flowers were counted and marked with individually identifiable wooden sticks between end of April until mid-May. Some measurements and picking of seeds occurred later in season. Individual marking is the only way to follow-up the changes in individuals and to define the exact population size and estimate different demographic parameters (such as survival rate, flowering, etc.). The individuals representing genets were mapped with GPS once again and the data were delivered as previously to the national vulnerable species database.

In order to explore the relationship between plant and their environments, we had two field campaigns in 2017 and in 2019. In 2017, we scored the flowering individuals and the light availabilities variables. In 2019, we scored the flowering individuals and measured edaphic conditions. A permit to diverge from the protected species protection

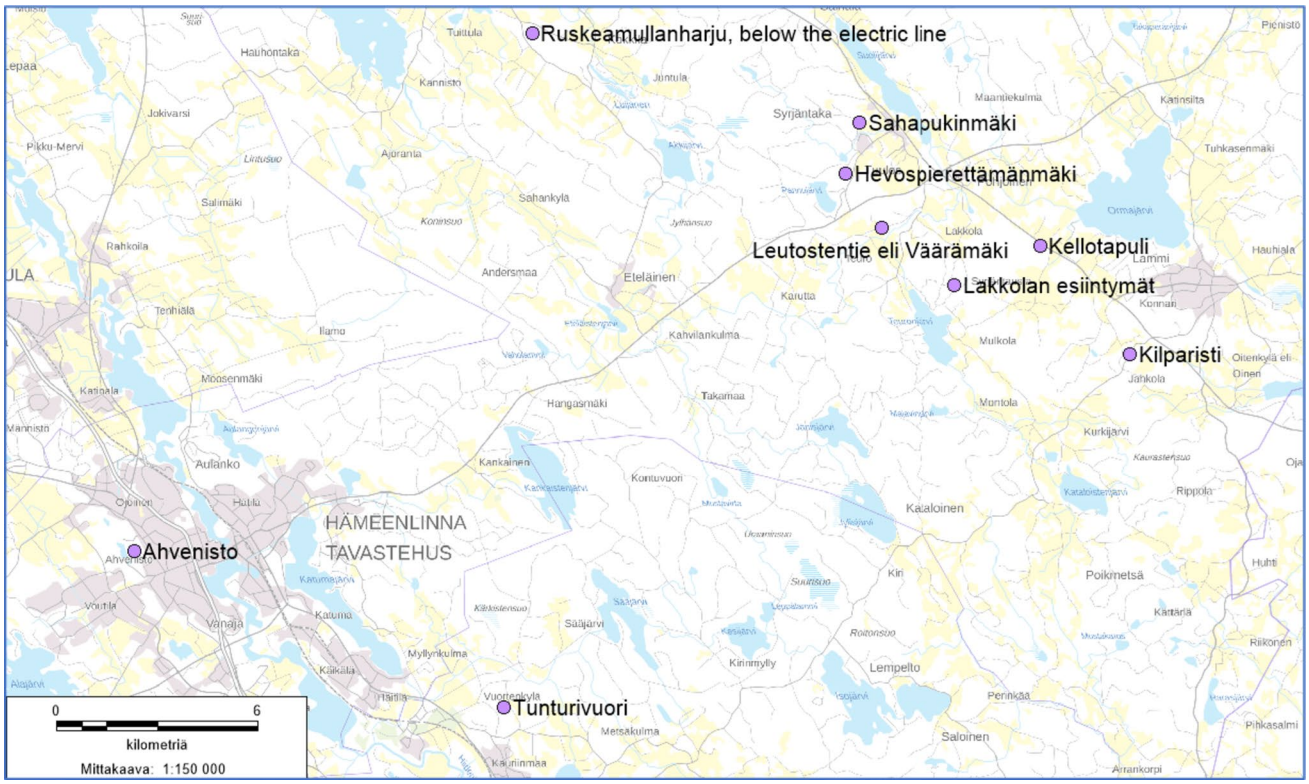


Fig. 1 A map of our eight *Pulsatilla* locations across the Hämeenlinna area (Finland)

regulations and a permit to diverge from the protection regulations of nature conservation areas were applied and granted for Heli Jutila and Katinka Vloon on 11.4.2017 and granted for Heli Jutila and Bastien Parisy on 8.5.2019 by the Centre for Economic Development, Transport and the Environment. Most of the collected seeds were returned to the source populations soon after measurements. These sown seeds were observed for half (collections of 2019) and two years (collections of 2017), but no germination of *P. patens* or hybrid seeds were detected. Some seeds were delivered to the botanical museum of Helsinki.

Plant measurements

In each population all individuals were recorded and classified into the following easily recognisable life cycle stages: generative (flowering) adult, vegetative adult and juvenile. A plant having one or two small leaves with a diameter less than 2.5 cm was recorded as a juvenile (age about 1 year). Following Kalliovirta et al. (2006), leaf rosettes closer than 10 cm from each other were interpreted as belonging to the same individual. In tussock-forming plants the identification of individual is somewhat difficult and this itself can be regarded a source of error when interpreting the results. Rosettes further apart from each other were treated as separate individuals if no other indication existed. The number of flower-stalks per flowering plant was counted each year from 2017 to 2019. Juveniles were recorded in May and in June, thus these were individuals that had germinated during the previous growing season and had survived one winter.

The diameter of each plant individual was also measured. It does not only indicate the age of the individual, which is the case in seedlings and very young plants, but it tells about the success of the individual. According to our observations the size of the individual does not correlate with the age, but the poor conditions can cause the plant to get smaller and better conditions allow it to thrive.

Flowering traits

Within the eight studied sites, the number of flowering individuals and the number of flowers per individuals were counted.

For flower trait measurements, populations were visited several times to survey the flowering evolution. The petal length and width (cm) were measured in 2017 for 15 flowering *P. patens* and 15 flowering *P. patens* × *vernalis* individuals at the point when flowers blossomed completely. If subpopulations were present, stratified systematic sampling was applied according to the total number of individuals in the subpopulations. For populations with less than 15 flowering individuals, also non-flowering individuals were

selected to attain a total of 15 individuals. If the total number of individuals in a population was lower than 15, all individuals were selected.

According to Krizek & Anderson 2013 (and references therein) flower size is a key ecological trait as it influences mating system evolution and reproductive success. Flower measurement was conducted with a flexible paper scale to avoid damaging the petals. For each flowering individual, three flowers were selected randomly and from them 2 or 3 of the outer petals were selected. From each petal, the length and the width at the widest point were measured. If individuals had less than three flowers, all flowers were selected. From these measurements, the average petal length and width were calculated for each individual.

