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THE EFFECT OF UNDERGROUND DRAINAGE ON PEAT MEADOWS AND INACTIVATION OF THE DRAINAGE IN AN ATTEMPT TO RESTORE THESE MEADOWS, WHICH FAILED AS IT REDUCED THE ABILITY OF SOILS TO RETAIN WATER

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ABSTRACT

Drainage is often used to increase agriculture production, but it has adverse effects on biodiversity and water retention. Here, the effect of subsurface pipe drainage on peat meadows near Senotín (Czechia), which were drained from the mid-1980s to 1990s, was studied. Attempts were made to restore the peat meadows by damming drainage pipes using clay-filled trenches in 1996. In this case study, the effect on the depth of the water table, soil water retention, infiltration and soil temperature were recorded. Measurements of the original peat meadow (undrained site), drained meadow (drained site) and restored meadow (restored site) before restoration and two decades after restoration were recorded. The water table in undrained areas was higher than at drained and restored sites, indicating that drainage had lasting effect on drained and restored sites. Infiltration was lowest at the undrained site, greater at the drained site, and highest at the restored sites. Field water capacity was lowest at the restored site, greater at the drained site and highest at the undrained site. Soil water content at maximum saturation was lowest at the restored site, greater at the drained site and highest at the undrained site. Soil temperature was highest at the restored site with no significant difference between the undrained and drained sites. Soil moisture levels were highest at the undrained site and lowest at the drained site. In addition, the undrained and restored sites did not differ significantly in soil moisture content. In conclusion, restoration did not have a significant effect on the level of the water table, initiation of peat formation or ability of soil to hold water.

Keywords: drainage; peatlands; restoration; soil water retention; soil surface temperature

Introduction

Peatlands are one of the most significant terrestrial carbon sinks and sources (Ussiri and Lal 2017). They comprise about 3% of the entire global terrestrial surface and contain about 25% of the total terrestrial stock of carbon (Waddington et al. 2015). In addition to carbon storage, they are also a significant source of water in the landscape. The perennial availability of water in this unique landscape has resulted in a specialized micro-ecosystem with a distinct biodiversity (Krejčová et al. 2021). Also, their high water table and high soil moisture content along with a low pH, low oxygen content and low soil surface temperatures are significant indicators of peatland development (Minayeva and Sirin 2012). There are a great variety of peaty habitats. Here the focus is on peat meadows where the layer of peat is usually just a few dozen cms thick (van Dijk et al. 2007; Querner et al. 2012; Krejčová et al. 2021).

The drainage of peat soil and consequent use for agriculture results in increases in the depth of the water table, aeration, and pH, which results in increased mineralization of organic matter in the soil and aerobic respiration that results in increased CO₂ emissions (Hergoualc'h and Verchot 2011; Menberu et al. 2016; Krejčová et al. 2021) and a reduction in biodiversity (Pfadenhauer and

Grootjans 1999; Krejčová et al. 2021). Peat meadows are particularly sensitive to drainage due to their thin peat layer, which mineralizes completely because of both drainage and subsequent use for agriculture (Krejčová et al. 2021).

Globally, it is estimated that about 15% of the world's peatlands have been drained (Buckmaster et al. 2014), mostly for agricultural purposes (Pfadenhauer and Grootjans 1999). In the Czech Republic, about 27% of the peat landscapes were converted to agricultural fields in the second half of the previous century (Frouz et al. 2010 a, b; Krejčová et al. 2021), which affected the hydrology and hydrochemistry of peatland (Holden et al. 2004; Menberu et al. 2017). Since the early 2000s ecological concerns have resulted in efforts to restore peatlands and several studies report the factors that determine the success of restoration in terms of depth of water table, increases in fauna and soil moisture (Buckmaster et al. 2014).

There is little knowledge on the physical properties of the soil in peatlands mainly due to the spatial variability in their physical and chemical characteristics (Cunliffe et al. 2013). In this study, changes in soil water retention of a peat meadow were recorded 20 years after it was restored by damming the sub-surface drainage system with clay. It was hypothesized that blocking the drainage sys-

tem will decrease the sub-surface runoff and cause the water table to rise to the level at undrained sites and result in the formation of a layer of peat and increase the capacity of the soil for retaining water.

Material and Methods

Study site

The study area is situated close to the village of Senotín in Czechia, South Bohemia (49.0640289°N, 15.1474864°E). The topography at the site is a gentle slope of between 2–10% at an altitude of 650 m a. s. l. The area has a mean annual temperature of 6 °C and mean annual precipitation of 700 mm (Krejčová et al. 2021). The soils at this site are mainly peaty along with historic cambisols and histosols. In the mid and late 1980s, wet meadows were drained for agriculture using underground drainage pipes (Frouz et al. 2010 a, b), although some remnants of undrained wet meadows remain. In 1993, studies were undertaken at this site to determine the effect of underground drainage on the hydrology and biodiversity. In 1996, about 3 ha of the drained meadows was reclaimed by constructing long 2 m deep trenches extending about 0.5 m below the surface. Trenches were filled with compacted clay as a sealant with soil above for infiltration. Trees were later planted on the clay barriers to reduce run-off and stabilize the field water capacity and raise the water table (Fig. 1). The success of peatland restoration was determined by comparing it with a wet undrained peaty meadow (a positive control) and drained meadow, which served as a negative control. Key hydrological parameters including depth of water table, field water capacity, soil water content at maximum sat-

uration and field water capacity were measured before restoration in 1995 (historical) and recently in 2018 and 2023. In addition, soil moisture and soil surface and air temperatures were measured in 2018–2020 and *in situ* infiltration in 2023 to provide a more comprehensive understanding of restoration.

Data collection

Water table depth was monitored at four locations at each of the three sites using perforated plastic pipes buried 60 cm below the soil. Sticks were inserted into the pipes and then withdrawn and length of stick that was wet was used to determine the depth of the water table. Measurements were collected in 1995 and 2018 at three monthly intervals from May 1995 to May 1996 and from May 2017 to June 2018, after which the average depths at each site and in each year were calculated.

Fifteen measurements of infiltration were recorded in 2023 at five different locations at each of the three sites using a mini-disk infiltrometer. Soil water content at maximum saturation and field water capacity of 15 samples of soil from three sites from an undisturbed area using a corer the inner diameter of which was 5 cm and length 5 cm in 2023. The initial weight of the samples was recorded. Water was extracted from the samples in the laboratory at room temperature for three days using a 1500F 15 bar pressure plate extractor (30 cm diameter). After three days, the samples were weighed to determine maximum saturation and then dewatered under a constant pressure of –0.33 bar, to determine field water capacity. The wet weight of the samples was recorded and then dried at 105 °C for 48 hours. Maximum water saturation and field water capacity were expressed in terms of volumetric water content. Volumetric soil

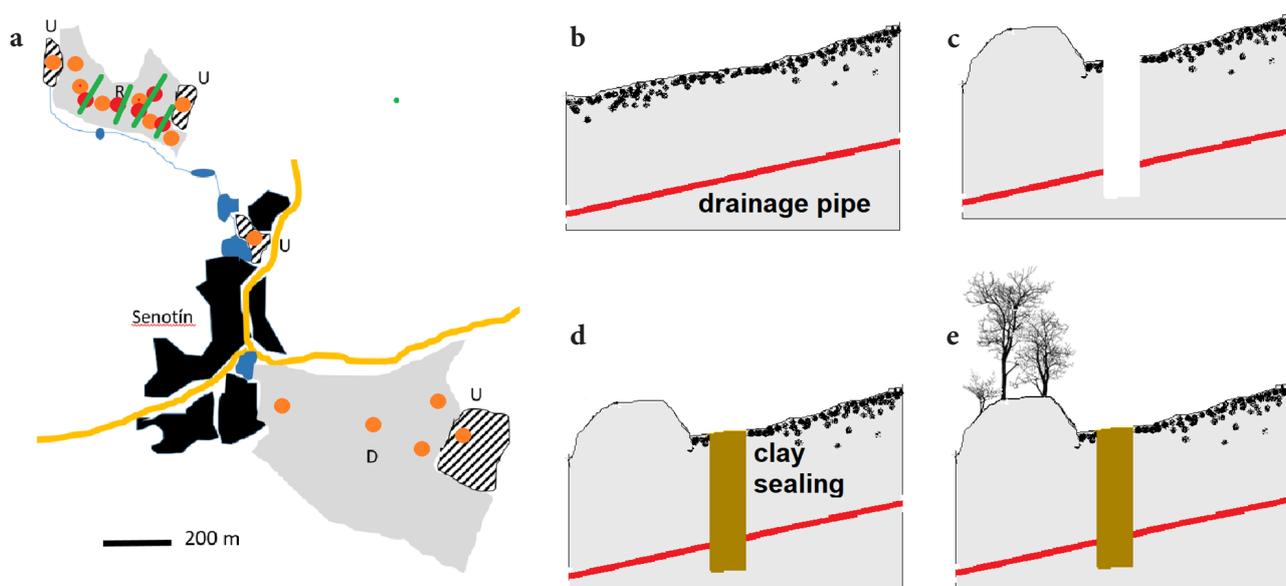


Fig. 1 Map of the sites sampled (a) near Senotín, showing position of undrained remnants of original site (U), drained site (D) and drained site that was restored (R), orange dots show where soil was sampled, red circles the location of soil moisture and soil surface temperature sensors, figures b–e show damming of the drainage pipes used in restoration.

moisture was measured throughout 2020 at three locations at each of the undrained, drained and restored sites. Measurements were taken at a depth of 15 cm using the SMT 100 and data recorded using a MicroLog SDI MP data logger.

The difference between air temperature and surface temperature at noon was used as a proxy for evapotranspiration. A big difference between surface temperature and soil surface temperature indicates that a greater proportion of incoming radiation was turned to sensible heat, whereas a small or even negative difference indicate a greater proportion was latent heat, i.e., from soil evaporation and plant transpiration processes. Soil surface temperature was measured using an infra-radiometer Apogee Instruments SI-411, while air temperature and humidity were recorded using EMS 33S. Data were collected using a MicroLog SDI MP data logger. Only noon data points were used, ensuring that all the locations were fully exposed to solar radiation and not shaded by surrounding vegetation.

Statistical analysis

A two-way ANOVA test was used to determine the effect of time of sampling (historical vs recent) and type of site (undrained, drained, and restored) on field water ca-

capacity, water saturation and water table depth. A one-way ANOVA test was used to determine the effect of type of site on the mean differences between soil surface and air temperature, soil moisture and infiltration. A Tukey post hoc analysis was used to determine whether the differences between types of sites were significant at a significance level (alpha level) of 0.05. All computations were done using Statistica 13.0.

Results

There was a significant difference in level of the water table at the individual sites ($p < 0.001$). The undrained site had a significantly higher water table than the drained and restored sites (Fig. 2a), with no significant difference between years. There was a statistically significant interaction between type of site and when sampled ($p < 0.001$). This is because the water table was closer to the soil surface in 2018 than in 1996 at the undrained site but not at the restored and drained sites.

Infiltration was lowest at the undrained site, higher at the drained site and highest at the restored site, which indicates a significant difference between sites ($p < 0.001$). Post hoc analysis revealed significant differences in in-

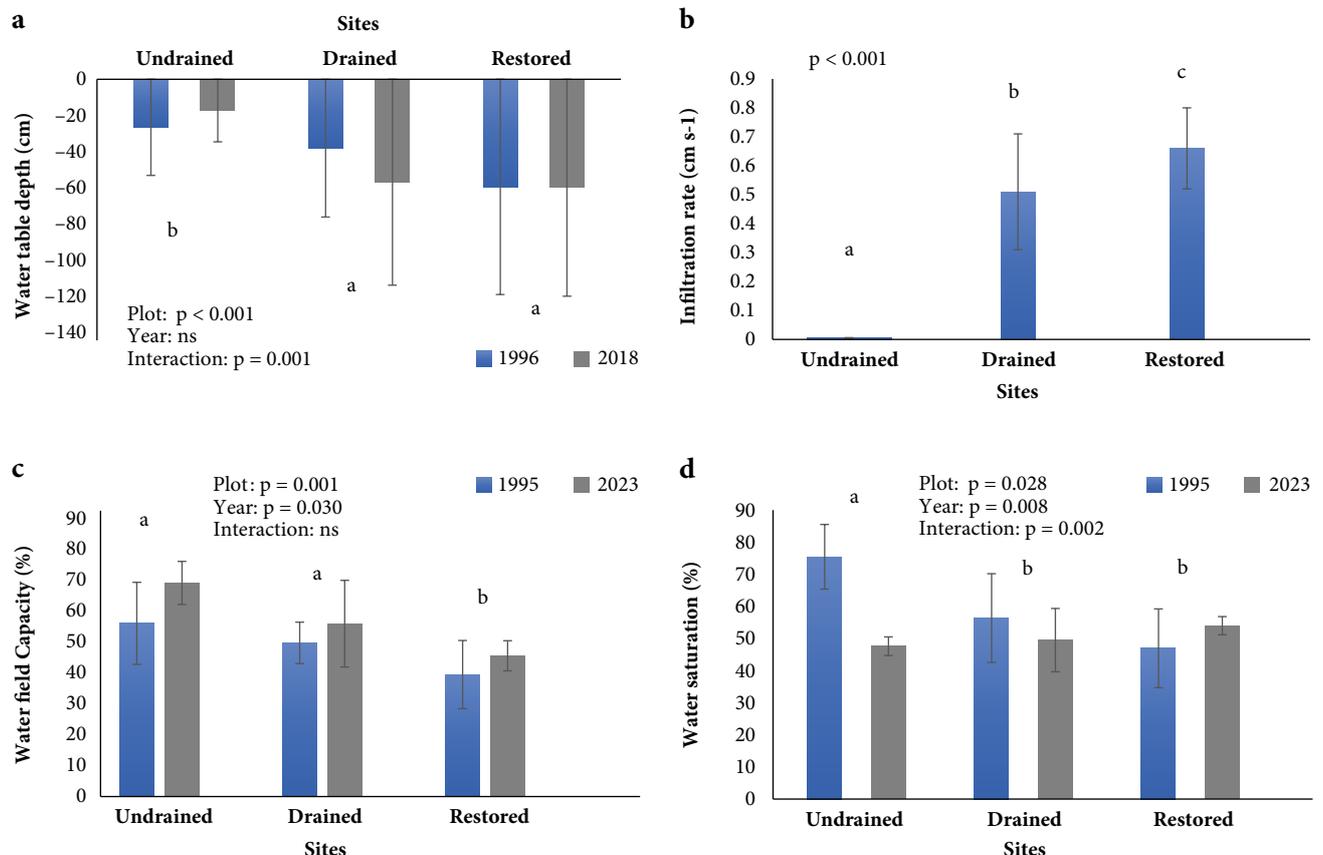


Fig. 2 Comparison of depth of water table (a), infiltration (b), field water capacity (c) and water saturation (d) at the three sites (undrained, drained, restored). Values are means \pm SD. For a, b and d, historical (1995) and recent (2018–2023) values are given and results of a two-way ANOVAs are in the tables on the figures. Infiltration was measured in 2023 and one-way ANOVA results are presented. Statistically homogeneous sites are indicated by the same letters (Tukey post hoc test, $p < 0.05$).

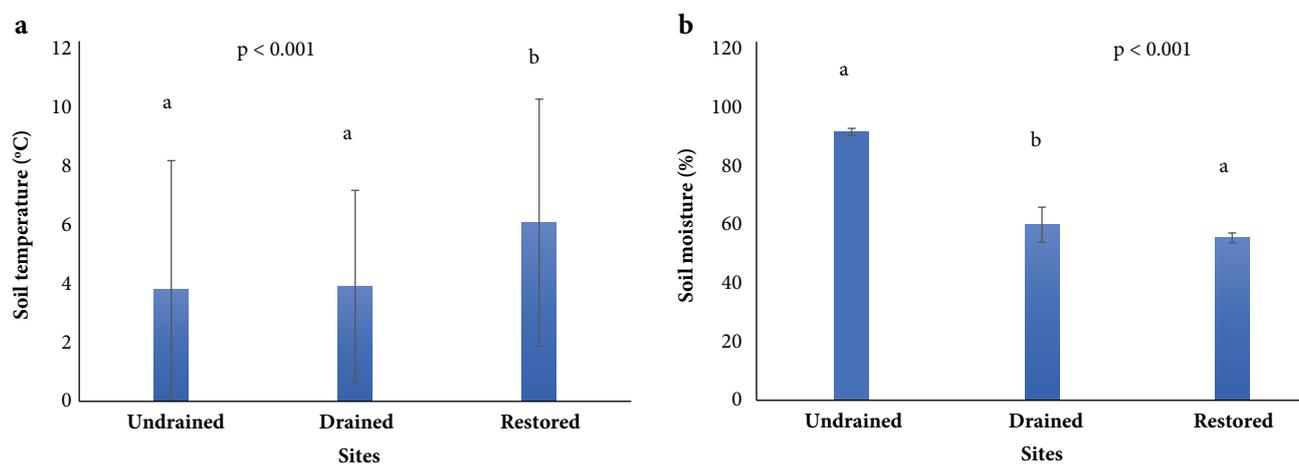


Fig. 3 Difference between soil surface temperature and air temperature (a) and soil moisture (b) in undrained, drained, and restored sites in 2020. Statistically homogeneous sites are indicated by the same letter (Tukey post hoc test, $p < 0.05$). The probability value on figures is the result of one-way ANOVA showing differences between sites.

filtration recorded for undrained and restored sites ($p < 0.01$), undrained and drained sites ($p < 0.01$) and restored and drained sites ($p < 0.05$) (Fig. 2b).

Field water capacity varied significantly across sites with the restored site having the lowest field water capacity, drained sites a higher value and undrained site the highest ($p < 0.01$). Significant difference in the field water capacity was recorded between the undrained and restored sites ($p < 0.001$) (Fig. 2c). The year of sampling had a significant effect on field water capacity ($p < 0.03$), with it being higher in 2023 than in 1995.

The degree of soil water content at saturation varied significantly between sites ($p < 0.001$) (Fig. 2d). The soil water content at saturation was low at the restored site, higher at the drained site and highest at the undrained site. Soil water content at saturation was significantly different between the undrained and restored sites ($p < 0.001$) and the undrained and drained sites ($p < 0.005$), with no significant interaction between site and when sampled on the degree of water saturation.

The difference in mean temperatures of air and soil differed significantly at the three sites ($p < 0.001$), with the greatest temperature recorded at the restored site and lowest at the drained site. There was a significant difference between undrained and restored sites ($p < 0.01$) and between drained and restored sites ($p < 0.05$). However, no significant difference was found between undrained and drained sites (Fig. 3a).

Soil moisture levels significantly differed at the three sites (undrained, drained, and restored) ($p < 0.001$) (Fig. 3b). There was a significant difference between the drained and undrained sites ($p < 0.01$), but not between the restored and drained sites (Fig. 3b).

Discussion

The results of this study indicate that draining resulted in a significant increase in the depth of the water table,

which is in accordance with previous studies (Price et al. 2003; Cunliffe et al. 2013; Menberu et al. 2018).

Despite restoration, there was no significant decrease in the depth of the water table or initiation of peat formation. This failure may be attributed to the hilly character of the sites (Price et al. 2003; Cunliffe et al. 2013; Menberu et al. 2018; Krejčová et al. 2021). Due to the slope a decrease in the depth of the water table only occurred around where infiltration was blocked and most of the area remained unrestored. At the same time, there was a loss of organic matter and water retention capacity due to drainage. A relatively low water table and high surface temperatures promote mineralization, prevent accumulation of organic matter and restoration of the retention capacity, which largely depend on organic matter (peat) restoration (Moskal et al. 2011). Alternative strategies that take into consideration the gradient of a site may be necessary for successful restoration, as suggested by Krejčová et al. (2021).

In some parameters, the restored site appears to be worse in terms of water retention than the drained site (control). It is likely that this is due to initial soil conditions. Although during the restoration in 1995 the comparability of the sites was considered, drainage increased the depth of the water table, water saturation and field water capacity at the restored site before restoration (in 1995) than at the control and drained sites (although the difference was not always significant, this trend appears in several parameters). Thus, it is likely that the poor initial conditions may magnify over time despite restoration.

The low level of infiltration at the undrained site was due to the proximity of the water table to the soil surface. Frouz et al. (2010a, b) report that tillage increase sand content of the surface layer of soil at drained sites, which may increase infiltration, which may be even enhanced by prevailing subsurface runoff, which is also likely to persist in restored drained sites. Moreover, grassy vegetation at the restored site, as reported by Krejčová et al. (2021), could affect the level of infiltration, as plant roots

support water percolation. Furthermore, the relatively higher level of infiltration at the drained site, compared to the restored site, could be attributed to the creation of cracks in the soil due to it drying out as a result draining the peat.

The consistently low levels of field water capacity and water saturation at the restored sites, historically and recently, may be linked to soil compaction during site restoration, which altered soil structure and inhibited the burrowing activities of soil micro fauna (Liu et al. 2022). In addition, the absence of native vegetation can be linked to low water absorption of restored peatland soils (Li et al. 2018). However, the observed gradual increase in field water capacity at all the sites from 1995–2023 can be attributed to several specific factors, such as absence of tillage and the natural succession of vegetation over time.

High infiltration, low water retention and low soil moisture are linked to reduced water availability in the surface layer of soil, which limit evaporative cooling and result in high soil temperatures (relative to air temperature), as recorded at the restored site (Ochsner et al. 2001; Lu et al. 2007; Mathur et al. 2014; Cawson et al. 2016). Lower temperatures recorded at the undrained sites could be linked to high field water capacity facilitating the cooling of the soil due to evapotranspiration (Bridgman and Richardson 1992). These variations in temperature indicate that draining peatland and subsequent alterations in the hydrologic regime have a significant effect on soil temperature dynamics (Tarnawski and Leong 2000; Hora 2011; Menberu et al. 2018). The higher the temperature of the soil, the faster the decomposition, which limits the build-up of soil organic matter (Swails et al. 2022).

Aerobic soil respiration which is linked to low soil moisture content and higher temperature may eventually contribute to the loss of peaty layers and subsequent drop in water table levels (Grayson et al. 2010; Lundin et al. 2017). Lower temperatures exhibited at the undrained and drained sites could be linked to the high-water field capacity and vegetation cover which facilitates cooling of the soil, hence, hindering aerobic respiration (Bridgman and Richardson 1992; Kluber et al. 2014; Wang et al. 2015; Drexler et al. 2017; Gutenberg et al. 2019; Huang et al. 2021).

Conclusion

In conclusion: restoration did not result in a significant decrease in the depth of the water table or in the initiation of peat formation. The hilly nature of the sites and the mineralization of organic matter post-drainage, coupled with low water retention, contributed to this failure. Factors such as proximity of the water table, early development of vegetation that obstructs the percolation of water and cracks in the soil due to draining the peat, influenced the level of infiltration. Persistently low field water capacity at restored sites was linked to soil com-

paction and destruction of native vegetation. However, a gradual increase in field water capacity from 1995–2023 was recorded, which is attributed to factors like absence of tillage and natural succession. High levels of infiltration, low water retention and soil moisture were associated with higher soil temperatures at the restored site than at undrained and drained sites, which are associated with a high field water capacity limiting aerobic respiration.

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ALBEDO ON A GLACIAL FORELAND AT GROUND LEVEL AND LANDSCAPE SCALE DRIVEN BY VEGETATION-SUBSTRATE PATTERNS

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ABSTRACT

Recent anthropogenic climate change has caused both glacial retreat and increased vegetative growth on Arctic and subarctic tundra landscapes resulting in changing albedo and energy budgets. Glacial forelands are topographically and ecologically heterogeneous landscapes comprising ice-contact and outwash deposits subject to primary succession. The most recent moraines on the foreland of the Skaftafellsjökull in southern Iceland are mostly unvegetated, but vegetation cover increases with the age in a general sense. Vegetated outwash channel terraces occur between the moraines, and a broad vegetated outwash plain occurs distal to the oldest moraine. Variations in albedo were measured at ground level to determine the specific role of vegetation types and varying substrates. Albedo and coverage by major plant groups were measured along transects established on moraines ranging in age from 20 to 130 years and the terrace of one outwash channel and three locations on the outwash plain. Total vegetation cover and coverage by mosses increases on the glacial moraines largely as a function of time but is subject to strong aspect effects. Total vegetation cover and moss cover are highest on outwash deposits, possibly due to a sheltered aspect and greater uniformity of the outwash surface. Measured albedo exhibits a modest positive correlation with total vegetation cover and a modest negative correlation with rock and soil exposure. The strongest positive correlation was found between albedo and moss cover. The differences in brightness between moraines and outwash deposits are evident visually at the landscape scale on satellite photographs and quantifiable by image-processing software.

Keywords: glacial foreland; glacial moraine; moss heath; outwash channel; outwash plain; primary succession

Introduction

It is well established that anthropogenic climate change over the last century has resulted in substantially greater warming at high latitudes compared to the global mean, causing the retreat of glaciers at both high latitudes and high altitudes (Hugonnet et al. 2021; Rounce et al. 2023). Moreover, the environmental impacts of this warming on Arctic regions include changes to the land energy budgets, decreasing snow cover and changing plant communities. Multiple studies have examined the transition of moss dominated tundras to shrub heaths and the increased growth of larger shrubs and trees, which presumably provide greater dark leaf area for absorption of solar radiation, thereby reducing albedo (Chapin et al. 2005; Sturm et al. 2005; Tape et al. 2006, 2012; Swann et al. 2009; Brown et al. 2010; Blok et al. 2011; Loranty et al. 2011; Pearson et al. 2013; Juszak et al. 2014; Williamson et al. 2016). However, the relationship between shrub vegetation and albedo is not always straightforward (Payette et al. 2001; Beck et al. 2011). Nonetheless, changes in the energy budget due to altered albedo have the potential to trigger climatic feedback, such as the oft-cited positive warming feedback in the Arctic climate system, which has the potential to cause accelerating permafrost thaw, glacial retreat and accelerated growth of shrubs on tundra (e.g. Blok et al. 2011; Pearson et al. 2013). At subarctic latitudes analogous studies have examined how anthropogenic climate change is causing the encroachment of boreal forest northward across the tundra-forest ecotone (Arnalds 1987; Robinson et al. 2008;

Berner et al. 2013). Here again, this shift in community structure lowers the albedo of the landscape through the replacement of light-toned mosses with dark coniferous trees, providing positive feedback with the potential to accelerate.

The subarctic setting of Iceland offers an opportunity to test the effects of changing plant communities on albedo in landscapes that are unique in one respect. As the bedrock of the island consists almost entirely of mafic volcanic rock, glacial and fluvial deposits and soils derived from them consist largely of volcanic detritus (Arnalds 2008) that is dark when fresh, but lighter when weathered. Tanner and Vandewarker (2019) studied the differences of albedo due to successional vegetation encroachment on the Skeiðarársandur, the glacial outwash plain of the Skeiðarárjökull in southern Iceland. They found a strong positive correlation between albedo and the coverage of the plain by a moss-dominated heath, in which mosses are the dominant early colonizing plant species. Because the moss heath community has a higher albedo than the dark substrate, colonization and succession is causing albedo to increase.

In contrast to the topographic uniformity of the outwash plains of the Icelandic glaciers, or sandur, the land areas exposed by the retreating glaciers, or glacial forelands, are topographically heterogeneous, comprising multiple geomorphic elements, including recessional moraines, push moraines, outwash terraces and outwash plains. The study described herein examines the albedo of a glacial foreland, that of the Skaftafellsjökull in southern Iceland, by measurement at close scale at ground



Fig. 1 Location of study area in southern Iceland (see arrow on inset map). Dates represent approximate locations of the ice front in the year indicated. Bold dashes represent specific transect locations. T = transect number. Brown areas are glacial moraines; pale green areas are moss-covered outwash deposits; dark green sinuous features are incised channels hosting downy birch; dark blue features are kettle ponds. Image downloaded from <https://satellites.pro> (by Google®).

level. We test the effect on albedo of the differences in the successional communities developed on the varying landforms in the glacial foreland and explore the causes for these differences. Additionally, we compare these ground-level measurements to observations of the landscape brightness as measured from satellite imagery.

Methods

Location

The study site is the foreland of the Skaftafellsjökull, an outlet glacier of the Vatnajökull ice cap in southeastern Iceland (Fig. 1). Modern climate for this area of the Icelandic south coast is a mean annual temperature of ca. 5 °C and mean annual precipitation of 1400–1800 mm (Roeloffs 2022). Like most glaciers worldwide, the Skaftafellsjökull, and the other outlet glaciers of the Vatnajökull have been in retreat since the end of the Little Ice Age. The Skaftafellsjökull reached its maximum modern extent at the close of the Little Ice Age in 1890 and has retreated ca. 3 km since (Baldursson et al. 2018). This retreat has exposed a foreland area with varying topog-

raphy. Through the middle 20th century, the Skaftafellsjökull retreated consistently due to continued anthropogenic warming, leaving recessional moraines that have been dated accurately through continuous monitoring of the glacier terminus (Sigurðsson 2005). The most distal moraine of the Skaftafellsjökull that is clearly identifiable is dated to the position of the ice front in 1890. A much more pronounced topography is formed by a set of nested moraines that date from the position of the glacial front in 1945 (Fig. 1). The more proximal moraines date to the positions of the glacial front in 1954, 1960, 1982 and 2002 (Perrson 1964; Sigurðsson 2005; Hannesdóttir et al. 2014; Evans et al. 2017). Between the moraines are flatter areas formed by alluvial outwash terraces and incised channels. These outwash surfaces differ from the moraines in both their topography – the outwash deposits generally have horizontal surfaces in contrast to the slopes of the moraines – and in composition; the moraines consist of very poorly sorted deposits with protruding boulders, while the outwash consists mainly of sorted gravel-size clasts. The ages of these inter-moraine outwash channel and outwash plain surfaces generally are not well constrained, other than by the moraines that

form their boundaries; i.e., the possibility of continued reactivation of the outwash channels over the course of many years removes age constraints. Consequently, the surfaces at Skaftafell can best be characterized as pre-1890, 1890–1904, 1904–1945, 1945–1960, 1960–1982, 1982–2002 and post-2002 (Fig. 1). Prior to 1967, the study area was used for sheep farming. This area was largely abandoned for grazing after the Skaftafell National Park (now incorporated within the Vatnajökull National Park) was established in 1967, and formally fenced off in 1987 (Vilmundardóttir et al. 2015).

Previous work

Multiple studies have been conducted on the foreland of the Skaftafellsjökull examining species diversity (Perrson 1964), soil properties (Tanner et al. 2013; Vilmundardóttir et al. 2015) and primary ecological succession (Glausen and Tanner 2019; Synan et al. 2021). Of these, Glausen and Tanner (2019), measured species richness on transects established on moraines at different distances from the current glacier terminus, representing different landscape ages, in addition to a glacial outwash channel terrace between moraines, and locations on the outwash plain distal to the oldest moraine. Their work suggested that following the pioneer stage, species richness and vegetation coverage both increased on the moraines through the mid-successional stages, but species richness decreased on the oldest portions of the landscape, even as total vegetation increased. In a general sense, total vegetative cover on the foreland increases with distance from the current glacial terminus. The most common component of the successional communities are mosses, primarily *Racomitrium lanuginosum* (hoary-fringe moss), followed by a low shrub community comprising *Empetrum nigrum* (black crowberry), *Calluna vulgaris* (scotch heather), *Arctostaphylos uva-ursi* (bearberry) and *Saxifraga oppositifolia* (purple saxifrage). Minor components include the dwarf trees *Betula pubescens* (downy birch), *Salix lanata* (wooly willow), *Salix phylicifolia* (tea-leaved willow), various graminoids, forbs, foliose and fruticose lichens and biological soil crusts.

Sampling design

This study was conducted in parallel with a reexamination of the study locations of Glausen and Tanner (2019) using the coordinates recorded by GPS in 2007 for the starting points of transects (Fig. 1). Transect 1 is the most proximal to the ice front, located on the glacial-facing slope (equals proximal aspect) of the most recent moraine, which is dated to the position of the ice front in 2002 (Fig. 2a; Hannesdóttir et al. 2014). Transect 2 is located southwest of Transect 1 on a flat at the top of a moraine associated with the ice position in 1982 (Fig. 2b). Transect 3 is situated on the northeast-facing slope (proximal aspect) of a moraine ridge that marks the position of the ice front ca. 1954 (Perrson 1964). Tran-

sect 4 is situated in a slight hollow on the southwest-facing slope (away from the glacier, or distal aspect) of the moraine ridge from 1954, overlooking a kettle pond in the 1945 moraine. Transect 5 is on a broad flat at the top of the 1945 moraine; the date of deglaciation of the site is estimated at 1938 (based on data from Sigurðsson 2005). Transect 6 is located on the terrace of a stream channel in a broad swale immediately east (toward the glacier) of the moraine associated with the ice position in 1904 (Sigurðsson 2005). However, this swale was occupied by an outwash channel active as recently as 1960 (Perrson 1964). Transect 7 is situated on the outwash plain adjacent to the margin of the oldest boulder moraine, associated with the position of the ice margin between 1904 and 1890 (Fig. 2d). Transects 8 and 9 are located on the outwash plain distal (west) of the 1890 ice front. The topography here is not entirely flat but consists instead of very subdued ridges and swales that are relics of the period when the outwash channels on the plain were occupied. Transect 10 (not studied by Glausen and Tanner 2019) is located to the south of the other transects on the distal side of the 1890 moraine (Fig. 2c).

At each site, a transect line was established parallel to the trend of the moraines with five measurement stations set 10 m apart. Starting points for stations were chosen without bias for substrate, i.e., individual stations may have included non-vegetated boulders. At each station, measurements were made using a 0.5 m × 0.5 m (= 0.25 m²) quadrat that was rotated spatially to provide 1.0 m² of continuous coverage per station. Within each quadrat, the percentage of cover by each of the major vegetation groups (mosses, shrubs, dwarf trees, graminoids, forbs, lichens and biological soil crust), plus non-vegetated area were estimated for comparison with the data collected in 2007.

Spot measurements of albedo were calculated from measurements of ambient and reflected light from the ground surface using a Reed Instruments® SD-1128 Datalogger with light sensor measuring in units of lux (= lumens m⁻²). The sensor was mounted on a flat board that was placed on the ground to measure ambient light intensity. Reflected light intensity was measured immediately following at the same location with the same sensor 1 m above the ground surface with the sensor oriented parallel to the ground surface. This measurement was repeated four times at each location (once in each quadrat). The mean of the resulting 20 measurements per transect presents an average value representative of the transect irrespective of the heterogeneity of the vegetation. Variations in intensity of ambient or reflected light due to cloud cover or time of day were effectively negated by the albedo calculation as incoming ambient and reflected light intensity were measured under identical conditions. The results are internally consistent with albedo varying as might be expected between sample sites on different types of surfaces. Moreover, they compare well with published values of albedo measured at ground



Fig. 2 Landscape features of the Skaftafellsjökull foreland. a) View of the 2002 moraine with negative aspect, or slope facing the ice front (Transect 1). Surface is covered mainly by soil and gravel. b) View near the crest of the 1982 moraine with positive aspect, or slope facing away from the ice (Transect 2). Surface is mostly vegetated, primarily by mosses. c) Location of Transect 10 on the 1890 moraine with view of positive aspect slope. Surface exposes protruding boulders but is largely covered by vegetation. d) Moss-covered bar top of outwash deposits at Transect 7. The downy birch to the right is mainly limited to the swale between the bars.

level on various landscape surfaces at high latitude (Petzold and Rencz 1975). The mean brightness of the land surface at each transect was approximated by analyzing the light intensity for an area approximating the transect area on a cloud-shadow free satellite photograph (Fig. 1; downloaded from <https://satellites.pro> by Google®) with ImageJ software, a public domain Java image processing program. The program performs measurements of mean brightness of an area defined on an image relative to the greyscale range of 0 (black) to 255 (white). Results for vegetative cover, moss cover, soil and rock exposure, albedo and mean transect brightness are presented in Table 1. Comparisons of albedo to vegetation surface cover data were analyzed by linear regression analysis using the statistical functions of Microsoft Excel®.

Results

Transect 1

The youngest land surface examined is the glacier-facing slope of a push moraine that is proximal to the present lagoon in front of Skaftafellsjökull (Figs 1, 2a). This surface is estimated to have been ice free since ca. 2002.