Seed mass, seed length and pappus

Seed variables were studied in three localities. Seeds were collected from 45 plant individuals, of which 34 were *P. patens* and 11 hybrids. A minimum of ten ripe ten seeds per individual were extracted to be weighed, making in total 452 seeds. In Hevospierettämänmäki seeds had been collected from nine *P. patens* individuals and 11 hybrid individuals. From the Ruskeamullanharju population seeds of 15 individuals were measured. From the Tunturivuori population seeds of nine individuals were measured. Seed and pappus were weighed in one entity and separately. The length measurements were similarly performed for both seed and pappus entity and separately. Because pappus could be sloping or curved the longest length with curves was measured, in addition to the short-cut measurement. The longest pappus length was added to the seed length to gain the total length of dispersule.

Environmental variable measurements

For each selected individual, several environmental factors were studied. These factors included the elevation, slope steepness, slope aspect, tree basal area, forest canopy density, general, direct and diffuse light availability, soil moisture and soil temperature of the location (Table 1).

The circular slope aspect was transformed into a linear variable ranging from 0 to 1 (Topographic Solar-Radiation Index (trasp) using the following formula by Roberts and Cooper (1989):

$$trasp = \frac{1 - \cos\left(\left(\frac{\pi}{180}\right)(\alpha - 30)\right)}{2},$$

where α is the circular aspect in degrees. In this newly created linear variable, a value of 0 represents a north-northeast facing slope (generally the wettest and coldest slopes), whereas a value of 1 represents a south-southwest facing slope (generally the warmest and driest). Because the variable 'slope aspect' does not exist in the absence of a slope and therefore does not have any meaning in itself, a new variable 'Slope Radiation Index' (SRI) was created by multiplying slope aspect and slope steepness. A high SRI value indicates the approximation of a perpendicular incidence to direct beam irradiance by the sun. Namely, when a slope is both steep and facing south, the longer it will receive direct beam irradiance with such an angle towards the sun that it will receive maximum PAR input (DeWalle & Rango 2008).

The light availability of studied individuals in 2017 was obtained from pictures taken with a Nikon D3200 and a Sigma 4.5 mm F2.8 EX DC HSM Circular Fisheye lens (Sigma). The camera was placed horizontally 30 cm above the individual. Camera settings regarding ISO, shutter speed and aperture were put to automatic, in order to get pictures with minor brightness differences. These pictures were taken before the flowering period, in April for logistical reasons. These pictures were analyzed with the software "Gap Light Analyzer" (Frazer et al. 1999). This software is able to convert pixels from objects into black pixels and to convert pixels from sky into white pixels. After that, it calculates the percentage of black pixels that correspond to the canopy (Frazer et al. 1999). From this canopy density, the percentage of diffuse light and indirect light transmission are calculated.

To investigate whether differences in photosynthetically active radiation (PAR) influence the fitness of *P. patens*, 3 Hoboware loggers with sensors were placed in the population of Ruskeanmullanharju in year 2017. This site was

Table 1 All the variables measured for this study across the years

Year	Number of flowers	Petal length and width	Petal area	Diameter of *P. patens* or hybrid	Seed characteristics	Canopy and light availability; tree basal area	Soil moisture and temperature	Atmospheric temperature and precipitation	Slope and aspect
2017	X	X	X	X*	X	X		X	X
2018	X								
2019	X	X		X*	X		X	X	

*Also height measurements for some individuals

selected since the South facing slope was steep and located under electric line. Sensor one was placed at a flat site, in the middle elevation of the hill, and covered by forest, where pasqueflowers had grown previously, but currently become extinct from the site. Sensors two and three were placed to a South facing, steep slope under an electric powerline, where two *P. patens* populations existed. Sensor two was placed in the lower subpopulation, where a considerable number of seedlings was found, and sensor three was placed in the higher elevation subpopulation. Each sensor was placed on a 50-centimetre high tripod, level to the horizon. PAR values were recorded during the beginning of the growing season from April 21 until May 31 (2017), with a sampling interval of one minute and a logging interval of fifteen minutes. From these values, the total amount of PAR per site per day was calculated. As one measurement per site type does not allow the data to be generalized to the entire population, these measurements were used as exploratory evidence to gain additional information about possible differences in PAR within a population and the relationship with fitness factors. To relate the PAR differences to fitness characteristics, 15 *P. patens* individuals from the higher elevation subpopulation and 15 *P. patens* individuals from the lower subpopulation were compared in terms of petal length, width and area.

Measurements of soil properties were performed in 2019 with a Watchdog 2450 mini station (Spectrum Technologies) when plants began flowering (from end of April to early May) under similar meteorological conditions, and during the same time period (between 9am–12 am). This mini station includes two sensors able to measure the Volumetric Water Content (VWC in %) of the soil, but also its temperature. Sensors were planted around 15 cm from plants to not impact the root branching.

These individual measurements were completed with atmospheric observation (during the day of plant measurement and during the month of flowering period, year 2019) obtained from a meteorological station (Finnish Meteorological Institute). From three stations one was selected according to the shortest distance to studied sites. The monthly mean temperature (corresponding to the 30 days before the visiting day of a flowering population) and the daily minimum and maximum temperatures were used as environmental parameters. Data from Hämeenlinna Katinen station were associated with two sites (Ahvenisto and Tunturivuori), Hattula Lepaa station with one site (Ruskeamullanharju), and Hämeenlinna Lammi Pappila station with four sites (Kilparisti, Kellotapuli, Hevospierettämänmäki and Leutostentie). Only one meteorological station collected precipitation data, thus, it was impossible to get exact precipitation data for all sites. To assess the differences in precipitation between sites, precipitation gauges were placed across our sites, with a thin layer of oil to limit evaporation. All the gauges were placed in the same day at the end of snow melting period (in mid-April). The site-based precipitation measurers seemed to have similar results as the data given by the meteorological station.

Statistical analyses

Different environmental variables were collected in 2017 and 2019 and analysed independently across our sites.

Analyses in 2017

Analyses were performed separately for *P. patens* and *P. patens* × *vernalis.* In total, 79 *P. patens* and 38 *P. patens* × *vernalis* individuals were included in the analyses. The analyses regarding petal length, width and area included 54 *P. patens* and 27 *P. patens* × *vernalis* individuals. Pearson correlations were used to test correlations between the environmental and fitness variables. If the variables were not normally distributed (Kolmogorov-Smirnov with $p > 0.05$), Spearman correlations were used. Subsequently, hierarchical multiple linear regression analyses were performed to evaluate the additive predictability of the independent variables on the flower traits of both species. It was expected that light availability, both direct and diffuse, would have the strongest influence on the fitness variables. The other variables were alternately added to see if the regression model would improve significantly. These variables were slope steepness and the Slope Radiation Index. Canopy density, direct and diffuse light availability and basal area could not be used in the same regression given their high intercorrelations. To reveal any differences in environmental and fitness variables between *P. patens* and *P. patens* × *vernalis*, independent samples t-tests were conducted. If the variables were not normally distributed for either species (Kolmogorov-Smirnov with $p > 0.05$), Mann-Whitney U tests were performed. For the exploratory analysis concerning the differences in PAR levels in different sites of the population of Ruskeanmullanharju, Kruskal-Wallis and Mann-Whitney U tests were performed. The data were analysed with the software SPSS Statistics (IBM, Version 20).