The surface of this transect is dominated by exposed gravel, although coverage varies from station to station, from 41.3% to 92.5% (mean = 72.1%; SD = 20.3). Vegetative cover (includes mosses, lichens, willows, forbs, graminoids and biological soil crusts) is also greatly variable, from 7.8% to 58.5%, averaging 26.6% across the five stations (SD = 20.9). The vegetative component is dominated by mosses (13.3%). Measurements of mean albedo for the individual stations range from 0.15 to 0.17 (mean = 0.158).

Transect 2

The transect line was established on the west-facing slope (aspect oriented away from the glacier) of the second prominent push moraine ridge from the current lagoon. This surface is estimated to have been ice free since 1982 (Figs 1, 2b). Vegetative cover is more pervasive and more diverse on the surface of this transect, averaging 73% across all stations (SD = 17.9). The vegetation is dominated by mosses (mean = 42.8%), which vary from 4.5% to 80.0% (SD = 33.9); forbs, graminoids, low shrubs, willows, lichens and biological soil crusts also are present. Albedo measured at the five sets in this transect ranges from 0.15 to 0.21 (mean = 0.182).

Table 1 Measurements of ground cover and albedo for all stations. Station = transect and station number (transect locations shown in figure 1, each station is the mean of four quadrats); VC = total vegetation cover %; moss = % ground cover by undifferentiated mosses; r&s = rock and soil cover %; SD = standard deviation of albedo measurements at each station; mean = the mean albedo for the five stations in the transect; brightness is measured relative a greyscale value range of 0 (black) to 255 (white) across the entire transect area shown in Fig. 1 as measured by ImageJ software.

Transect	VC	moss	r&s	albedo	SD	mean albedo	Brightness
1-1	58.5	36.3	41.5	0.15	0.008	0.158	78.72
1-2	9.8	7.8	90.3	0.16	0.042		
1-3	16.3	2.1	83.3	0.15	0.006		
1-4	30.3	16.3	69.0	0.16	0.015		
1-5	7.8	4.0	92.8	0.17	0.019		
2-1	40.5	4.5	60.0	0.15	0.010	0.182	85.73
2-2	58.5	16.8	40.0	0.16	0.011		
2-3	75.8	37.5	27.5	0.18	0.004		
2-4	65.3	80.0	11.8	0.21	0.016		
2-5	88.0	75.0	12.0	0.21	0.019		
3-1	55.0	27.5	46.5	0.19	0.008	0.186	86
3-2	59.5	17.5	43.8	0.18	0.012		
3-3	58.0	37.5	43.8	0.19	0.009		
3-4	0.3	0.0	100.0	0.19	0.006		
3-5	87.5	62.5	13.0	0.18	0.010		
4-1	97.5	48.8	2.3	0.19	0.014	0.184	82.36
4-2	99.5	58.8	0.2	0.2	0.015		
4-3	70.5	20.0	29.5	0.17	0.010		
4-4	92.5	32.5	7.5	0.15	0.011		
4-5	99.0	75.0	1.0	0.21	0.008		
5-1	85.0	57.5	20.8	0.18	0.010	0.188	78.53
5-2	38.3	23.0	72.5	0.19	0.011		
5-3	100.0	77.0	17.3	0.2	0.010		
5-4	7.8	3.0	97.3	0.18	0.011		
5-5	26.3	11.3	75.0	0.19	0.014		
6-1	99.3	73.3	0.3	0.2	0.037	0.192	93.14
6-2	99.8	82.5	0.1	0.2	0.022		
6-3	87.3	48.3	13.0	0.21	0.024		
6-4	99.8	77.5	0.3	0.18	0.015		
6-5	97.3	93.8	2.6	0.17	0.011		
7-1	79.8	79.8	20.3	0.23	0.021	0.238	101.56
7-2	97.3	93.0	4.0	0.24	0.007		
7-3	97.8	87.0	2.0	0.24	0.024		
7-4	99.8	38.8	0.0	0.23	0.017		
7-5	87.5	78.8	13.8	0.25	0.037		
8-1	99.8	99.3	0.3	0.18	0.019	0.206	98.8
8-2	95.8	93.0	4.0	0.25	0.030		
8-3	95.3	84.0	7.3	0.2	0.016		
8-4	97.0	87.3	3.0	0.23	0.027		
8-5	25.0	9.8	75.0	0.17	0.022		
9-1	100.0	96.3	0.0	0.26	0.026	0.2666	99.12
9-2	100.0	91.8	0.0	0.27	0.019		
9-3	100.0	98.5	0.0	0.28	0.019		
9-4	98.8	61.3	1.3	0.23	0.004		
9-5	98.8	98.3	1.3	0.29	0.011		
10-1	95.8	35.0	9.0	0.18	0.023	0.194	84.57
10-2	96.5	45.0	4.8	0.19	0.014		
10-3	91.8	11.3	13.3	0.16	0.008		
10-4	70.0	31.3	59.3	0.22	0.023		
10-5	92.0	73.8	15.5	0.22	0.019		

Transect 3

This transect line is located near the crest of an older push moraine located between the glacial lake and several prominent kettles. The location is estimated to have been ice free since ca. 1954. Total vegetative cover (mean = 53.5%) slightly exceeds that of the exposed rock and soil surface but is highly variable (SD = 31.7%). Mosses account for most of the vegetation, ranging from absent (at station 4) to 62.5% (mean = 29%; SD = 23.3); the remaining vegetative components are similar to those in Transect 2. The non-vegetated surface comprises gravel to boulder size rock (37.3%) and bare soil (12.2%). Measurements of albedo on this transect occupy a narrow range of 0.18 to 0.19 (mean = 0.0.186).

Transect 4

Transect 4 is located proximal to a prominent kettle pond on the slope of the most distal push moraine facing away from the glacier. The date of exposure of this surface is estimated at 1945. All stations in this transect are well-vegetated, ranging from 70.5% to 99% (mean = 99.4%; SD = 12.2). Mosses are the dominant component of the vegetation, ranging from 20% to 75% (mean = 47%; SD = 21.6), with contributions by the groups observed at the younger transects in addition to downy birch (*B. pubescens*). Albedo measured at the stations ranges from 0.15 to 0.21 (mean = 0.184).

Transect 5

This transect is situated at the crest of a broad, gently sloping moraine distal to the younger push moraines and intervening kettles. The date of glacial retreat from this location is estimated at 1938. Vegetative cover is inconsistent between stations, ranging from 7.8% to 100% (mean = 53.5%; SD = 44.2). The cover is dominated by mosses, which vary from 3% to 77% of cover (mean = 34.4%; SD = 31.6), but exhibits similar diversity as Transect 4. Albedo measured at the stations in this transect ranges from 0.18 to 0.2 (mean = 0.188).

Transect 6

Transect 6 is located on the fluvial terrace of a glacial outwash channel between the broad moraine of Transect 5 and an older arcuate moraine dated to 1904. However, historical imagery suggests the area between the moraines was occupied by a glacial outwash stream as recently 1960 (Perrson 1964). The surface of the terrace is well-vegetated (mean = 100%; SD = 5.4) and dominated by hummocky mosses (mean = 75.1%; SD = 16.8). All other vegetation groups are present, although aside from low shrubs (mean = 18.5%), most are very minor components. The terrace is dissected by a narrow channel. In contrast to the terrace surface, the channel floor is occupied by a thicket of downy birch, visible on Fig. 1. Albedo measurements at Transect 6 stations range from 0.17 to 0.21, but cluster near 0.2 (mean = 0.195).

Transect 7

This transect is located on the outwash plain proximal and to the west of the oldest of the Skaftafellsjökull arcuate moraines, which dates to the most distal position of the glacier in 1890 (Figs 1, 2d). Although the age of the moraine is well established, there are no means for dating directly various locations on the outwash plain in the distal foreland. Satellite imagery demonstrates conclusively that the channels have been inactive at least since 1985, and the figures from Perrson (1964) illustrate this area of the outwash plain as free of active streams. Therefore, we embrace the interpretation that most of the outwash plain derives from the early stages of glacial retreat. In general, the outwash plain consists of a series of diamond to linguoid-shaped bars with relatively flat surfaces separated by narrow channels, the floor of which are commonly occupied by thickets of downy birch, as described for Transect 6. Presumably, the age of exposure of the bar surfaces equates to the date of deposition of the outwash. The surface at the stations in Transect 7 is consistently vegetated (mean = 100%; SD = 8.5) and dominated by mosses (mean = 75.5%; SD = 21.3). As on Transect 6, low shrubs are a significant component (mean = 16.5%), while other components are minor. Albedo measurements occupy a narrow range between 0.23 and 0.25 (mean = 0.238).

Transect 8

This transect, located approximately 125 m to the north-northwest of Transect 7, is also situated on the outwash plain of Skaftafellsjökull and shares a similar bar and channel topography. As with Transect 7, the age of exposure of the land surface is ambiguous.

The surface here is mostly vegetated, although total vegetative cover is variable, ranging from 23.8% to 99.8% (mean = 99.6%; SD = 32.2). Mosses dominate the vegetation, ranging from 9.8% to 99.3% of surface cover (mean = 74.7%; SD = 36.7), with low shrubs next most abundant (mean = 11.0%). Albedo measurements at individual stations range from 0.17 to 0.25 (mean = 0.206).

Transect 9

This transect is the most distal transect in the study, located on the outwash plain approximately 120 m west-northwest of Transect 8. Once again, the age of exposure of the surface is unknown, although hypothetically it could be older than the transects located closer to the oldest moraine (7 and 8). The surface at all stations in this transect are nearly fully vegetated (mean = 100%; SD = 0.7). The vegetative cover is dominantly moss (mean = 89.2%; SD = 15.9%), with low shrubs subordinate (mean = 8.1%). Measurements of albedo at the five stations range from 0.23 to 0.29 (mean = 0.266).

Transect 10

The transect is located on the distal side of the oldest arcuate moraine of the Skaftafellsjökull, dated to the max-

imum Little Ice Age glacial extent in 1890 (Figs 1, 2c). Vegetation covers most of the surface at all stations in the transect, ranging from 54% to 95.8% (mean = 95.7%; SD = 10.9). Mosses are common, but do not dominate the surface, ranging from 11.3% to 73.8% (mean = 39.3%; SD = 22.9), subequal with low shrubs (mean = 30.4%). Albedo at the stations in this transect varies from 0.16 to 0.22 (mean = 0.194).

Discussion

Primary succession on glacial deposits initiates by colonization, which as described by Glausen and Tanner (2019) for the Skaftafelljökull foreland, is dominated in the earliest stages by graminoids and mosses. Vegetative cover increases with time but is also subject to local conditions of favorable or unfavorable aspect. The vegetative cover on the youngest moraine on the foreland, from 2002 (Transect 1), is sparse (Fig. 2a) due to the young age of the surface and the aspect (facing the glacier) that exposes the surface to katabatic winds from the ice sheet. The aspect at Transect 2, facing away from the ice sheet, provides partial shelter to the surface (Fig. 2b), which combined with the greater age of exposure (from 1982) allows for vegetation coverage over the majority of the surface. As reported by Glausen and Tanner (2019), this transect is the youngest location studied in which the low shrub component of the community becomes common. The ground surface at Transect 3, near the crest of a push moraine from 1960, varies from horizontal to sloping toward the ice. Consequently, the location is poorly sheltered, accounting for the lower vegetative cover as compared to Transect 2, despite the greater time of exposure. The location of Transect 4 has a favorable aspect (i.e., facing away from the ice) for vegetation growth and as a result exhibits more complete vegetative cover than Transect 3 despite having been exposed for less than a decade longer. Transect 5 lies near the crest of a broad, gently sloping moraine where vegetation is poorly sheltered from the wind. Hence, surface cover by vegetation is incomplete here. The oldest moraine on the foreland, and most distal (ca. 3 km) from the ice front, was studied at Transect 10. The surface is well vegetated, but mosses constitute less than half of the community; most of the remainder consists of shrubs and lichens. The four transects on outwash deposits (6, 7, 8 and 9) are similar to each other in exhibiting nearly completely vegetated surfaces that are dominated (> 75%) by mosses.

Across all stations, albedo measured at the metre-scale exhibits a modest positive correlation with total vegetation cover ($R^2 = 0.25$; Fig. 3a). This stems from the fact that the glacial debris of the Skaftafellsjökull, both ice-contact and outwash deposits, are darker, because they are derived from mafic bedrock, than most of the successional vegetation. Therefore, the overall trend on the moraines of the Skaftafellsjökull foreland over time is

of increasing albedo due to the increase in vegetative cover through plant colonization and primary succession. This is further demonstrated by the modest negative correlation between albedo and total rock and soil surface exposure ($R^2 = 0.25$; Fig. 3c). This is contrary to the trends on most Arctic and sub-Arctic landscapes where the conversion of moss heath to shrub heath lowers albedo (cf. Chapin et al. 2005; Blok et al. 2011; Loranty et al. 2011; Pearson et al. 2013; Juszak et al. 2014; Williamson et al. 2016). The strongest positive correlation was found between albedo and cover by mosses, which are primarily *Racomitrium* species ($R^2 = 0.50$; Fig. 3b), due the fact that the mosses generally are lighter in colour than the other components of the vegetative community. Total vegetation coverage and coverage by mosses increases on the glacial moraines largely as a function of time but is subject to aspect effects; surfaces exposed to the katabatic winds are less vegetated than surfaces on the sheltered. Hence, time and/or distance from the glacier of a moraine surface control albedo but are constrained by aspect.

Notably, albedo is higher on the outwash deposits (transects 6 through 9) than on the moraines, with the exception of Transect 10, which is nearly equal to the lowest mean albedo from an outwash location (Transect 6). The outwash surfaces have higher mean vegetative cover than the moraines, possibly due to the neutral aspect of the nearly horizontal outwash channel terrace and bar surfaces and the nature of the substrate. Glausen and Tanner (2019) speculated that colonization by mosses on the outwash surfaces limits recruitment of other vegetation by restricting seeding sites, thereby limiting community diversity. However, Burga et al. (2010) noted the importance of microsite variation, including substrate, in controlling successional trends. We point out here that the poorly sorted substrate of the moraines, in which fine-grained material occurs in the spaces between gravel and boulders, provides more seeding sites for vascular plants than the gravel-covered surfaces of the outwash bars and terraces. Hence, vascular plants have more opportunities for colonization and competing with mosses on the moraines. As mosses lack root systems, they can effectively grow over gravel-covered surfaces and dominate the outwash deposits to the exclusion of most vascular plants (i.e., low shrubs and dwarf trees).

In summary, the differences in the landforms and their substrates control the successional communities, which in turn control variations in albedo at the landscape scale. These differences are recognizable from satellite photographs as distinct variations in brightness between moraines and outwash deposits due specifically to the greater coverage by mosses. These brightness variations can be measured semi-quantitatively by image-processing software (Table 1). The mean greyscale brightness measured for all of the outwash transect locations (transects 6 through 9) have values of > 90, while all of the moraines (transects 1 through 5 and 10) have values

of < 90. The brightness values obtained from the satellite photograph (Fig. 1) correlate well with the mean albedo for each transect as measured at ground level ($R^2 = 0.66$; Fig. 3d). These results demonstrate that measuring albedo at ground level can be used to establish the vegetative control of metre-scale albedo, and also show that these small-scale measurements can be related to observations at the landscape-scale by remote sensing.

Conclusions

The foreland of the Skaftafellsjökull consists of moraines with varied slopes and relatively horizontal outwash deposits. Primary succession since the retreat of the ice starting in 1890 has resulted in differences in the vegetation communities on the moraines compared to the outwash deposits, which also causes measurable differences in albedo. The moraines, which consist of poorly sorted materials, are incompletely vegetated after 130 years of exposure. Vegetation on the older moraines comprises a mix mainly of mosses, low shrubs, and dwarf trees. In a general sense, albedo of the moraines increases with time as vegetation increases, subject to local aspect controls, because most of the vegetation

is lighter than the substrate of mafic composition. The deposits of outwash channel terraces and the distal outwash plain consist primarily of water-transported, gravel-size clasts. The outwash deposits are nearly completely vegetated by a low diversity community dominated by mosses that are lighter in colour than most other plants in the successional communities. Consequently, the outwash deposits have a higher mean albedo than the glacial moraines. The difference between the landforms in albedo as measured at ground level are also measurable as differences in greyscale brightness on satellite images, demonstrating that albedo measurements made at ground level are representative of differences in albedo at the landscape scale.