Analysis in 2019

From all the environmental variables measured in 2017 and 2019, a Principal Component Analysis (PCA) were used to reduce the number of variables. Bioclimatic variables are projected on two axes that explained 46.7% of the total variance. From the components with a clear relation to particular axes, we selected the variables that contributed the most to the principal component. Out of our 15 variables available, only 7 were kept in order to explore environmental factors that explained the number of flowers

and the flowering probability. The flowering probability is here represented as a binary value (0 as non-flowering and 1 as flowering). If the individual flowered, it was marked as 1 and if not, it was marked as 0. This aims to assess whether the mechanism of flowering can be modulated by external environmental factors. Thus, the flowering probability also takes into account the energy spent in 2018 on the flowering of 2019.

To predict flowering probability, we used the diameter of the rosette (cm), the mean of monthly air temperature, monthly precipitation, soil moisture, soil temperature, and canopy openness.

To test flowering probability, we built a piecewise structural equation model (SEM, Lefcheck 2015) in order to unite the suite of variables in a single network and explore causal relationships among variables described in a series of equations. However, considering the relatively small size of our data, a flexible method developed by Lefcheck in 2015 was chosen. We built two piecewise structural equation models (SEM, Lefcheck 2015) based on the number of flowers and based on the flowering probability observed in 2019 across our study sites. In the SEM, we fitted separate general linear models (GLM) with the number of flower as a response variable (with Poisson distribution family) and the environmental, biological factors as explanatory variables. Based on our a priori, we created interactions between specific biological and environmental variables. For instance, a relationship between the monthly mean temperature measured in 2019 was considered as independent of the breadth of the individual measured in 2019. We also fitted separate general linear model (GLM) with the flowering event (Binomial distribution family) with the same explanatory variables. All the statistical analysis and the creation of the SEM analysis were performed on R (software version; Lefcheck 2015).

Results

Number of flowers

Annual comparisons from 2017–2019 show that the number of flowers decreased significantly each year for *P. patens* (Kruskal–Wallis: p-value < 0.05; Pairwise Wilcoxon test: p-value < 0.05, see Fig. 2), while for the hybrid the number of flowers decreased significantly after 2018 (Pairwise Wilcoxon test: p-value < 0.05; Fig. 2).

For *P. patens*, the mean number of flowers varied significantly across sites (Kruskal–Wallis: chi-squared = 15.293, $df = 6$, p-value < 0.05). The decrease has been observed in almost all the sites (Kruskal–Wallis, p-value < 0.05). Only in Kilparisti and Kellotapuli, the decrease was not significant (online Appendix 1).

For the hybrid *P. patens* × *vernalis*, the mean number of flowers did not vary significantly across sites (Kruskal–Wallis, p-value < 0.05).

Spatially, since 2017 the number of sites where *P. patens* produced a lot of flowers (> 7) reduced during the years. Indeed, eastern sites and sites close to the city of Hämeenlinna show a reduction in number of flowers while the northern and southern sites maintain a high production of flowers.

A hybrid individual produced on average more flowers than a *P. patens* individual (Wilcox test, p-value = 0.0504). In addition, the share of flowering individuals was higher in hybrids than in pure pasqueflowers.

Comparison of *Pulsatilla patens* and hybrid habitat preferences and flowering characteristics

Generally, *P. patens* and the hybrid inhabit somewhat open sites (at least 30% of openness), but the canopy openness varied significantly across sites (Kruskal–Wallis: $df = 7$, p-value < 0.001; $df = 4$, p-value < 0.001). In Tunturivuori *P. patens* occupies the habitat with the largest variance of site openness. Mann-Whitney U tests showed that the canopy was denser above *P. patens* than above *P. patens* × *vernalis* ($p = 0.031$) and that *P. patens* received a lower amount of diffuse light ($p = 0.042$). *P. patens* occurred on steeper slopes ($p = 0.001$), and on slopes with an aspect closer to the optimal trasp value (south-southeast) ($p = 0.018$) and somewhat oddly at lower elevations ($p = 0.014$) than *P. patens* × *vernalis*. No significant differences were found for direct light transmission ($p = 0.375$) and tree basal area ($p = 0.433$) between the species.

Independent samples t-tests showed a smaller petal width (p < 0.001) for *P. patens* than for *P. patens* × *vernalis*. Wilcoxon tests showed no significant difference in petal length ($p = 0.57$) between the species. Petal dimensions of *P. patens* × *vernalis* showed no significant correlation with any of the predictors, while there was correlation for *P. patens*. Canopy openness was negatively correlated with petals length and width for *P. patens*. (Pearson: $p < 0.05$). However, there were no relationships between environmental conditions and petal length and petal width for the both species together (GLM: p-value > 0.05, link family = poisson). The average length of petals in *P. patens* is not significantly different across sites (TukeyHSD: p-value > 0.05), but the variance is significantly different across sites (Levene: Test Statistic = 1.2708, p-value = 0.264). For *P. patens* × *vernalis*, the same pattern was observed for the average width of petal across sites (Kruskal–Wallis: chi-squared = 8.2482, $df = 5$, p-value = 0.1431) and for the variances (Levene: Test Statistic = 0.052571, p-value = 0.8194).