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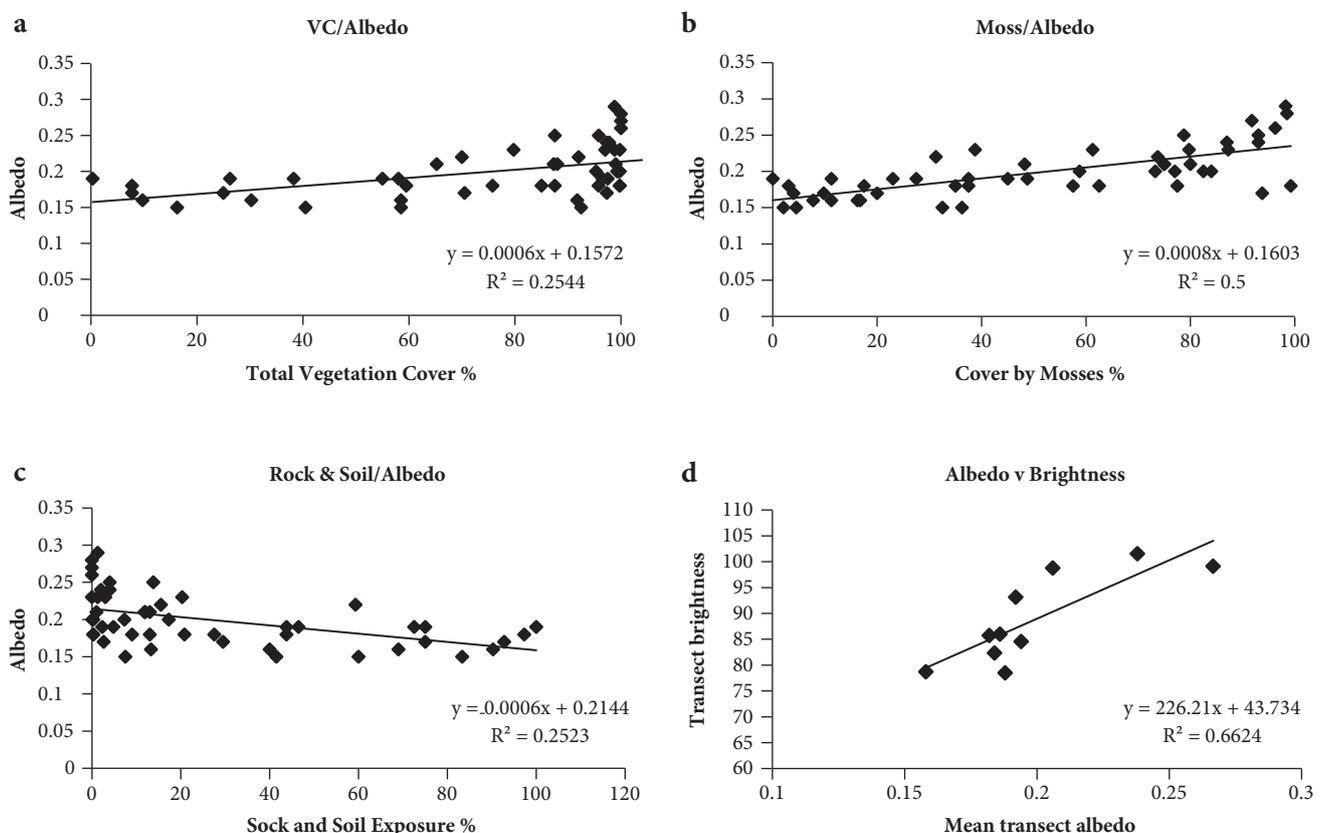


Fig. 3 Albedo scatter plots with linear regression. Each data point represents mean for 1 m² (four quadrats) at one station in transect. a) Albedo plotted against total vegetative cover (VC). b) Albedo plotted against percent of surface area covered by mosses. c) Albedo plotted against a total surface area consisting of exposed rock and unvegetated soil. d) Mean albedo for each transect (mean of five stations) plotted against greyscale brightness measured for transect location on satellite photograph using ImageJ software.

Data availability

The raw data generated in this study are available from the corresponding author on request. Summary data for all plant groups measured are presented as a Supplement.

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DISTRIBUTION AND INVASIVENESS OF FOUR NON-NATIVE SPECIES OF PLANTS IN ECOSYSTEMS IN THE CHOROKHI DELTA (SW GEORGIA)

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ABSTRACT

Chorokhi Delta is known for its high diversity and many habitats, which however are being threatened by invasive plants. Here, the effects of four invasive species of plants, namely *Ambrosia artemisiifolia*, *Sicyos angulatus*, *Solidago canadensis* and *Verbena brasiliensis* were studied. These species were recorded in the coastal area of the Black Sea and in particular in Georgia for the first time in the first half of the last century and *S. angulatus* is a very recent arrival. Currently, these species constitute a significant threat to biodiversity at local, national and global levels. These invasive species were monitored from 2021 to 2022. During this period, sites with high densities of the invasive species were identified. For each species, 5 transects were randomly set, and along each of them, 10 plots (1 × 1 m) were surveyed. The density, frequency, coverage and average height of the invasive plants were measured in each plot. These measurements were recorded twice per year for two years (2021–2022). All this information will be used to develop management plans aimed at preventing their further spread or control their abundance. The results indicate that *Ambrosia artemisiifolia* is the most invasive and widely distributed. *Verbena brasiliensis* and *Sicyos angulatus* are also highly competitive species that can seriously affect semi-natural habitats in the Chorokhi Delta and in agricultural land located close to the Delta. Unlike these species, *Solidago canadensis* is not widely distributed in the area studied. However, its ability to survive in a wide range of habitats and clonal growth indicate that it is potentially a highly dangerous invasive species, which in the future is expected to expand its range and severely affect the semi-natural ecosystems and agricultural land in the Chorokhi Delta. The results of the present study demonstrate the high adaptability of the species studied and their potential for spreading further in the near future.

Keywords: environmental effect; foreign origin; Georgia; invasive species (IAS); Kolkheti

Introduction

Currently, one of the most important threats to biodiversity are non-native invasive species (IAS), which are locally threatening the survival of certain species, specific populations and natural habitats. As the distribution of most invasive plants is climate-driven, they can colonize areas and habitats outside of their natural distribution, where they may thrive and cause ecological, economic and human health problems (Pyšek 1995; Richardson et al. 2000; Pimentel 2005; Pimentel et al. 2005; Richardson and Pyšek 2006; Simberloff et al. 2013; Blackburn et al. 2014; Colautti et al. 2014; Regulation EU 1141/2014; Mikeladze 2015; Aderoju et al. 2020).

The occurrence of non-native invasive plants (IASs) in Georgia was first recorded many years ago and new ones are still arriving. They are particularly abundant in the Kolkheti lowlands, which is in the Adjara floristic region that has a very favourable climate and diverse flora (Davitadze 2001; Davitadze 2002; Kikodze et al. 2010; Fisher et al. 2018; Mikeladze et al. 2019; Mikeladze and Sharabidze 2020; Mikeladze et al. 2021). From 1975 to 2000, 50 new non-native species of plants were recorded by Davitadze (2001) in the Adjara region, including c. 20 that were new for the flora of Georgia (*Tradescantia*

fluminensis Vell., *T. virginiana* L., *Ophiopogon japonicus* (L.f.) ker. *Solanum pseudocapsicum* L., etc.). Moreover, between 2010 and 2020, several new IASs (*Sicyos angulatus* L., *Verbena brasiliensis* Vell., *Maclura tricuspidata* Carriere., *Lobelia urens* L., *Solidago canadensis* L., *Mazus pumilus* (Burm. f.) Steenis) were found for the first time in the Adjara region (Mikeladze et al. 2015; Mikeladze et al. 2017; Mikeladze et al. 2019; Mikeladze and Sharabidze 2020; Mikeladze and Bolkvadze 2021).

The increase in the distribution of IASs is closely related to the rapid increase in cross-border traffic that has greatly facilitated the transport of IASs from one region to another (Davitadze 2001; Simberloff 2013). The study of their distribution and effects on the native flora was significantly limited in restricted areas for decades, which resulted in little scientific data on their origin, rate of spread and the consequences up to now. The valley of the Chorokhi river and Chorokhi Delta in Georgia was a restricted area for political reasons.

The valley of Chorokhi river, as a cross-border crossing between Turkey and Georgia, was closed for most people for centuries (16th–19th). In the last century, there was a Soviet military base in the area, which covered more than 300 ha of the Chorokhi Delta. For military purposes, this place, as well as the surrounding areas

Table 1 Identified target invasive alien species in the study areas.

Scientific name	Common Name	Family	Life form	Origin	Pathway of introduction
<i>Ambrosia artemisiifolia</i> L.	Common ragweed	Asteraceae	Therophyte	North America	Unintentional by human
<i>Verbena brasiliensis</i> Vell.	Brazilian vervain	Verbenaceae	Chamaephyte	South America	As an ornamental plant, by human
<i>Sicyos angulatus</i> L.	Bur cucumber	Cucurbitaceae	Therophyte	North America	Accidental
<i>Solidago canadensis</i> L.	Canadian goldenrod	Asteraceae	Hemicryptophyte	North America	As an ornamental plant, by human

were restricted and thus inaccessible for most people, including even for scientific purposes. Today, the territory of the former military base is now a pasture, hunting and entertainment facility. There are car parks nearby for vehicles transporting cargo through Turkey to various countries in Asia and Europe via the Sarpi customs point, which is the main trade and highway corridor through the Caucasus and main route by which many plants of foreign origin spread and became established in the surrounding areas.

Chorokhi Delta is known for its high diversity of plants and many habitats, which is why it is part of the “Emerald Network” – Emerald site: Chorokhi Delta-GE0000054 (Evans et al. 2019; Archuadze et al. 2021) and a Special Protection Area (SPA) for birds in Georgia (SPA 15) and also an Important Bird and Biodiversity Area (IBA; GEO32) (Paposhvili et al. 2016; Archuadze et al. 2021). The delta is an important area for overwintering and migrating birds. Specifically, the large numbers of fish that are present at the mouth of the river are a rich source of food for birds for replenishing the energy used during migration. As a result, Chorokhi Delta is a very important habitat for a large number of birds. The delta is also where many local, rare and endangered plants occur (*Trapa colchica* Albov, *Marsilea quadrifolia* L., *Paliurus spina-christi* Mill., *Ruscus colchicus* Yeo, *Pancreatium maritimum* L., *Periploca graeca* L., *Rubus adzharicus* Sanadze, and many others).

Currently, there are four highly invasive ISAs recorded in the Chorokhi Delta; the common ragweed (*Ambrosia artemisiifolia*), bur cucumber (*Sicyos angulatus*), Brazilian vervain (*Verbena brasiliensis*) and Canadian goldenrod (*Solidago canadensis*). Of these species, three are of North American origin and one of South American origin (Table 1).

A. artemisiifolia, *S. canadensis* and *V. brasiliensis* were first recorded in Georgia in the first half of the last century along coastal areas of the Black Sea. Of these species, *A. artemisiifolia* and *S. canadensis* are widely distributed in both natural habitats and agroecosystems. *V. brasiliensis* began to spread rapidly in the early decades of the current century. *S. angulatus* is a recently recorded ISA in Georgia as it was first recorded 15 years ago in the Chorokhi valley and has now been growing for 5 years in the Chorokhi Delta.

A. artemisiifolia was first recorded in Georgia at the beginning of the last century and in the Adjara (Georgia) floristic area in 1938 (Davitadze 2001). *V. brasiliensis*

was first recorded by Verloove (2006) based on a specimen of *Verbena* in the herbarium of the Belgium Botanical Garden, which was collected in 1979 by Vladimir Vashak near Sokhumi, who first identified it as *V. hastata*, but after studying the herbarium specimen identified it as *V. brasiliensis*. Early, in 1945–1946, this plant was recorded by Kolakovskii (1986) and over the last ten years, *V. brasiliensis* has been recorded growing near most highways and railway stations, at the edges of canals (waterway), along the sides of rivers in the lowlands of Western Georgia. In 2014–2015, *V. brasiliensis* was widely recorded in both humid and dry places (Mikeladze et al. 2017) mainly along the coast, roads, railroads, in ruderal areas and near canals and rivers, at deserted building sites and landfills sites. It grows in a variety of soils: red, black, shingle and sandy soils etc., especially in humid and secondarily damaged habitats.

Bur cucumber (*Sicyos angulatus*) was first recorded in Georgia in 2012 growing in agricultural land in the Chorokhi River Valley (Mikeladze et al. 2015). Its abundance and frequency of occurrence indicates it must have been present much earlier than when first recorded. Based on information provided by local residents, this species was present 10–15 years ago, that is around 2000, initially in small colonies at the edges of the river and then spread into agricultural areas. In 2014, a number of individuals of *Sicyos* were reported in the Chorokhi Delta. Currently it is widespread in the western part of Georgia, especially on moist soils along the edges of rivers, in agricultural land and in semi-natural habitats.

Solidago canadensis was first recorded in Georgia in the surroundings of Ochamchire in 1920s. After that, it spread into the other floristic districts in western Georgia and was widely distributed at the beginning of the 21st century. The first individuals of *S. canadensis* in the Adjara floristic region were recorded in 2011. In 2019, few more individuals were recorded at the Batumi landfill and in the Chorokhi Delta (Mikeladze and Bolkvadze 2021). This species is widely distributed in South Kolkheti, occurring along roadsides, railways, in ruderal areas, along edges of canals and rivers, in abandoned construction sites, wetlands and degraded cleared forests.

Based on the above, the aim of the present study is to present the results of monitoring the above mentioned IASs, which are common in the Chorokhi Delta. Knowledge of their current status is very important as it can be used to develop management plans for preventing their further spread and reducing their abundance.

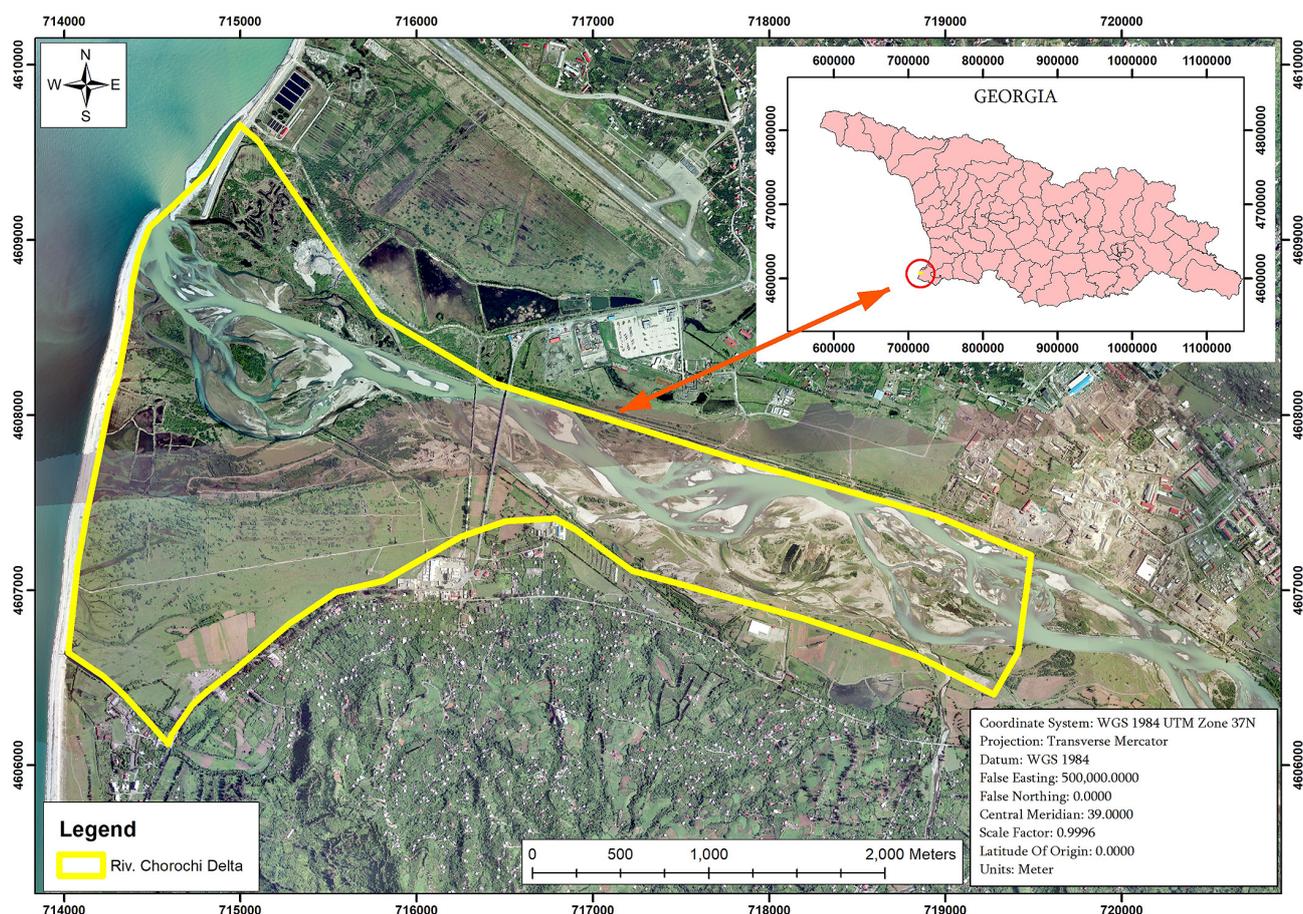


Fig. 1 Map showing the location outlined in yellow of the Chorokhi Delata where the 4 IASs were studied (700ha).

Material and Methods

Study area

The area studied is in the southwestern part of Georgia (floristic area of Adjara) and includes the Delta of the Chorokhi river, which is in the area between the border with Turkey and the Korolistskali river. In this area is a Special Protection Area (SPA) for birds for which the geographical coordinates are: 41°35.844'N / 41°33.922'E, which encompasses approximately 2,232.337 ha with an altitudinal gradient of 0 to 30 m (Paposhvili et al. 2016). The area surveyed for IASs included only semi-natural habitats in which the plant communities were only slightly altered. The total area surveyed was approximately 700 ha (Fig. 1).

Climate

Geographically, Adjara consists of two parts one close to the coast and the other inland. The coastal area mainly consists of lowlands surrounded in the east by foothills and the climate is humid subtropical. Most of the year the prevailing wind is from the west and is humid, whereas in winter it comes from inland. The average annual temperature is 13–15 °C and that of the coldest month is 5.9–7.5 °C and is rarely less than –8 °C. In the

warmest month (July–August), the average temperature is 22–23 °C, and highest temperature 39–40 °C. The average humidity is 80–87% (Manjavidze 1982; Climate change strategy of Adjara 2013).

Soils

Litoral and river detritus occurs throughout the Chorokhi Delta on the seashore and on riverside terraces (*Haplic Arenosols*, according to the WRB – World Reference Base for Soil Resources classification (FAO 2023). Alluvial meadow soils (Distric Fluvisols) are abundant here. These are non-carbon, loamy, medium soils. In addition to alluvial meadow soils, Mollic Fluvisols and Alluvial meadow marshy (Umbric Fluvisols) soils occur in fragments of forest vegetation in the lowlands. Furthermore, alluvial forest-meadow soils are non-carbon, light, medium and heavy loams, and alluvial meadow marshy soils differ in being sandy or clayey. On old river terraces the soils are usually Podzolic-glei (Gleic Podzol) or Podzolic (Distric Podzoluvisols) (Urushadze 1997).

Flora and vegetation

They have changed greatly as a result of human activity. On the banks of the Chorokhi River and edges of the canal, there is fragmented seaside lowland forest, where

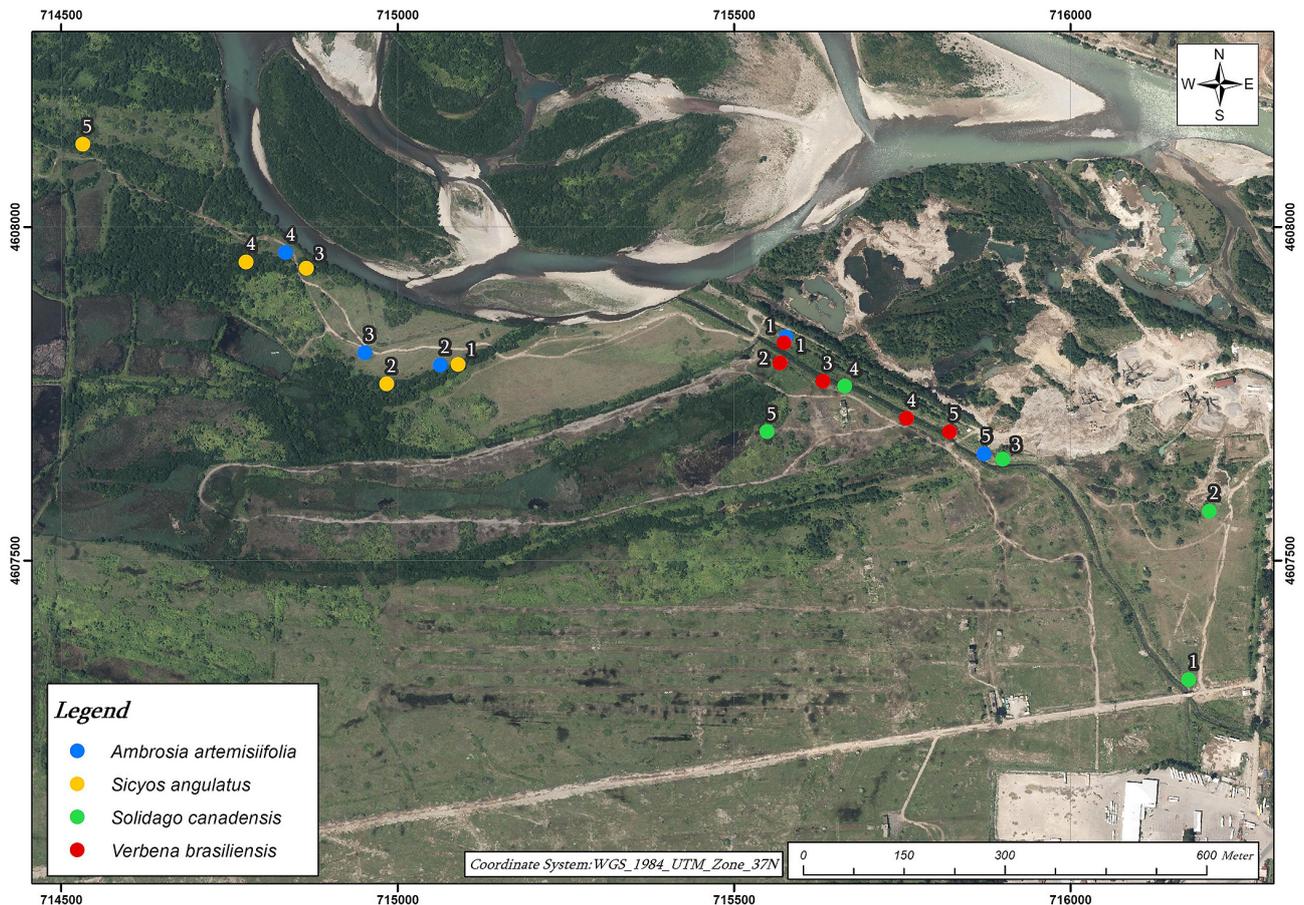


Fig. 2 Map showing the locations of the plots in the Chorokhi Delta where the four IASs were studied.

Alder (*Alnus barbata*) is the main tree. Goat willow (*Salix caprea*), white willow (*Salix alba*), Christ's thorn (*Paliurus spina-christi*), common hornbeam (*Carpinus caucasica*), Caucasian wingnut (*Pterocarya pterocarpum*), chestnut (*Castanea sativa*) and figs (*Ficus colchica*) also occur here, but are uncommon. Small clusters of bushes of Sea buckthorn (*Hippophae rhamnoides*) and more commonly species of raspberry (*Rubus anatolicus*, *R. caesius*, *R. hirtus*, *R. serpens*) occur in the delta. There are also many small ponds, in which Trapa (*Trapa colchica*, *T. Maleevi*), yellow water-lily (*Nuphar luteum*), bladderwort (*Utricularia minor*), duckweed (*Lemna minor*) occur. Around the ponds and at the edges of the canals, there are wetland-loving plants, such as slender tufted sedge (*Carex acuta*), broadleaf cattail (*Typha latifolia*), common reed (*Phragmites australis*), soft rush (*Juncus effuses*), European water-plantain (*Alisma plantago-aquatica*), bur-reed (*Sparganium neglectum*), water flag (*Iris pseudocorus*) etc.

Among the non-native woody plants, Japanese walnut (*Juglans ailanthifolia*), heart-seed walnut (*J. cordiformis*) and honeysuckle (*Lonicera japonica*) are common in this area, whereas black locust (*Robinia pseudoacacia*), many-flowered rose (*Rosa multiflora*), Japanese spirea (*Spiraea japonica*), tree of heaven (*Ailanthus altissima*) and

boxelder maple (*Acer negundo*) are uncommon. There are also numerous non-native herbaceous plants.

Fieldwork

The four IASs (*Ambrosia artemisiifolia*, *Sicyos angulatus*, *Verbena brasiliensis* and *Solidago canadensis*) that are widely distributed in the Chorokhi Delta were surveyed in 2021–2022 during visits to the Chorokhi Delta. During these visits sites where the IASs were abundant were identified. At these sites, 5 transects were established in semi-natural habitats, for each of the ISAs (2 m wide × 50 m long) (Fig. 2). To determine the density (expressed as the number of individuals per plot), frequency, coverage and average height of the IASs, 10 plots (1 × 1 m) five meters apart were surveyed. A total of 200 plots were surveyed for the four IASs. This was done twice during the vegetative period first at the beginning and then when the IASs were flowering and fruiting. A total of 800 records were collected during the two years of the study. Based on these results, the mean values for each species and period were calculated.

The nomenclature for the species of plant follows the Plants of the World Online (POWO 2021). Field information for mapping was collected using a handheld Garmin GPS MAP 64st. The maps were compiled using

Table 2 Monitoring data of the studied invasive alien species in the Chorokhi Delta in the period 2021–2022.

Species	Year	Av. height (m)		Density		Frequency %		Cover %	
		I	II	I	II	I	II	I	II
<i>Ambrosia artemisiifolia</i> L.	Period								
	2021	0.1	0.47	77	40	96	100	46	20
	2022	0.1	0.48	99	57	96	100	55	44
<i>Verbena brasiliensis</i> Vell.	2021	1.24	1.35	6	10	90	90	40	59
	2022	1	1.28	13	16	70	94	65	83
<i>Sicyos angulatus</i> L.	2021	3.94	7.56	11	17	84	92	52	76
	2022	0.3	6.32	6	11	83	90	14	85
<i>Solidago canadensis</i> L.	2021	0.45	62.5	12	18	6	46	40	65
	2022	0.13	0.6	40	44	44	64	45	74

geographic information systems (GIS) software ArcMap. Maps were produced using the UTM WGS 1984 Zone 37N coordinate system (Fig. 2).

Results

The results of the fieldwork done during the period 2021–2022, on the frequency, density, coverage, annual growth, flowering-fruiting and rates of spread of the IASs studied are presented in Table 2.

Ambrosia artemisiifolia

Based on the results of the field surveys, *A. artemisiifolia* occurs almost everywhere (except ponds) and on all types of soils, being especially abundant along the edges of canals, in ruderal areas and edges of forests. It inhibits the growth of other species. The average height of the plants measured in the plots were 0.47–0.48 m. The height of the plants in wet woodland and along banks of canals ranges from 1.5–1.8 m (Fig. 3). New growths are abundant in meadows, but smaller due to competition from other plants and the effects of other factors (cattle grazing).

As recorded in Table 2, the average number of individuals of *A. artemisiifolia* in the first period of 2021

**Fig. 3** Photograph of *Ambrosia artemisiifolia* L.

(period I) was higher (77 individuals) than in the second period (period II) (40 individuals). In the following year, the number of individuals recorded was higher with 99 individuals in period 1 and 57 in period 2 (Table 2, Fig. 4a). The reason for the difference in numbers recorded in the two periods each year is that this plant is an annual and ceases growing and produces a large number of seeds, the percentage germination of which is very high. In the given case, in the plots in the first period contained a large number of seedlings, many of which subsequently died due to competition within and between species. Another reason for the decrease was that the plots were subject to grazing.

The percentage cover was correlated with density. Specifically, in 2021 the coverage in the first period was 46%, whereas in the second it was 20%. In 2022, the cover values were 55% and 44%, respectively (Table 2, Fig. 4b). *A. artemisiifolia* was one of the most common IASs in the Adjara area, its frequency of occurrence in plots was 100%.

In the Adjara area (study area), *A. artemisiifolia* started germinating in March and ended in April, flowered from July to September and dispersed seed from September to October.

Verbena brasiliensis

Unlike *A. artemisiifolia*, the density of *V. brasiliensis* increased from the first period to the second in both 2021 and 2022 (Table 2, Fig. 5). The average number of individuals per plot increased from 6 in the first period of 2021 to 16 in the second period of 2022, (Table 2, Fig. 6a). The percentage cover of *V. brasiliensis* was positively correlated with its density. In 2021, the percentage cover in the first period was 40% and in the second 59%. In 2022, the percentage cover increased to on average 65% in the first period and 83% in the second (Table 2, Figs 6a, 6b). Based on these results percentage cover increased by 45% and frequency by 94%.

The average height of *V. brasiliensis* was 1.33 m, although some were 2.0–2.5 m tall. It is noteworthy that the frequency, percentage cover and density increased continuously over the period of the study, as shoots pro-

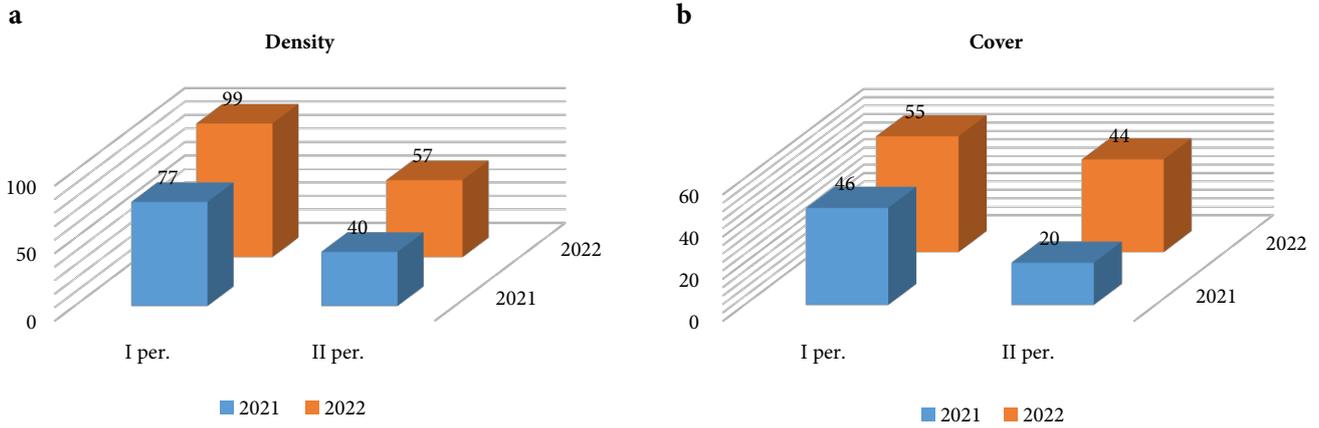


Fig. 4 *Ambrosia artemisiifolia*, a – Density; b – Percentage cover.

duced lateral shoots. Although *V. brasiliensis* in its area of origin is an annual or short-lived perennial, here it is a perennial, which enables it to dominate. Although cattle consume the thin stems, this species has a well-developed root system that can produce additional suckers, side shoots and lateral branches.

This species can produce up to 100,000 seeds in its second and subsequent years, a high percentage of which germinate, so increasing the probability of spreading further. However, this species does not occur in all types of habitats. It is especially abundant along the edges of canals, roadsides, in ruderal areas, wetlands and other places. In the Chorokhi Delta it is common on the banks of rivers, along roadsides, edges of canals, in places covered with litter and ruderal areas, where it forms large and extensive colonies.

Sicyos angulatus

The results for this species indicate it is quite abundant in the Chorokhi Delta (Table 2, Fig. 7). The spring of 2021 was particularly favourable for seed germination and it was abundant in transects and plots. In first period of 2021, average density was 11 individuals per plot and 17 in the second period. this increase was due seed

germinating in summer. The spring of 2022 was colder and in the first period, the average density per plot was 6, and in the second 11. Thus, an increasing trend was recorded in both years. The percentage cover in the first period of 2021 was 52% and 76% in the second (Table 2, Figs 8a, 8b). Its frequency was 92%.

Sicyos angulatus is a climbing plant, the average length of a stem ranges from 6.5–7.5 meters, with some individ-



Fig. 5 Photograph of *Verbena brasiliensis* Vell.

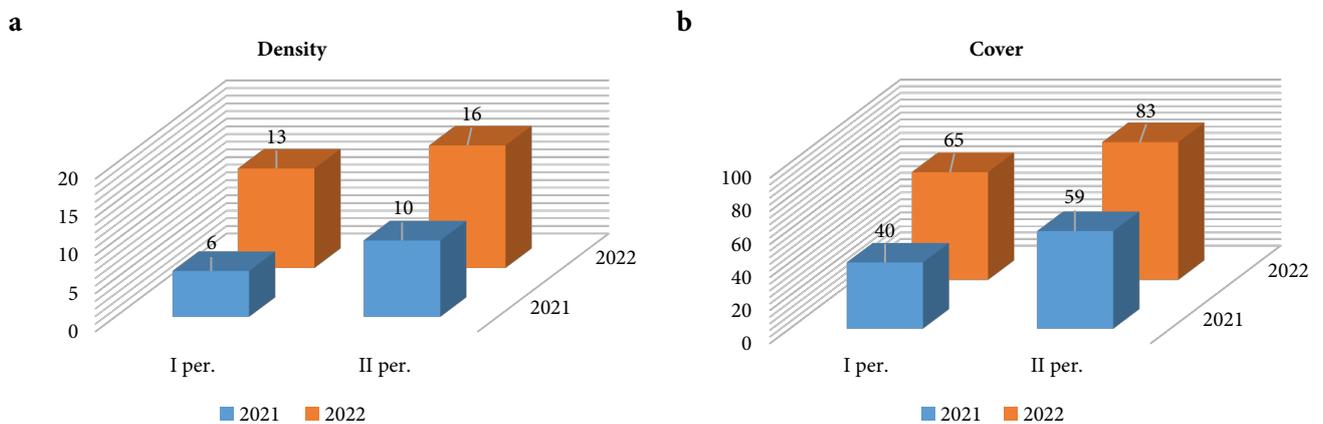


Fig. 6 *Verbena brasiliensis*, a – Density; b – Percentage cover.



Fig. 7 Photograph of *Sicyos angulatus* L.

uals reaching 12–16 m. This plant produces many lateral shoots; thus, its percentage cover increases. This species is spreading in the area studied and colonizing areas with other types of soils. *S. angulatus* mainly occurs in wetlands, along canals and rivers in the Chorokhi Delta and especially in *Alder* forests.

Solidago canadensis

This species was first recorded in the Chorokhi Delta in 2019 since then it has been spreading, but not to the extent of the other IASs (Fig. 9). In the 5 transects, plants were recorded in 3 plots in 2 transects in the first period of 2021 and 12 plots in 3 transects in the second period and in the first period in 2022 in 22 plots in 4 transects and in the second period in 32 plots in 5 transects.

In 2021, in the first period, the average number per plot was 12 and in the second period 18. In 2022, in the first period, the average number per plot was 40 and in the second period 44 (Table 2, Fig. 10a). Percentage cover of this species in the first period of 2021 was 40%, and in the second 65% and in 2022 it was 45% in the first period and 74% in the second (Table 2, Figs 10a, 10b). Based on these results, the percentage cover increased by 34% over the period of this study. Frequency of occurrence at the beginning of the study was 6% and 64% at the end, which

is an indication of rate at which this species is spreading (Fig. 11).

Discussion

The geographical position of the valley of the Chorokhi river has favoured the spread of several IASs, but because of restrictions related to the military status of this area scientists were unable to study the non-native flora and the effects it was having on native plants. This is the first study on the growth, development, propagation and spread of four IASs (*Ambrosia artemisiifolia*, *Solidago canadensis*, *Verbena brasiliensis*, *Sicyos angulatus*) in the Chorokhi Delta. This study revealed that these IASs are spreading, occupying new habitats and degrading the area.

There are many papers and articles in newspapers on the effects of IASs on native fauna and flora, which are based on visual and literary descriptions (e.g., Kikodze et al. 2010; Beridze 2020). Of the four IASs studied, *A. artemisiifolia* is the most invasive. It is known for its allelopathic properties, which can prevent the growth and development of neighbouring plants (Vidotto et al. 2013). According to Bretagnolle and Chauvel (2009), *A. artemisiifolia* is among the 100 most invasive non-native plants in Europe and is continuing to spread in all European countries, where it is mostly found in agricultural fields, along roadsides and on riverbanks. In this study, it was recorded growing almost everywhere and in all types of soils. The effect of this species on the biodiversity in the Chorokhi Delta is high, especially in semi-natural ecosystems. It reduces the fertility and changes the structure of soil.