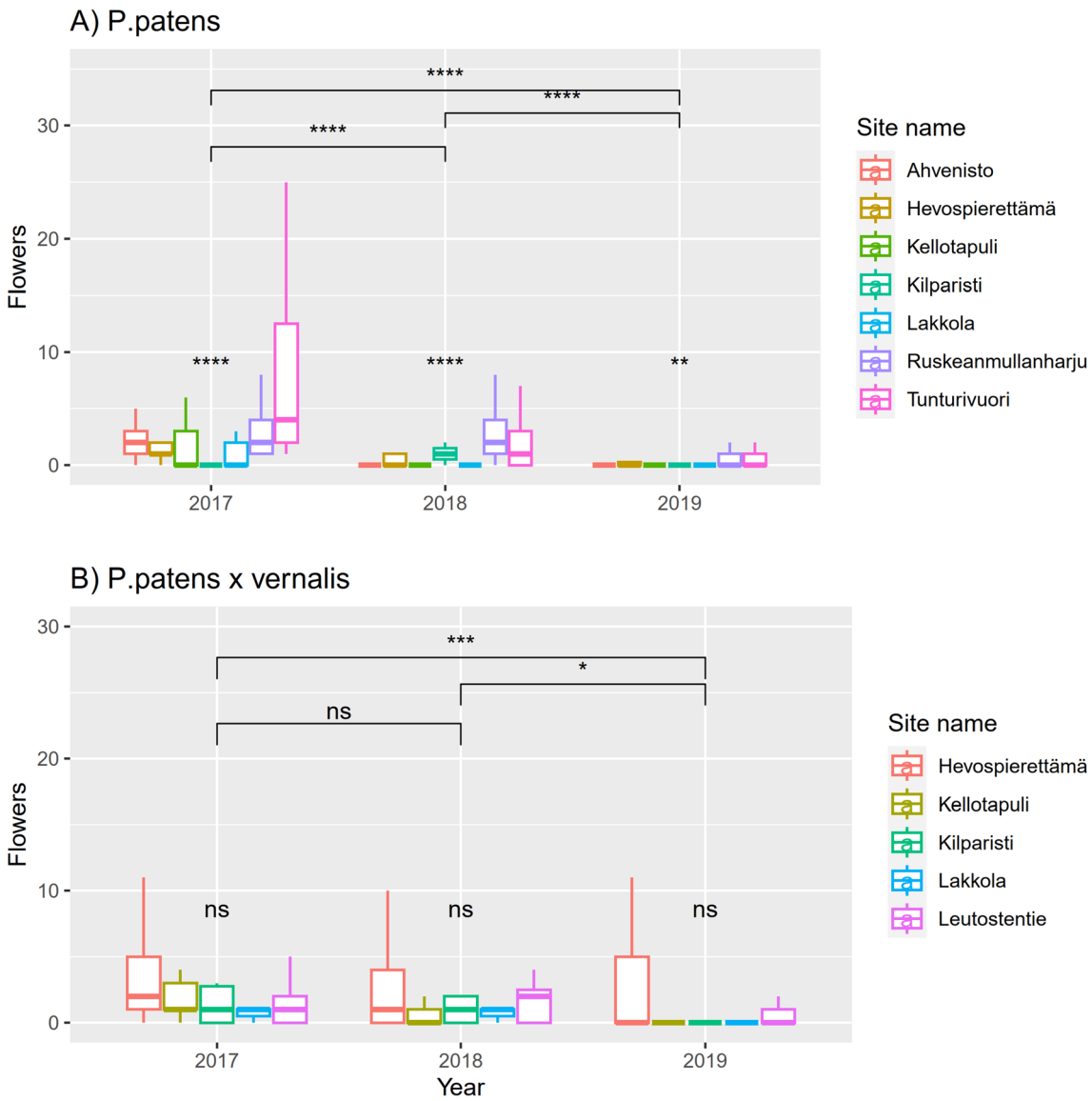


Fig. 2 Yearly production flowers for *Pulsatilla patens* (**A**) and *Pulsatilla patens* × *vernalis* (**B**) across population. The bracket indicates the level of significancy between years (Paired Wilcoxon test, p-value < 0.05) and the significance levels at the bottom part represents the differences between populations for each year. (Kruskal–Wallis test, p-value < 0.05)

Effect of photosynthetically active radiation on number of flowers and petal area

A Kruskal-Wallis test revealed a significant difference in PAR levels across the three different sites in Ruskeamullanharju. The site of the extinct individual had a considerably lower median score (Mdn = 16,407 µE, $n = 41$) than the lower elevation subpopulations (Mdn = 26,190 µE, $n = 41$) and higher elevation (Mdn = 26,975 µE, $n = 41$), $\chi 2$ (2, $n = 123$) = 27.36, $p < 0.001$. The lower and higher elevation subpopulations did not differ significantly from each other (Mann-Whitney U, $U = 789.00$, $z = -0.48$, $p = 0.633$, $r = 0.05$).

Independent samples t-tests revealed that the *P. patens* individuals in the higher and lower elevation subpopulations differed significantly in petal length, width and area. The lower elevation petals were shorter ($M = 3.76$, $SD = 0.25$), narrower ($M = 1.48$, $SD = 0.14$) and smaller in area ($M = 4.17$, $SD = 0.60$) than the individuals uphill ($M = 4.28$, $SD = 0.45$, t (22.30) = − 3.84, $p = 0.001$; $M = 1.74$, $SD = 0.20$, t (26) = − 3.87, $p = 0.001$; $M = 5.16$, $SD = 1.63$, t (26) = − 2.18, $p = 0.043$, respectively). The lower elevation population had more seedlings in the area than the higher elevation population.

SEM-model on number of flowers and flowering probability for *P. patens* and hybrid

The a priori model, which corresponds to a list of GLM, were implemented into a piecewise SEM. 69% of the total variation of the number of flowers in *P. patens* is explained by the plant's diameter, the mean of monthly air temperature, the soil moisture and canopy openness (p-value < 0.05; Fig. 3). The relationships between the monthly air temperature and the soil moisture and the number of flowers are negatively correlated and the relationship is stronger than the canopy openness or the diameter of the rosette. Thus, yearly conditions seem to have a stronger impact on the number of flowers than the conditions of the plant or light availability.

For the hybrid *P. patens* × *vernalis*, 94% of the total variation of the number of flowers is explained by the plant's diameter, the soil moisture, canopy openness, precipitation and by the flowering probability from the previous year (p-value < 0.05; Fig. 3). The edaphic conditions as such as precipitation have an effect on the number of flowers produced for the hybrid. The flowering of the

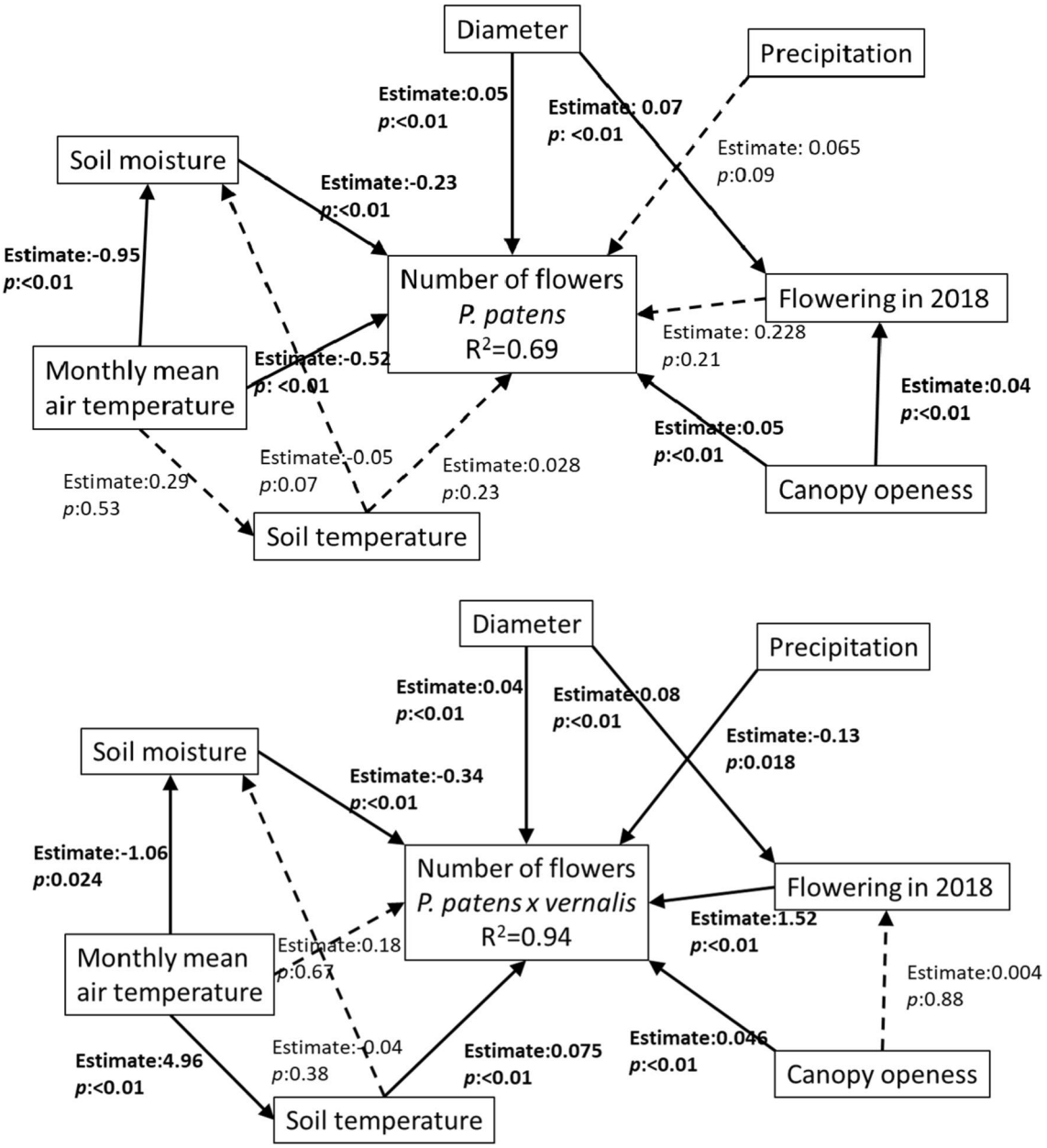


Fig. 3 The piecewise SEM-model that represents environmental variables effecting the number of flowers in *P. patens* (top) and hybrid (bottom). Bold arrows show significant relationships between two variables. Dotted lines represent non-significant relationships

previous year has the strongest relationship with the number of flowers produced.

For *P. patens*, the piecewise SEM approach showed that 35% of the total variation of the flowering probability is explained by the plant's diameter, the mean of monthly air temperature, the soil moisture and the canopy openness (p-value < 0.05; Fig. 4). The monthly temperature as such as soil moisture has the strongest relationship with the flowering probability. However, it seems that the current variables explained only a small part of the flowering probability.

In the hybrid, 56% of the flowering probability is explained by the soil moisture and by inherent factors such as the diameter and the flowering in 2018 (Fig. 4). For the hybrid, the conditions of the individual are main drivers that shape the flowering state. 44% of the variation are not explained by the current variables measured in this study.

The negative relationship between soil moisture and flowering probability is stronger for the hybrid than for *P. patens*. The flowering probability is more affected by environmental conditions for *P. patens* where the flowering probability of