V. brasiliensis is an annual or short-lived perennial (Yeo 1990; GISD 2023). In the area studied it is a perennial and present throughout the year, with only the top of the shoots withering in winter. It propagates by means of seed and shoots from underground roots, which enables it to spread rapidly. According to the Global Invasive Species Database (GISD 2023) it is a significant inva-

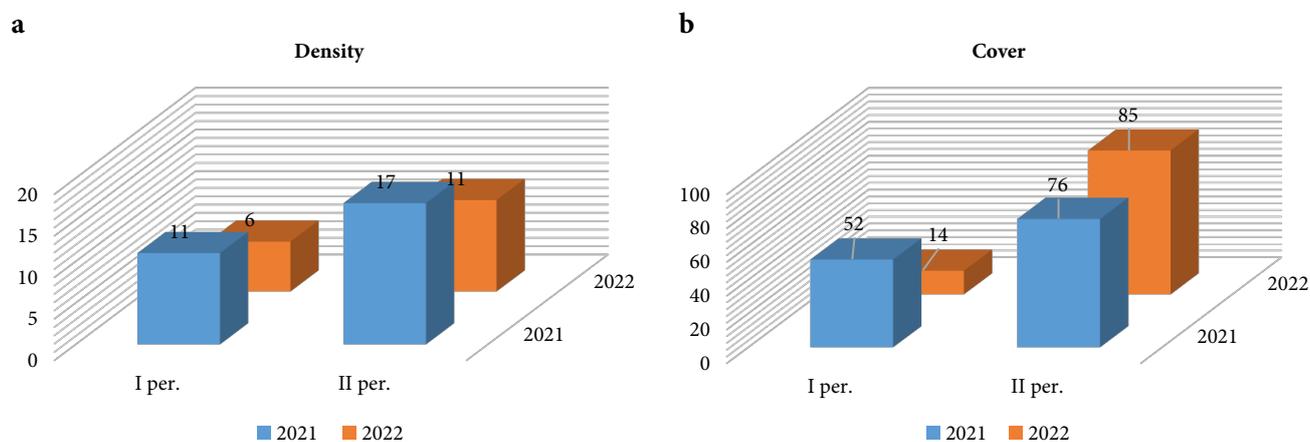


Fig. 8 *Sicyos angulatus*, a – Density; b – Percentage cover.



Fig. 9 Photograph of *Solidago canadensis* L.

sive species in the mid-south of the United States. It was first recorded in Georgia several decades ago and is now widely distributed in the lowlands in Adjara, where it is mostly found in ruderal areas, in agricultural land and near canals and rivers (Mikeladze et al. 2017). At colonized sites, this highly competitive species can alter the floristic composition. Its invasion capability is well illustrated by it being recently recorded in the northern part of Turkey (Tunçkol 2022). This species is much favoured by the local population for its beautiful flowers. As indi-

vidual plant bear flower buds, flowers and mature seeds at the same time, then once it is used as an ornamental plant, people disperse large quantities of its seed, which germinates in new environments.

S. angulatus is another IAS that is recorded in a number of countries including Georgia (Tzonev 2005; Zhao et al. 2019). It was recorded in Georgia a decade ago and is considered to be one of the most aggressive IASs and currently is widespread in the western part of the country (Mikeladze et al. 2015). The seedlings of *S. angulatus* are very similar to cucumber seedlings and it only differs when flowering and fruiting, when it has many stems that completely cover surrounding plants. The lengths of the stems range from 10–12 meters (Terzioglu and Ansin 1999; Tzonev 2005). During the current study, stems up to 20 m long were recorded. Therefore, it needs to be eradicated early in its development.

The negative effect of *S. angulatus* on the native flora and vegetation in the Chorokhi Delta is very obvious in areas with moist soils where there are large populations that engulf all other plants in the area and inhibit their growth and development by producing thick and heavy mats that cover the existing vegetation. In the future, it is very likely that it will greatly damage agricultural crops, especially corn crops and citrus trees, by markedly reducing the yield and quality of these crops. The soil-climatic

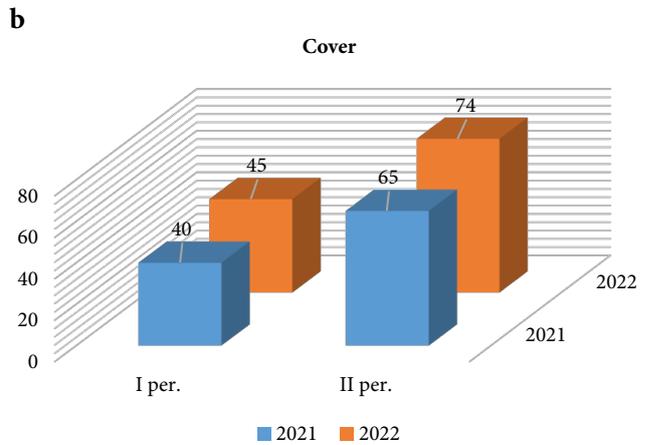
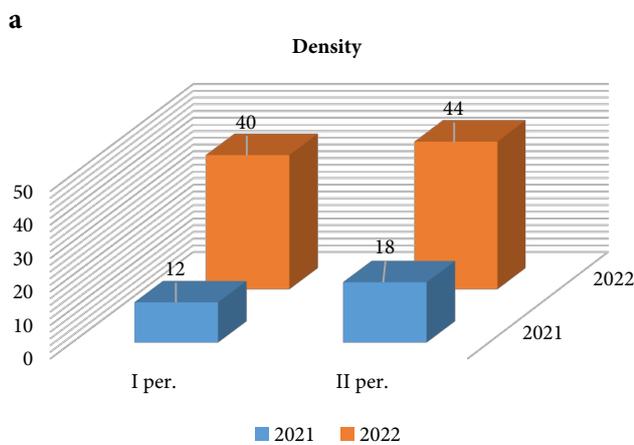


Fig. 10 *Solidago canadensis*, a – Density; b – Percentage cover.

conditions in Western Georgia, are particularly favourable for this species as it is difficult to control because after the destruction of the above-ground parts of the plant its seed can survive in the soil for at least three years.

In many European countries, *S. canadensis* is common and is continuing to spread (Kabuce and Priede 2010). However, although it is not widely spread in the Chorokhi Delta, its broad ecological tolerance and clonal growth leaves no doubt that it is a highly dangerous invasive species. By vegetative and generative propagation, it spreads rapidly producing stable and resistant colonies, which can colonize new areas. Thus, *S. canadensis* can

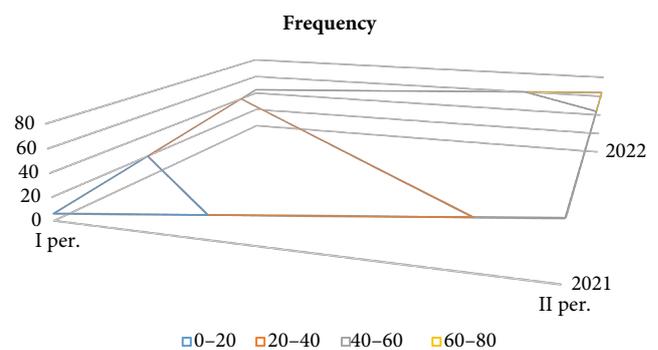


Fig. 11 Frequency of occurrence of *Solidago canadensis*.

become and remain dominant for a long time after becoming established (Kabuce and Priede 2010). Although it has a negative effect on semi-natural ecosystems and agricultural landscapes it cannot become dominant in natural ecosystems where only single individuals occasionally occur. *S. canadensis* and *V. brasiliensis* were first recorded in the flora of Georgia in the first half of the 20th century when they were present in a small area (Kolakovskii 1982). The great increase in their distribution in the Kolkheti lowlands at the beginning of the current century and the record of first individuals in the Chorokhi Delta 5–15 years ago (Mikeladze et al. 2017; Mikeladze and Bolkvadze 2021) is attributed to global changes in climate.

Although the present study focused on four IASs in the Chorokhi Delta, an Emerald Network site and Special Protection territory for birds, which is likely to have also been affected not only by other non-native plants as there are other potentially invasive plants of foreign origin in the Chorokhi Delta that were not included in this study. This fact highlights the need for further research on the distribution and effects of non-native plants on natural and semi-natural ecosystems. Moreover, the results obtained in this study can be used to predict the current and future potential distribution of the species studied (Aderoju et al. 2020). In this way, the general public could be informed of the negative consequences associated with these IASs now and in the future. Thus, although the species studied have some positive features (e.g., people use them for decoration, birds feed on the fruits), their potential invasiveness should be taken into consideration when developing programs aimed at preventing their spread and minimizing their effect on ecosystems.

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IN VITRO ASYMBIOTIC PROPAGATION OF THE VULNERABLE SLIPPER ORCHID *CYPRIPEDIUM CORDIGERUM* D. DON

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ABSTRACT

This paper presents an attempt to establish a protocol for the conservation of *Cypripedium cordigerum* D. Don a vulnerable species of orchid using *in vitro* symbiotic seed germination. The suitability of four orchids seed germination media [Terrestrial orchid medium (BM), modified terrestrial orchid seed germination medium (BM-1), Malmgren modified terrestrial orchid medium (MM), Knudson C medium (KC)] was tested using different photoperiods i.e. 24-h dark or 12-h light per day. The seed capsules were harvested at two different stages of development in order to determine the effect of capsule maturity on seed germination. The maximum percentage germination of seed from intact capsules in the dark was $65.00 \pm 0.12\%$ on the BM-1 medium. Release of brownish exudates was stopped by the addition of activated charcoal to the cultures. Seedlings developed in 26.17 ± 0.17 weeks. The current study is the first to report the *ex-situ* conservation of *C. cordigerum*.

Keywords: asymbiotic; *in vitro*; ornamental; terrestrial; vulnerable orchids

Abbreviations: BM – Terrestrial orchid medium; BM-1 – Modified terrestrial orchid medium; MM – Malmgren modified terrestrial orchid medium; KC – Knudson C medium

Introduction

The genus *Cypripedium* includes 56 species and 4 varieties and belongs to the family Orchidaceae (subfamily: Cypripedioideae Lindl.) (Cribb 1997; Wu et al. 2009). It is a horticulturally important genus. The subfamily Cypripedioideae includes the following genera: *Cypripedium*, *Paphiopedilum*, *Phragmipedium* and *Selenipedium*. *In vitro* initiation and establishment of cultures of *Cypripedium* is difficult. Among these genera, *Cypripedium* is reported to be the most difficult (Arditti 2008). The only and most common method for the *in vitro* propagation of species of *Cypripedium* is a symbiotic seed germination. One of the species, *Cypripedium cordigerum*, is a vulnerable species. It is also known as heart-lip lady-slipper orchid. *C. cordigerum* grows at an altitude of 2,500–3,000 m a. s. l. in small areas in terrestrial habitats in Bhutan, Nepal and temperate Indian Himalayas (Bose and Bhattacharjee 1980). This species has beautiful, solitary, white flowers with heart-shaped labellum and plicate leaves (Fig. 1). However, although *C. cordigerum* is well known for its beautiful flowers, its therapeutic utility is unexploited, but its tender leaves are cooked as a vegetable by local rural communities (www.ionopsis.com/edible_orchids.htm).

Currently, *Cypripedium cordigerum* grows sporadically in small groups consisting of 4–6 plants in humus rich soil under partial shade from *Cedrus* trees. Previously, dense populations of this species occurred in habitats in north-western Himalayas (Jalal et al. 2009). Several factors are associated with the decline in the abundance of this species: destruction of its natural habitat due to deforestation for residential areas and agricultural pur-

poses, logging, overgrazing, trampling, climate change and indiscriminate collecting. As a consequence, the rate of decline of this species is increasing. According to the current conservation status, *C. cordigerum* is categorized as vulnerable (VU) and is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023). It is categorized as vulnerable species in the Red Data Book of Indian Plants volume 1 (www.iucnredlist.org; Jalal et al. 2009).

This paper proposes that mass propagation of this species, using tissue culture techniques, be used to assist in the conservation of *C. cordigerum*. Currently, there is no information on *in vitro* symbiotic seed germination of *C. cordigerum*. The objective of this study is to establish a protocol for *in vitro* symbiotic seed germination using immature/mature seed, four types of media kept in the dark or under a 12-h photoperiod.

Percentage germination of slipper orchids in both *in vivo* and *in vitro* conditions is very low, particularly so *in vitro* (Pierik et al. 1988). They lose the ability to germinate shortly after harvesting (De Pauw and Remphrey 1993). Although, a single capsule contains millions of seeds, very few germinate in the wild.

The use of *in vitro* culture techniques greatly enhances the ability to propagate, multiply and conserve the gene pool of endangered and commercially viable species. *In vitro* symbiotic seed germination enables the conservation of endangered germplasms as it can be used to rescue embryos that would be aborted in natural habitats and shortens the breeding cycle (Knudson 1922; Arditti et al. 1982). In addition, *in vitro* symbiotic seed germination can also increase the number of mature seeds that

germinate successfully (Lauzer et al. 1994; Whigham et al. 2006). For these reasons, a symbiotic seed germination is utilized here to initiate cultures *in vitro*.

Terrestrial species of orchids from temperate regions are more difficult to germinate *in vitro* than tropical epiphytic species (Oliva and Arditti 1984; Hossain et al. 2013). Among terrestrial species of orchids, *Cypripedium* have specific microhabitat requirements for germination (Kull 1999). They are a source of material for horticulture and medicine (Zhu 1989). Unlike many other orchids, species of *Cypripedium* have stringent needs for germination. There is little in the literature on their specific requirements (Arditti and Ernst 1993). Several physical and nutritional factors affect seed germination in *Cypripedium* (Zeng et al. 2014). In nature, germination and propagation of species of *Cypripedium* is quite low due to ecological constraints. Currently, there are only a few reports on the a symbiotic seed germination of *Cypripedium* species *in vitro* (De Pauw and Remphey 1993; Hsu and Lee 2012; Huh et al. 2016) and no information exists on *in vitro* symbiotic seed germination of *Cypripedium cordigerum*. There is a need for more studies on seed germination in *Cypripedium*. For that reason, the present study provides information on the germination of *Cypripedium cordigerum in vitro*, a protocol for a symbiotic seed germination, and identifies the optimum (1) capsule age, (2) medium for germination and (3) photoperiod.

Materials and Methods

Plant collection

A plant of *Cypripedium cordigerum* with capsules growing on a roadside slope (altitude 2,276 m a. s. l.) at Chabra, Shimla forest hills, Himachal Pradesh, India (Latitude range 31°6'12"N 77°10'20"E) was collected in summer (May–June). The plant was replanted in a pot (diameter 27.5 cm × 22.4 cm) containing soil and kept in a greenhouse under natural light conditions, 70% relative humidity and 22 °C / 15 °C day/night temperatures. The seed from dehisced pods was collected on paper.

Culture medium for *in vitro* seed germination

To initiate the cultures, four media: BM (Terrestrial orchid medium; van Waes 1984), BM-1 (Modified terrestrial orchid medium; van Waes and Debergh 1986), KC (Knudson 1946), MM (Malmgren modified terrestrial orchid medium; Malmgren 1996), were used. The pH of each medium was adjusted before autoclaving using 1N NaOH and 1N HCl. The autoclaved media were kept at 37 °C to check for any contamination. Seed viability was assessed by staining with TTC 1% (2,3,5-triphenyl-2H-tetrazolium chloride) at a pH of 6.5 adjusted using 1N NaOH (van Waes and Debergh 1986; Lauzer et al. 1994). The seed was stained for 40–48 h at 30 °C in the dark. Viable seeds contained ovoid embryos that ap-



Fig. 1 Plant of *Cypripedium cordigerum*.

peared healthy and were a pink-red in colour. To reduce the release of brownish exudates by growing seedlings, activated charcoal (2 g / l) was added to the BM-1 medium.

Surface sterilization of green capsules

Each capsule was first scrubbed with a soft brush in running tap water to remove any debris and rinsed thoroughly in a detergent solution. They were swabbed with ethyl alcohol under sterile (laminar) hood and surface sterilized with an aqueous solution of 0.1% mercuric chloride (HgCl₂; Qualigens, Mumbai, India) containing 1–2 drops of Teepol as a wetting agent for 7 min, then rinsed with sterilized distilled water to remove any traces of mercuric chloride on its surface. Thereafter, the capsule was flamed on a burner, slit open longitudinally and the seed scooped out into a petri dish.

Sterilization of seeds from dehisced capsule

The seed from a dehisced capsule was collected on a thin sheet of glazed paper. Inside a laminar air-flow hood, with great care, the seed was poured in 100 ml flask and treated with sodium hypochlorite (4%) (Merck, Mumbai, India) for 7–8 minutes. The solution was then filtered and washed twice with sterilized distilled water. The sterilized seeds were evenly spread thinly over the media in culture tubes with the help of a spatula.

Inoculation and incubation conditions

The inoculations were done under aseptic conditions in a laminar air-flow cabinet. The culture vessels were incubated either in the dark or under a 12-h light photoperiod of 40 μmol⁻² s⁻¹ light intensity (Fluorescent tubes, Philips India Ltd, India) at 25 ± 2 °C. There were eight replicates of each experiment. To check the reproducibility of the protocol, the experiment was repeated twice. The cultures were sub-cultured as and when required.

Percentage germination

Four weeks after of inoculation, a few seeds were scooped-out of the culture vessel. These were dispersed in a drop of water on a glass slide and observed under a light microscope. Percentage germination was calculated using the following formula:

Germination (%) = (Number of enlarged seeds with swollen embryos/Total number of seeds) × 100. This was recorded weekly in order to determine differences in the stage of development of the cultures, using a stereo zoom microscope (Nikon H600L, Japan).

Observation and statistical analysis

This experiment was of complete randomized design (CRD). For each treatment there were four replicates. The cultures were observed regularly under a binocular microscope (Olympus SZX10, Japan). Photographs were taken using a digital camera, Nikon, Digit Sight, DS, Ril Nikon, and data recorded accordingly. The results were tested using one-way ANOVA and analysed using Tukey's Multiple Comparison test in SPSS (Version 17) software package (SPSS Inc., Chicago, corporation, US).

Results

Effect of capsule age and photoperiod on a symbiotic seed germination

The age of the capsule determined the percentage germination of the seed, with the seed from the immature capsule germinating more readily, $65.05 \pm 0.13\%$, than that from the mature capsule (Table 1). The mature seeds took longer to germinate and the percentage germination was lower, $18.02 \pm 0.02\%$ (Table 1).

Seeds started germinating after 7.15 weeks, when embryos started to swell (Table 2) and achlorophyllous spherules ruptured the testa. These grew in size while

still attached to the seed coat. During the next 4 weeks, the conical, achlorophyllous protocorms developed (Fig. 2a). A shoot-tip became evident on the apical part of the protocorm and leaf primordia developed after 15.95 ± 0.25 weeks. The protocorms remained achlorophyllous under both light conditions (12-h light / 24-h dark). The chlorophyll developed at 1st leaf stage. Just below the shoot-tip a root primordium developed after 21.88 ± 0.25 weeks (Fig. 2a). The root was off-white in colour and densely hairy (Fig. 2b). Morphogenetic events were more advanced in those cultures kept in the dark than in those kept under a 12-h photoperiod. Near the shoot primordia, a horizontally growing positively phototropic root developed after 26.17 ± 0.17 weeks.