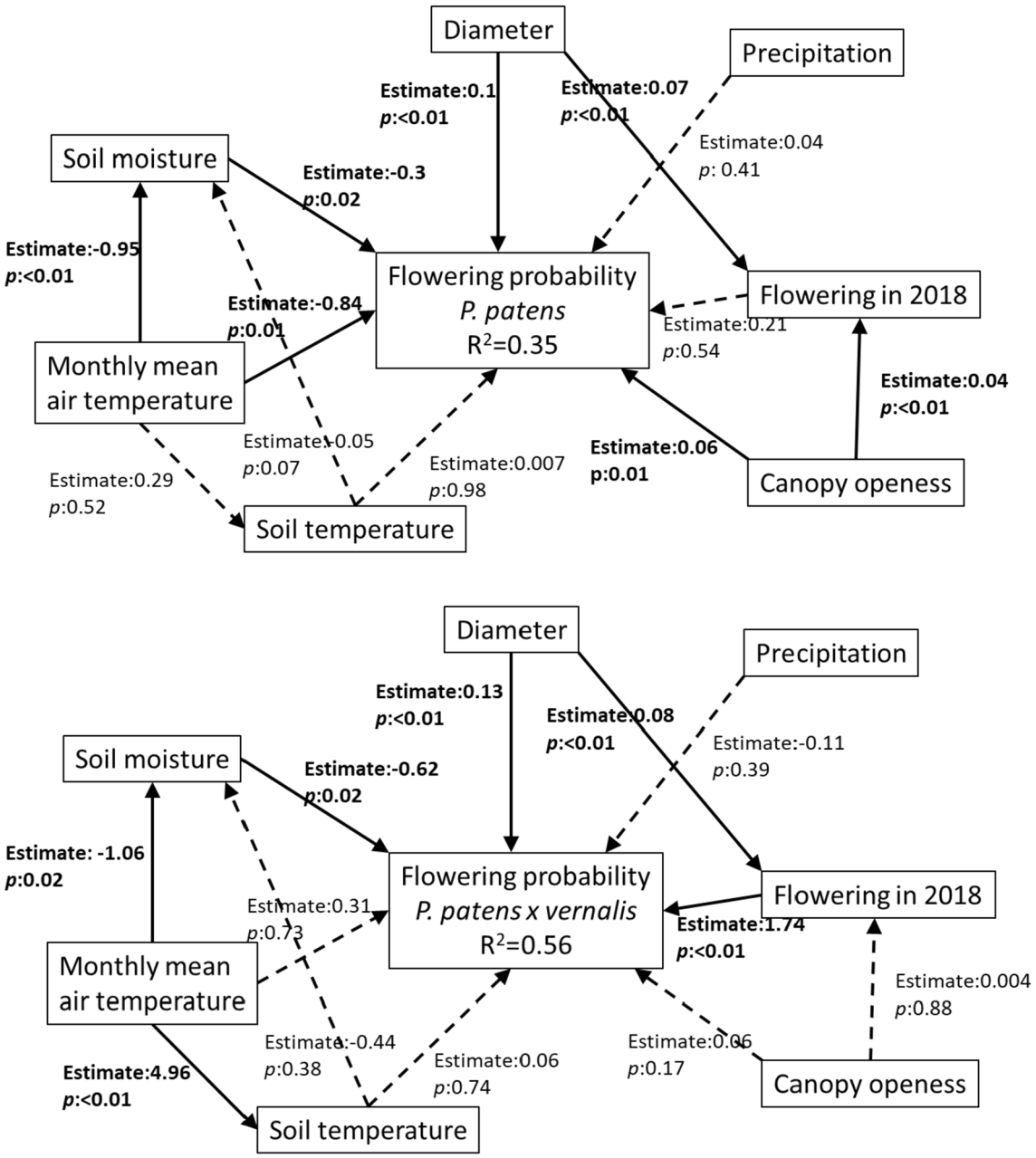


Fig. 4 The piecewise SEM-model that represents environmental variables affecting the flowering probability in *P. patens* (top) and *P. patens* × *vernalis* (bottom) in 2019

2019 and 2018 shows a relationship with the monthly mean temperature and canopy openness. Whereas the flowering probability for the hybrid seems to be mainly explained by inherent factors.

Seed variables

The heaviest seeds without pappus of *P. patens* were found in Tunturivuori, but variation was greater within the populations than between them and no significant differences could be detected (Fig. 5). The mean seed mass with pappus was 17, 6 mg and without pappus 09, 0 mg. Seeds of *P. patens* were heavier than the ones of hybrid. (Wilcoxon test, p-value = < 0.05; online Appendix 2).

Pure seed mass and the length (both without pappus) correlated clearly in the *P. patens* data ($R^2 = 0{,}48$; Fig. 6), but seed mass with pappus and seed length with pappus correlated only in Hevospierettämä's *P. patens* population. One possible reason might be that some individuals defined as pure pasqueflowers had been pollinated by hybrids producing small and possibly badly germinating seeds since the hybrid is more abundant in Hevospierettämä than *P. patens*. Indeed, for the hybrids seed mass and seed length correlated even more clearly than in *P. patens* ($R^2 = 0{,}73$), since unfertilized seeds remain long and thin. The average seed length (3, 9 mm; with pappus 30, 9 mm) varied little between populations. The biggest variation inside population was observed in Hevospierettämä.

Flower stem length correlated positively with seed plus pappus mass in *P. patens* (Fig. 7) in the 2017 data and furthermore, in populations of Tunturivuori and Ruskeamullanharju, but not in Hevospierettämä. The longer the individual's flower stem, the bigger the seeds. However, this correlation was no longer evident in 2019 data. We tested also whether bigger flowers produced bigger seeds. With the small data we could get only a slight positive correlation (0,0996). There was a lot of variation in the data (Fig. 7).

When studying the relationships between the seed morphologic traits and the environmental conditions, we did not find any correlation nor relationships between pure seed mass and seed length with the canopy openness (Pearson correlation test, p-value = 0.26; General Additive model, p-value > 0.05) for the two species. In this case, we tested only the canopy openness as the soil moisture or soil temperature (measured only in spring) were not considered as meaningful variables here. Thus, the environmental variables at our disposal does not explain the variation of the seed size here.

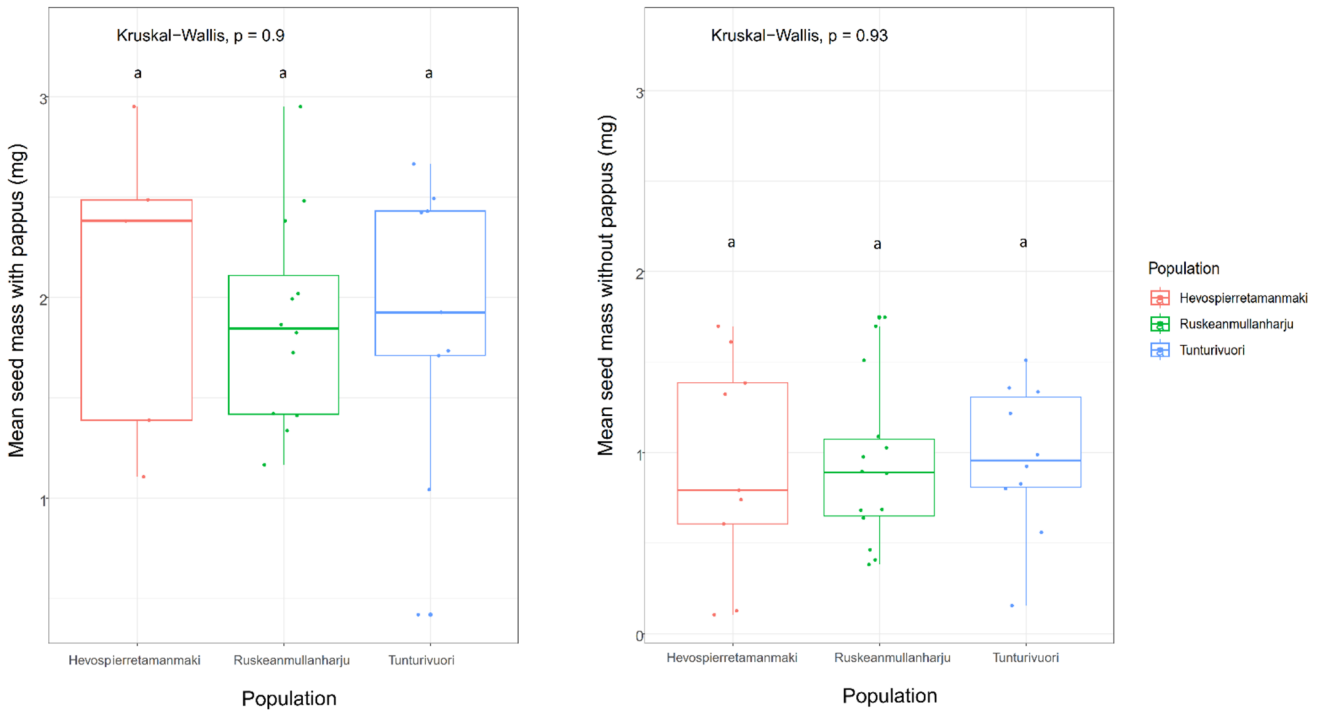


Fig. 5 Seed mass with and without pappus in three *P. patens* populations. Letters a within the figures indicates that the differences between plots are non-significant

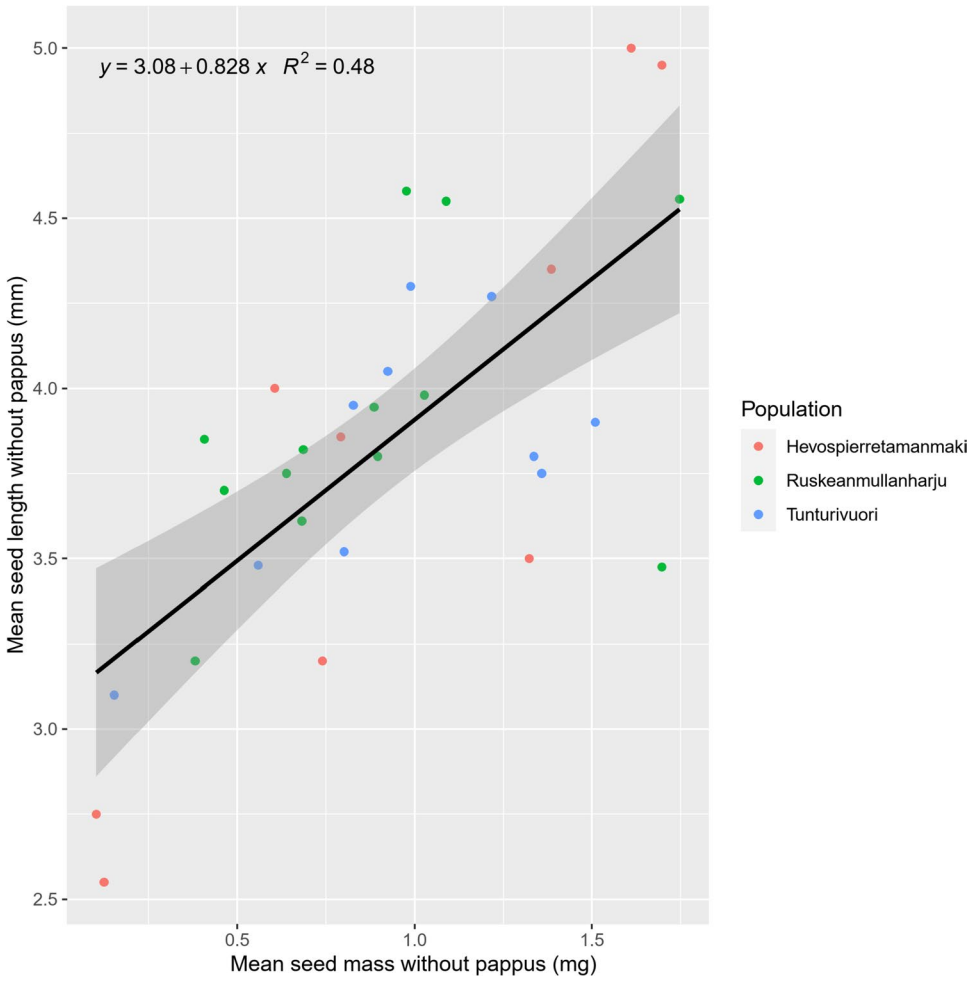


Fig. 6 Seed mass and length correlate in *P. patens* populations. A minimum of 10 seeds were collected for each individual. Dots are means of seed mass and length measurements

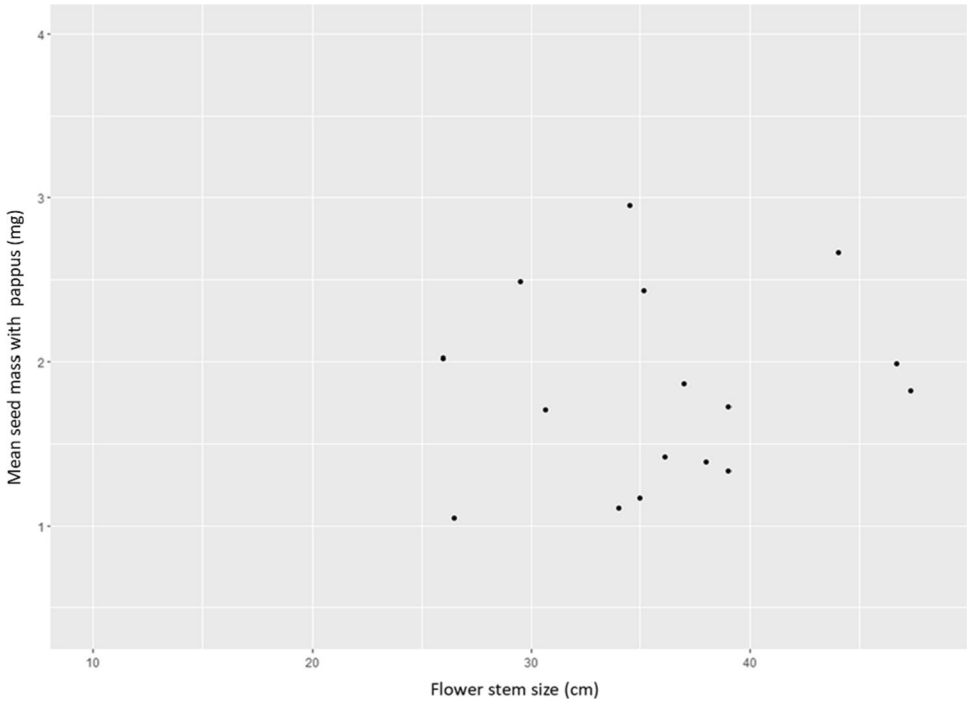


Fig. 7 Mean seed mass and flower stem length correlated in *P. patens*

Discussion

Based on previous studies, it was hypothesized that light availability influences the fitness-related traits of both *P. patens* and *P. patens* × *vernalis* (Juśkievicz-Swaczyna & Grzybowski 2013, Kalliovirta et al. 2006). Canopy openness predicted number of flowers for both *P. patens* and *P. patens* × *vernalis*. For the other environmental variables that were measured, we found that the number of flowers benefitted in the hybrid and in *P. patens* from drier and cooler conditions.

Hybrids have wider and thus, larger petals than *P. patens* (5.91 cm^2 versus 4.97 cm^2) and the ratio of flowering to non-flowering individuals is higher for *P. patens* × *vernalis* (2.6:1) than for *P. patens* (1.5:1). It seems that the species have different flowering strategies. *P. patens* × *vernalis* flowers more abundantly and invests more energy in flowering than *P. patens*. However, the huge investment in flowering of the hybrid is counterintuitive since the seeds produced from flowers are thin, likely empty and unlikely able to germinate.