Effect of media on percentage germination and seedling development

The quickest response and highest percentage germination of $65.05 \pm 0.13\%$ was recorded in the dark 7.15 ± 0.17 weeks on the modified terrestrial orchid medium (Fig. 3). For the BM and KC media, the percentage germination was lower, with only 30.10 ± 0.10 and 20.75 ± 0.05 of the seeds germinating, respectively. Under a 12-h photoperiod, the percentage germination was lower. The seeds took longer to start germinating and the percentage germination was also lower for all other media (Table 2).

Those cultures that were initially kept in the dark were illuminated for 12-h per on their respective media in order to prevent the protocorms becoming necrotic and dying. The development of seedlings, within 26.17 ± 0.17 weeks, was recorded for the BM-1 medium. The seeds cultured on MM medium did not germinate in the dark or under a 12-h photoperiod. The seedlings that developed on the KC medium were quite weak and did not grow, and were transferred to BM-1 medium.

Table 1 Effect of capsule age on the percentage germination of *Cypripedium cordigerum* on the BM-1 medium.

Capsule stage	Germination %
Mature Seed (Dehisced)	18.02 ± 0.02^a
Immature (Undehisced)	65.05 ± 0.13^b

Values in columns with the same superscript are not significantly different at $p \leq 0.05$ according to Tukey's test.

Table 2 *In vitro* germination of immature seed of *Cypripedium cordigerum* on different media under a 12-h photoperiod.

Media	Germination percentage	Initiation of response (weeks)	Development of (weeks)			Seedlings (weeks)
			Protocorm	1st leaf	1st root	
BM	30.10 ± 0.10^{bd}	11.12 ± 0.14^{bcd}	17.00 ± 0.00^{bcd}	21.03 ± 0.64^{bcd}	24.68 ± 0.09^{bcd}	29.20 ± 0.27^{bcd}
BM-1	65.05 ± 0.13^{acd}	7.15 ± 0.17^{acd}	11.10 ± 0.15^{acd}	15.95 ± 0.25^{acd}	21.88 ± 0.25^{acd}	26.17 ± 0.17^{acd}
MM	0.00^{abc}	0.00^{abc}	0.00^{abc}	0.00^{abc}	0.00^{abc}	0.00^{abc}
KC	20.75 ± 0.05^{bd}	15.12 ± 0.15^{abd}	20.05 ± 0.11^{abd}	25.42 ± 0.21^{abd}	27.05 ± 0.19^{abd}	31.12 ± 0.09^{abd}

BM-1 – terrestrial orchid medium (modified); BM – terrestrial orchid medium; MM – Malmgren modified terrestrial orchid medium; KC – Knudson C medium. In a column, the values followed by the same letter superscripts are not significantly different at $p \leq 0.05$ according to Tukey's test.



Fig. 2 *In vitro* symbiotic seed germination and seedling development of *Cypripedium cordigerum* (a) Different stages in the germination and development, (b) Development of 1st leaf, (c) Seedling formation.

In culture the seedlings produced brownish exudates that inhibited the growth of leaves. In order to promote growth activated charcoal (2%) was added to the BM-1 medium, in which the seedlings produced shoots (Fig. 2c).

Discussion

The success of a symbiotic seed germination depends on the maturity of the seed and a suitable culture medium. In the current study, percentage germination was significantly affected by the maturity (harvesting time) of the capsule, culture medium and photoperiod.

A higher percentage of the seed obtained from green capsules germinated than that from dehisced capsules. Similar responses are reported for other species of *Cypripedium*, *Paphiopedilum spicerianum* and *P. venustum*, in which immature seed germinated better than mature seed (Kaur and Bhutani 2013, 2016; Zeng et al. 2014). According to Lee et al. (2007) and Ding et al. (2004) there is a positive correlation between metabolic activity of the embryos and percentage germination. Although the earliest stage at which immature seed can germinate was not determined, previous studies indicate that the decline in percentage germination is primarily due to accumulation of inhibitory substances that lead to rapid loss of viability and induces dormancy in the seeds (Stoutamire 1974; Linden 1980; van Waes and Debergh 1986; Miyoshi and Mii 1988; De Pauw and Remphrey 1993; Rasmussen 1995). Lauzer et al. (1994) state that mature seed of *Cypripedium acaule* is extremely difficult to germinate. Zeng et al. (2014) indicate that mature seed has an impermeable testa and the suspensor channel is also closed and consequently, the embryo desiccates, which accounts for the reduction in the percentage germination in species of *Cypripedium*. Harvais (1980) correlate the reduced percentage germination of seeds of *Cypripedium* with the accumulation of suberin in the testa, which make mature

seed hydrophobic and prevents absorption water and nutrients by the embryo, which prevents mature seed from germinating in *in vitro* (Lee et al. 2008).

In the current experiment, there were significant differences in percentage germination and time taken by seed to initiate germination on variety of different media. The maximum percentage germination was recorded on the BM-1 medium, followed by the BM and KC media. A comparative analysis of the composition of media indicates that seed of *C. cordigerum* require organic nitrogen. The available organic nitrogen in the different media differed, with the most in BM-1. In this experiment, the inorganic nitrogen content of KC may have reduced percentage germination due to the lower activity of nitrate reductase as earlier suggested by Raghavan and Torrey (1964), van Waes and Debergh (1986) and Malmgren (1992). The results presented corroborate earlier results for *Habenaria macroceratitis* (Stewart and Kane 2006), *Paphiopedilum spicerianum* (Kaur and Bhutani 2013) and *Paphiopedilum venustum* (Kaur and Bhutani 2016) that

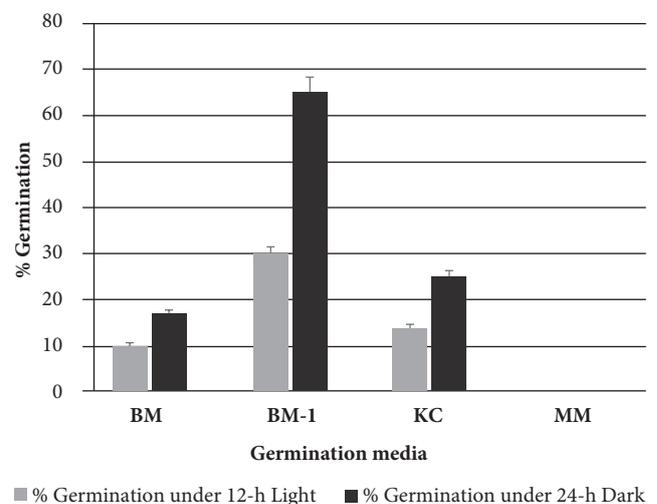


Fig. 3 Percentage germination of *Cypripedium cordigerum* recorded on different media and under different photoperiods.

an organic form of nitrogen induces high levels of germination and growth in cultures. Organic compounds present in culture media promotes seed germination in several species of slipper orchids (Curtis 1947; Stewart and Kane 2006; Dutra et al. 2008; Kaur and Bhutani 2016) and *C. cordigerum* does not appear to be an exception, as its seed failed to germinate in the MM medium, which is also an organic nitrogen-based culture medium, but the MM medium contains the amino acid glycine, whereas, BM-1 medium contains amino acid L-glutamine. Thus, the amino acid glycine could have inhibited the germination of *C. cordigerum* on the MM medium.

In *C. cordigerum*, darkness (24-h) favoured the early onset of seed germination and a high percentage germination. These results are in accord with earlier results on the germination of various species of terrestrial orchids, in which germination was inhibited when illuminated (Arditti et al. 1981; van Waes and Debergh 1986; Pierik et al. 1988; Yamazaki and Miyoshi 2006; Zeng et al. 2012; Kaur and Bhutani 2013). In contrast, germination in the slipper orchid *Paphiopedilum venustum* is higher when illuminated (Kaur and Bhutani 2016). Previous studies indicate that the responses of terrestrial orchids to illumination in terms of germination vary and are species specific (Arditti et al. 1981).

The highest percentage germination for *C. cordigerum* was recorded in the dark. In nature, *C. cordigerum* grows mainly in shaded areas; thus, this appears to be an adaptation, which affects its germination *in vitro*. The findings of this experiment and descriptions in previous studies support the inhibitory effect of illumination and darkness, on percentage germination and seedling development in terrestrial species of orchids and the response to these conditions appear to be an intrinsic trait (genotype) of each species.

In *C. cordigerum*, roots developed after the formation of protocorms similar to that reported earlier for *Cypripedium reginae* (Zeng et al. 2014). Previous studies reveal that the development of roots in species of *Cypripedium* vary; it is species specific and according to Oliva and Arditti (1984), in the majority of other species of *Cypripedium* there is simultaneous formation of shoots and roots. However, interestingly in *C. acaule* the shoots developed after the formation of roots, whereas it is the reverse in *C. californicum*.

In *C. cordigerum*, the production of chlorophyll occurs after the formation of a protocorm. Stoutamire (1974) states that protocorms lacking chlorophyll are a characteristic feature of species growing in well-drained soils. Since *C. cordigerum* also grows in soil, protocorms lacking chlorophyll appear to be a genetic trait.

In culture, the exudation of phenolic compounds was successfully stopped by the addition of activated charcoal to the medium. Addition of activated charcoal also promoted an increase in the number of shoots. The beneficial effects of activated charcoal is that it adsorbes inhibitory substances (Phenolics) as reported earlier for seedling

cultures of *Paphiopedilum* and *Phalaenopsis* (Butcher and Marlow 1989; Hicks and Lynn 2010) *Paphiopedilum spicerianum* (Kaur and Bhutani 2013) and *P. venustum* (Kaur and Bhutani 2016). Pacek-Bieniek et al. (2010) in their study on *Zygostales grandiflora* state that activated charcoal promotes germination, seedling development and growth of aerial roots. There are reports of the supportive effect of activated charcoal on the growth of seedlings of *Cypripedium* spp. (Ernst 1974, 1975; Yan et al. 2006; Bae et al. 2009).

Conclusion

In vitro symbiotic seed culture facilitates the conservation of germplasm of vulnerable species of orchids of floricultural importance. Modified terrestrial orchid medium proved the best in terms of germination in the dark and seedling development under 12-h photoperiod. For the differentiation of protocorms into seedlings, the cultures initially kept in the dark required exposure to a 12-h photoperiod. The results of the present study should facilitate future improvements in the *ex-situ* conservation of this vulnerable species and the restoration of *C. cordigerum* in its natural habitat. Further research is also needed on improving the *in vitro* and *ex vitro* methods for saving the germplasm of this horticulturally important species, especially the role of mycorrhiza in establishing symbiotic cultures, initiating *in vitro* flowering and acclimatization of *in vitro* cultures.

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DIFFICULTIES IN DETERMINING DISTRIBUTION OF POPULATION SIZES WITHIN DIFFERENT ORCHID METAPOPULATIONS

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ABSTRACT

When examining the probability of extinction of a given orchid species, the species must be viewed as a metapopulation composed of many individual populations connected by migration. In biology, much attention has been paid to the dynamics of metapopulations, especially in the situation where metapopulation dynamics are affected by active migration of individuals between populations. However, this is not the case with orchids. Their seeds are passively spread by wind, and therefore are unable to actively choose the point where they land (passive migrants, unlike, e.g., butterflies, which can actively look for a suitable site that hosts, hosted or can potentially host a population of their species (active migrants)). Thus, while active migrants can often find a suitable destination for their migration, passive migrants often die after landing at an unsuitable site. One would therefore expect that, other things being equal, the proportion of suitable sites inhabited by active migrants is larger than that inhabited by passive migrants. In passive migrants (orchids) we may therefore meet metapopulation dynamics of a different, yet unexplored type, in which some existing localities die out and new localities appear, in the vast majority where no orchids have ever grown before. This type of dynamics has not yet been studied anywhere and this paper is intended to be the first step in this direction. The main goal here is therefore empirical determination of actual distributions of population sizes in different metapopulations. We do it in four regions of the Czech Republic and for four species of orchids, considering the factors that influence it.

Keywords: distribution of population sizes; metapopulations; orchids; random migration

Introduction

One of the problems for organisms in general and for orchids in particular, is a very significant decrease in their biodiversity. According to the 2019 International Platform for Biodiversity and Ecosystem Services (IPBES) State of Biodiversity Report (Díaz et al. 2019), the sixth mass extinction of species is currently underway, reaching up to a hundred times the rate compared to the situation over the last ten million years. Most terrestrial and aquatic organisms are threatened primarily by the loss of natural habitats and this is also the case for orchids (Štípková and Kindlmann 2021).

When examining the probability of extinction of a given orchid species, the species must be viewed as a metapopulation composed of many individual populations connected by migration (Hanski and Gilpin 1997; Hanski 1999). Much attention has been paid to the dynamics of metapopulations, especially in the situation where metapopulation dynamics are affected by the migration of individuals between populations (Hanski and Gilpin 1997; Hanski 1999).

However, this is not the case with orchids. Their seeds are passively spread by wind, and therefore are unable to actively choose the point where they land (passive migrants), unlike, e.g., butterflies, which can actively look for a suitable site that hosts, hosted or can potentially

host a population of their species (active migrants). Thus, while active migrants can often find a suitable destination for their migration, passive migrants often die after landing at an unsuitable site. One would therefore expect that, other things being equal, the proportion of suitable sites inhabited by active migrants is larger than that inhabited by passive migrants. In passive migrants (orchids) we may therefore meet metapopulation dynamics of a different, yet unexplored type, in which some existing localities die out and new localities appear, in the vast majority where no orchids have ever grown before.

It is therefore a different and unexplored type of metapopulation dynamics, in which occurrence at some localities cease and new localities are occupied, which in the vast majority of cases are where no orchids may have ever grown before. This type of dynamics has not yet been studied and this paper is intended to be the first step in this direction. In defining a new type of metapopulation dynamics, it is first necessary to determine the distributions of population sizes within different metapopulations and determine the factors associated with these distributions for different species of orchid in the field. Thus the main objective of this study is to determine the distributions of population sizes in different metapopulations. This was done in four regions of the Czech Republic and for four species of orchids, taking into consideration the factors associated with it.

Materials and Methods

Study area

For this study, four regions in the Czech Republic were selected: Polabí (a lowland area), South Bohemia (area of low foothills), Bohemian-Moravian Highlands (high foothills) and Jeseníky Mountains (mountainous area). A map showing the areas studied is presented in Fig. 1.

Polabí is in the vicinity of the towns of Jaroměř and Lovosice. This lowland area is located around the river Elbe and consists of a plain and a slightly hilly area with an altitude of 150–300 m a. s. l. It includes an area of approximately 5,000 km² and is mainly an intensively managed agricultural landscape, with little woodland.

The South Bohemian region is an area of low foothills in the southern part of the Czech Republic with many aquatic biotopes, which is surrounded by highlands. The highest point in the region is the Plechý peak (1378 m a. s. l.) in the Šumava region, while the lowest is the level of the Orlik dam (350 m a. s. l.). On average, the altitude is around 400–600 m a. s. l. It includes an area of approximately 10,000 km².

Bohemian-Moravian Highlands is a high foothill area mainly in the Vysočina region, but also extends into the Pardubický region. This area is in the northern part of the geomorphological region of the Bohemian-Moravian Highlands, specifically in the vicinity of Žďárské vrchy

and Železné hory. In this area an area of approximately 1300 km² was studied and many orchid localities recorded. The average height of this area is approximately 660 m a. s. l.

Jeseníky is a mountainous area in the northern part of Moravia in the region of Silesia. The Hrubý and Nízky Jeseník mountains, Rychlebské hory and Králický Sněžník are in this area. In the Jeseníky Mountains the highest altitude is at Praděd (1491 m a. s. l.).

Species of orchid studied

Four species of orchids were studied: *Dactylorhiza majalis*, *Platanthera bifolia*, *Neottia ovata* and *Dactylorhiza fuchsii* (Figs 2a–d). These species are among the most abundant in the areas studied.

Dactylorhiza majalis (Fig. 2a) is relatively widely distributed. It is found in Western Europe, Scandinavia, the Balkan Peninsula and Siberia. Until the second half of the 20th century, *D. majalis* was a widespread species, especially in wet meadows and wetland habitats. It is now designated an endangered species, specially protected species, and a vulnerable taxon (category C3) in the framework of the Red List (Grulich and Chobot 2017). In the lower regions of the Bohemian-Moravian Highlands, this species is almost extinct, or the populations are very small (Čech et al. 2017b).