P. patens is less successful in flowering than *P. patens* × *vernalis*. The higher proportion of flowering individuals for *P. patens* × *vernalis* compared to *P. patens* and the fact that hybrid produces more flowers per individual, suggests that the hybrid is more efficient in spending energy on flowering. We suppose that the hybrid might represent a reproductive competitor for *P. patens* particularly in future with climate change warming up winter temperatures and increasing precipitation (that could be a reason of its higher abundance on common sites). Nevertheless, niche differences that promote coexistence between these species could only be described by combinations of various traits, corresponding to differentiation between species in multiple ecological dimensions (Kraft et al. 2015). Then, this hypothesis based only on the number of flowers and number of flowering individuals must be supplemented by an analysis of other flowering traits across population such as pollen and nectar quantity (Juillet & Scopece 2010; Weber & Strauss 2016).

Interestingly, the size of the plant individual proved to be important for both *P. patens* and hybrids in flowering success. For *P. patens* seed size also had a slight correlation with the size of the mother plant and even the length of the flower stem. It is likely that bigger individuals reproduce better than smaller ones. However, a long-term study would be needed to reveal all the aspects related to seed production.

Pasqueflowers are quite rare, long-living perennials, persisting in sites, which have often changed from suitable to less suitable to them during the years. The old individuals can persist in shaded conditions, which seem not to be suitable for their flowering and seed germination. Indeed, many of the sites are managed by removing bushes and opening canopy now and then to avoid the slow death of the pasqueflower individuals. The photosynthetically active radiation (PAR) levels at the site where a *P. patens* individual had previously become extinct in Ruskeanmullanharju were, indeed, considerably lower than in those more successful parts of the population. This suggests that low PAR levels –in combination with other unfavourable environmental factors—can be disadvantageous for the species directly or indirectly, e.g., in the form of lack of pollinators (Groom 1998), and might eventually lead to extinction.

Measurements of local environmental variables showed that the habitat preferences of *P. patens* and the hybrid *P. patens* × *vernalis* are overlapping to some extent, but the hybrid is found in somewhat more open, drier and colder soils than *P. patens*. *P. patens* having a weak preference for more humid, somewhat denser canopy, nutrient- and herb-richer sites than the hybrid fit with the literature (Uotila 1969, 1980; Pilt & Kukk 2002). Since we know that *P. vernalis* inhabits drier and poorer soils than *P. patens* (Uotila 1980), it is not surprising that this trait of the hybrid is intermediate between the parents' characters.

It is important to note that the soil condition measurements done in this study represent only a specific time point. Influence of winter conditions and snow melting on soil property dynamics even at longer temporal scale could be interesting topic to study and link it to the species' persistence and flowering success.

Indeed, it seems that the flower abundance of some arctic and alpine species are influenced by environmental conditions just prior to flowering (Cooper et al. 2011; Panchen 2016). In the present study, it is illustrated by the monthly mean air temperature of the thirty days prior to flowering which is important factor in influencing the number of flowers and flowering probability. In *P. patens* interestingly the lower temperature before flowering would indicate more flowers while for hybrid the correlation is positive. The influence of temperature of the flowering year should be studied further to find out more. In previous studies also the role of the previous growing season and the numbers of flower buds produced in previous autumn play an important role in flowering success. Our studies provide evidence that the reproduction of *P. patens* also depends on the soil moisture that probably results from the habitat type occupied (soil type, moss layer, etc.).

Decades of studies have revealed that flowering is initiated in response to both environmental and inherent factors (Amasino & Michaels 2010). These "endogenous pathways" function independently from environmental conditions and depend on the developmental state of the plant (McDaniel and Hsu 1976). It has been noticed in the study area that

after several very good flowering years, the regenerative pasqueflowers take a break from flowering (or produce only a small number of flowers, Jutila 2019). The relative contributions of these endogenous and environmental processes to the "flowering decision" vary among, and even within, species (Amasino & Michaels 2010). Then, to increase the understanding of flowering probability on our two species, other factors not measured in the present study such as soil chemistry and its dynamic, inherent factors such as genetic diversity within populations could be crucial.

Physiological mechanisms behind flower size-depend partially on plant's developmental stages at the time of the environmental stress (Wahid et al. 2007; Scaven & Rafferty 2013). It is interesting to notice that in our case, among the sites there was only a slight variation in the petal size and that environmental factors did not influence petal morphology. This is not unexpected since petal size like seed size is often quite strictly genetically defined character. From the fact that populations in our study are relatively small, our results might suggest that small flower size variation could reflect a low level of genetic diversity across populations. Information about genetic variation and partitioning of genetic variation across populations showed that small populations are prone to extinctions (Hedrick and Hurt 2012; Betz et al. 2013). However, further work to measure the genetic diversity in our study populations and better understand if there is a link between genetic diversity and flower morphology may help to develop future conservation strategies for *P. patens*.

Conclusions

The overall historical trend of population decline in *P. patens* is a conservation concern not only for populations in Finland, but also across Europe. In this research, we wanted to understand the environmental and intrinsic factors that affect the fitness-related traits of the species and in turn give clues to the viability of the few remaining *P. patens* populations in Finland. We found that the number of flowers decreased between 2017–2019 and that a large portion of the variance in number of flowers and probability of flowering was explained by intrinsic (larger plants produced more flowers) and environmental factors. In general, cooler and drier conditions and greater canopy openness increased the number of flowers and flowering probability. However, petal and seed size were not affected by environmental variables. A potential concern for the future conservation of *P. patens* is the presence of the *P. patens* × *vernalis* hybrid. Flowering probability in the hybrid was explained by similar environmental factors as *P. patens*, indicating the possibility for competition between them. However, the microhabitat that they were found in differed by slope steepness and light characteristics. Currently, we do not regard the hybrid as an imminent threat since the sites where hybrid and *P. patens* coexist are few and the hybrid is restricted to a fairly limited area. However, we recommend continued monitoring of population trends to assess changes in the relationship between *P. patens* and the hybrid over time.

Current management activities in the pasqueflower sites include tree thinning, small clear cuttings, removal of the ground layer and other competing plant species and the sowing of *P. patens* seeds. The thinning of forests is supported by the results of our research, as enhanced light conditions improved flowering. Thinning also decreases competition from other species and should help improve the viability of the *P. patens* populations. However, some caution is recommended as there may be a threshold in which a forest becomes too open, and the large amount of direct light could be above the optimum for this species, resulting in photoinhibition or periodically dry conditions unfavourable for seed germination.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11258-024-01400-1.

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Author contributions HJ, BP and JL: conceived the idea and designed the study. HJ and BP: collected the data; HJ and JL: supervised and BP: performed the statistical analysis; HJ: wrote the first draft of the manuscript with specific contributions from BP and JL, and all authors contributed substantially to the revisions.

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Data availability The data that support the findings (raw measurements) of this study are openly available in "figshare" at https://doi.org/10.6084/m9.figshare.25162811

Declarations

Conflict of interest The authors declare no conflict of interest.

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