Platanthera bifolia (Fig. 2b) is perennial orchid. On the Red List, it is in category C3, i.e., a vulnerable tax-

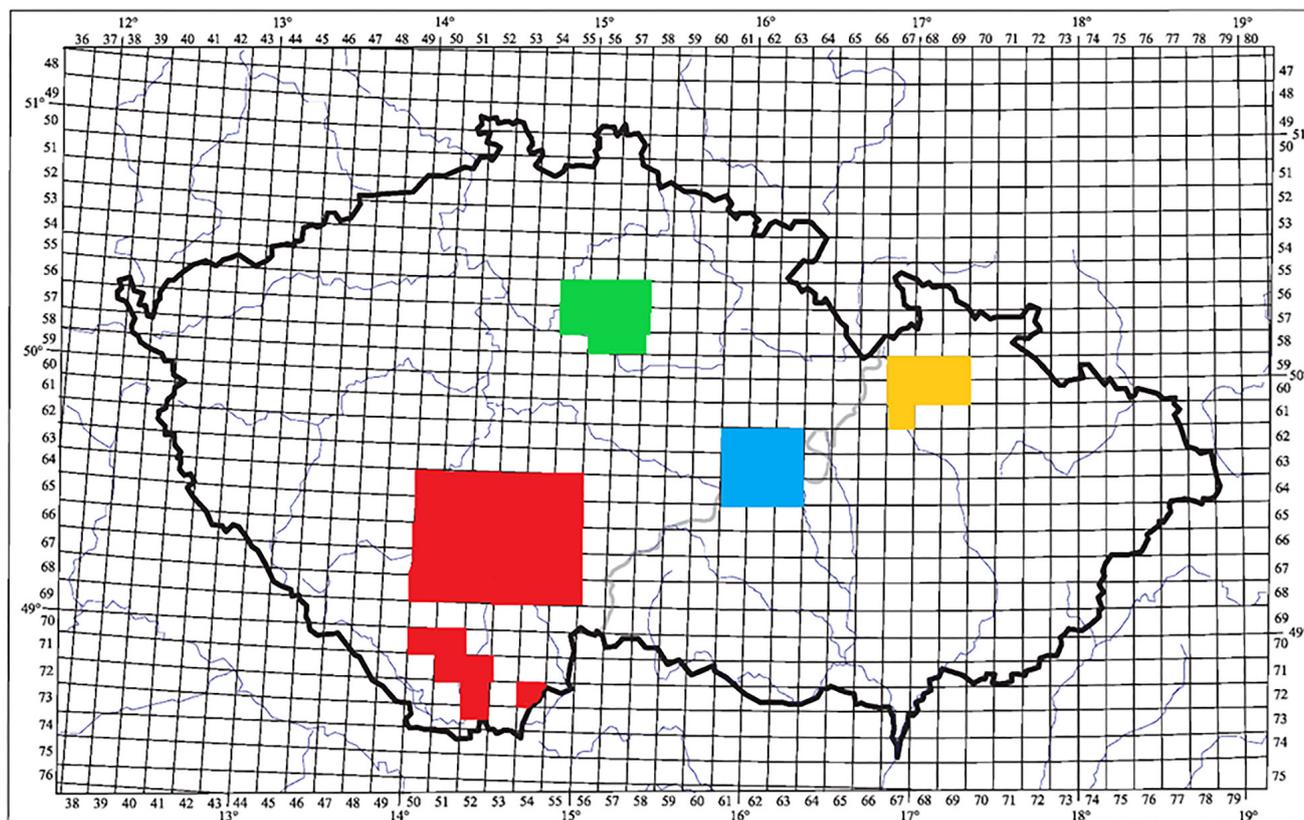


Fig. 1 Map of the Czech Republic divided into vegetation squares showing the areas studied: Polabí (green), South Bohemia (red), Bohemian-Moravian Highlands (blue) and Jeseníky (yellow). Source: <https://www.entospol.cz/sit-mapovych-ctvercu/>.



Fig. 2 Species of orchids studied:

(a) *Dactylorhiza majalis* (Čech et al. 2017b), (b) *Platanthera bifolia* (Čech et al. 2017d), (c) *Neottia ovata* (Čech et al. 2017c), (d) *Dactylorhiza fuchsii* (Čech et al. 2017a).

on (Grulich and Chobot 2017). It occurs mainly in forest and non-forest biotopes, such as dry lawns including fennel meadows, bushy areas, at the edges of woodland and in deciduous forests, but especially large meadows and deciduous habitats (Čech et al. 2017d).

Neottia ovata (Fig. 2c) is designated a near-threatened taxon (category C4a) in the Red List of the Czech Republic (Grulich and Chobot 2017). It has a wide ecological amplitude and is recorded in most of Europe. It occurs mainly in moist habitats with a higher nutrient content. In the Bohemian-Moravian Highlands, it occurs in mesophilic environments, such as wet meadows and peatlands. However, it is most likely to occur in very small populations (Čech et al. 2017c).

Dactylorhiza fuchsii (Fig. 2d) like *N. ovata*, is a near-threatened taxon (C4a) according to the Red List (Grulich and Chobot 2017). It occurs throughout Europe its range extends to Asia in the vicinity of Lake Baikal. In the Bohemian-Moravian Highlands, it occurs mainly at high altitudes, especially in oligotrophic sedge peat meadows, very wet sedge meadows, or ephemeral wet habitats. It also occurs in forest habitats, such as spruce-alder forests, forest meadows or around springs (Čech et al. 2017a).

Database and its updates

As part of this study, data from the Discovery Database of the Agency for Nature and Landscape Protec-

tion (NDOP), which collects data on the occurrence of plants in the Czech Republic, was used. This database is not accessible to the public, therefore only part of it was made available for the purposes of this project, i.e., data concerning the four species of orchids in the four regions studied. In total, 1466 records were listed in the database, for which the name of the plant, GPS coordinates of occurrence and name of the locality, number of flowering individuals at the locality, dates of visits, numerical designation of the vegetation square, description of the locality, possibly a note on management at that locality and the name of the finder.

Field study

Data on the frequency of the species of orchids studied at localities located in the Bohemian-Moravian Highlands in the NDOP were often not up to date (often more than twenty-year-old records) or the number of flowering individuals at the locality was not indicated. Therefore, as part of the field research, the data was updated in 2021 (for all four above-mentioned species of orchids) and in 2022 and 2023 (only for *D. majalis*).

Data collection in the field always took place between the end of May and the beginning of June, i.e., during the peak flowering period of all the four orchids monitored. Using the GPS coordinates listed in the NDOP, orchid localities in the Bohemian-Moravian Highlands were visited. Flowering individuals of orchids studied were counted at these localities. In addition, the date of the visit and data on the state of the site were recorded (any management of the orchid species studied, surrounding vegetation and water regime). A total of 114 orchid localities were visited: 73 localities with possible occurrence of *D. majalis*, 15 localities of *D. fuchsii*, 16 localities of *P. bifolia* and 10 localities of *N. ovata*. The data were compared with existing records in the NDOP, which were then updated.

Data processing

The data were first classified in terms of regions (Polabí, Jeseníky, South Bohemia and the Czech-Moravian Highlands) and then in terms of species (*D. majalis*, *D. fuchsii*, *P. bifolia* and *N. ovata*). Data on the number of flowering orchids were checked. If there were multiple records of the number of flowering individuals at the same location in the database, the average and standard deviation of the number of flowering individuals from all findings were calculated for further analyses.

Unfortunately, some data on the number of individuals at a locality are missing from the original records in the NDOP, so it is not clear whether they only indicate the occurrence of a species without a census, or no individuals. These data were removed from our calculations and only data with a specifically marked number of flowering individuals were used.

Using the "FREQUENCY" matrix function in Microsoft Excel, the sites were categorized according to

the number of flowering individuals into groups of 0, 1–10, 11–30, 31–100, 101–300, 301–1000, 1001–3000, 3001–10,000 flowering individuals (i.e., using a log scale of population abundances).

From these data, bar graphs of the relationships between the number of populations (y-axis) and category according to the number of flowering individuals in the population (x-axis), i.e., the distribution of population sizes in the region were plotted for each species and region.

Due to the insufficient suitable data for comparing the four species of orchids in the four regions 2022 and 2023 only the species *D. majalis* occurring in the Bohemian-Moravian Highlands was analysed in those years.

The analysis of three-years of data on the frequency of *D. majalis* at locations in the Czech-Moravian Highlands was carried out using correlation on a logarithmic scale when graphs of the frequency of flowering individuals of *D. majalis* in individual populations were plotted in the Microsoft Excel program. The frequencies were compared between years.

Results

Images highlighted in grey were not used for further analyses, as there were less than 10 sites in each category with the number of flowering plants, which were not considered to be enough for statistical analyses.

South Bohemia

In South Bohemia, *D. majalis* was the most common of all four orchid species monitored (Fig. 3a). Data for 399 sites were analysed. Most often, the population of *D. majalis* consisted of 1–10 flowering individuals. For *N. ovata* (Fig. 3c), at 18 out of 37 localities there were no flowering individuals, with the populations most often consisting of 11–30 individuals. For *P. bifolia*, populations of 1–10 individuals prevailed (Fig. 3b).

Jeseniky Mountains

In Jeseníky Mountains, *D. fuchsii* was the most common orchid (Fig. 4d), the sizes of its populations ranged from 1 to 300 individuals. Very small populations of *D. majalis* were not recorded in this region (Fig. 4a). For *P. bifolia* (Fig. 4b), localities with 1–10 flowering individuals predominated.

Polabí

Of the four species of orchids, *N. ovata* (Fig. 5c) occurred most often in Polabí, and most often in populations of 11–30 individuals, as well as *P. bifolia* (Fig. 5b). The species *D. fuchsii* was not recorded in this region.

Bohemian-Moravian Highlands

The orchid *D. fuchsii* (Fig. 6d) was not as abundant as *D. majalis* (Fig. 6a) in this region. There were no flower-

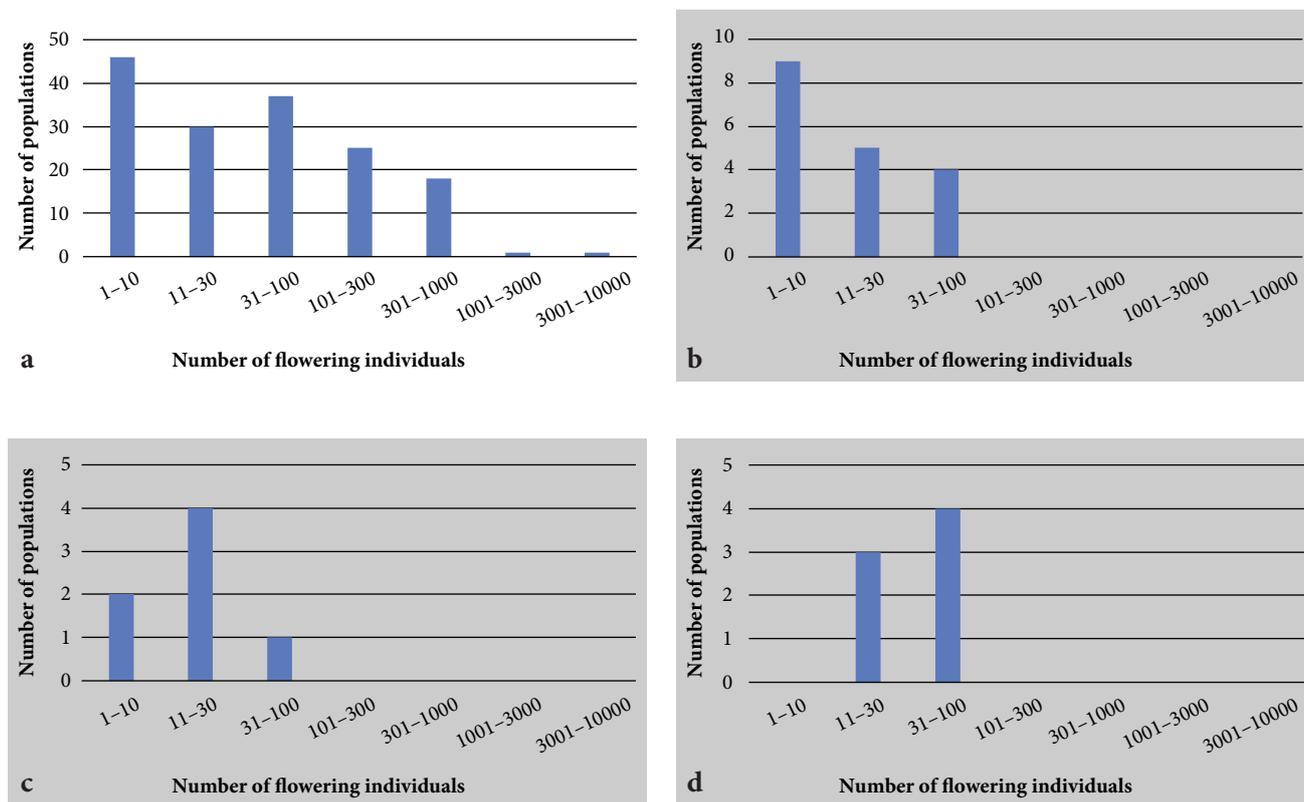


Fig. 3 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in South Bohemia. For grey images, the populations in all categories consisted of less than ten individuals.

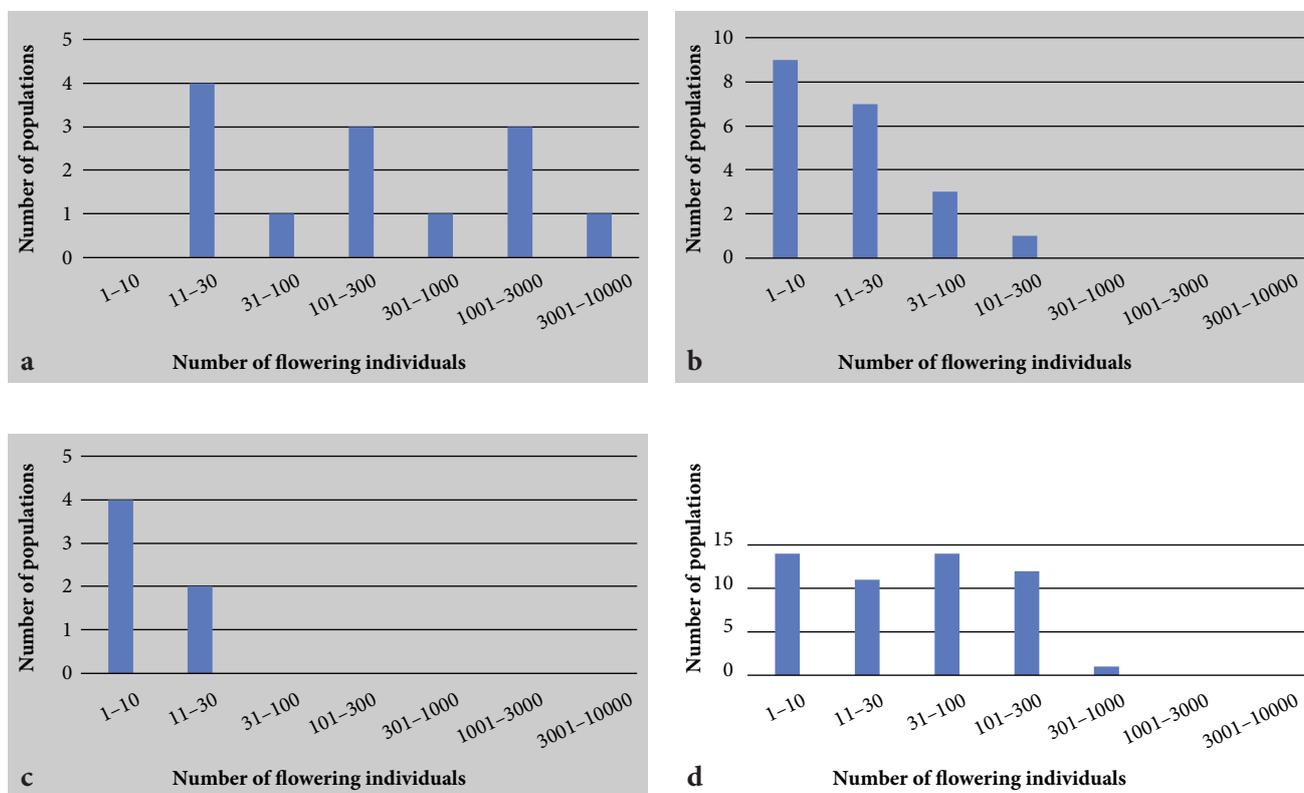


Fig. 4 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in Jeseníky Mountains. For grey images, the number of populations for all categories of number of individuals is less than ten.

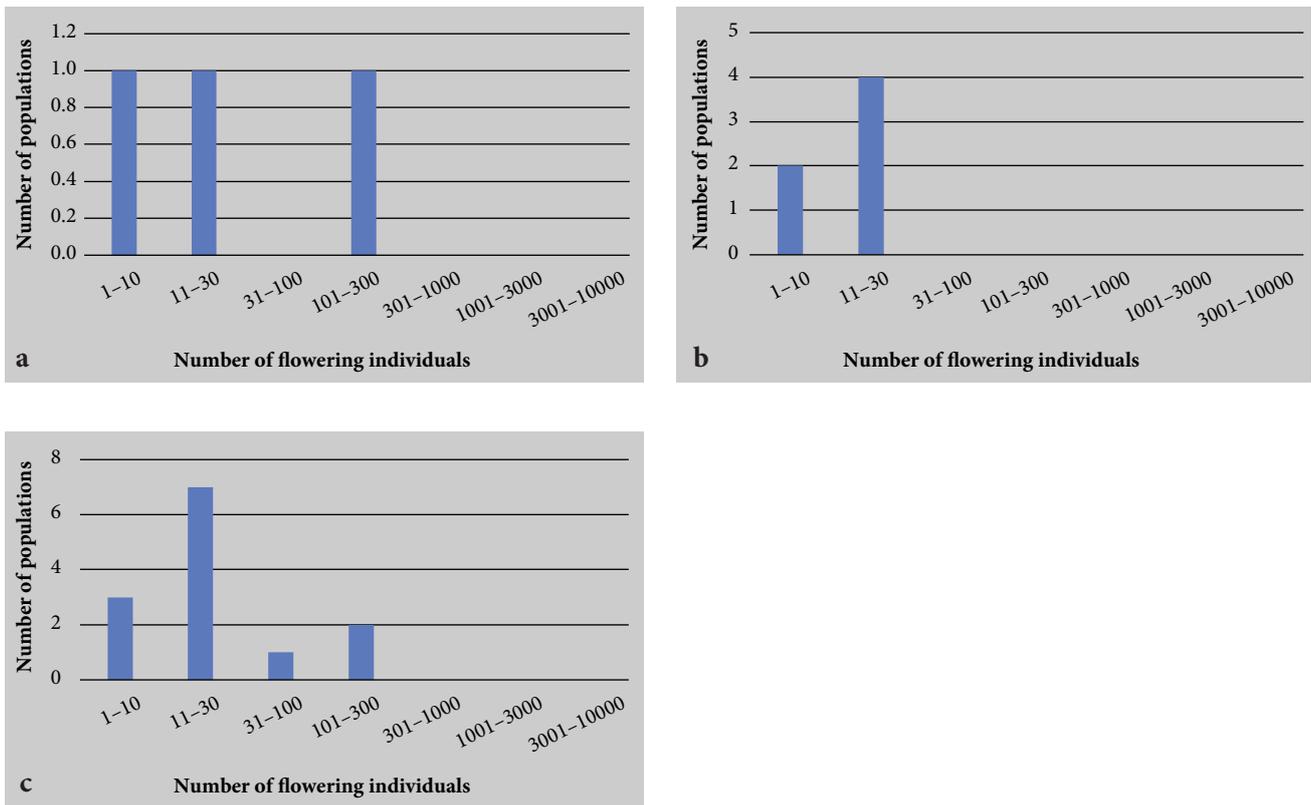


Fig. 5 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b) and *N. ovata* (c) in the Polabí region. For grey images, the number of populations for all categories of number of individuals is less than ten.

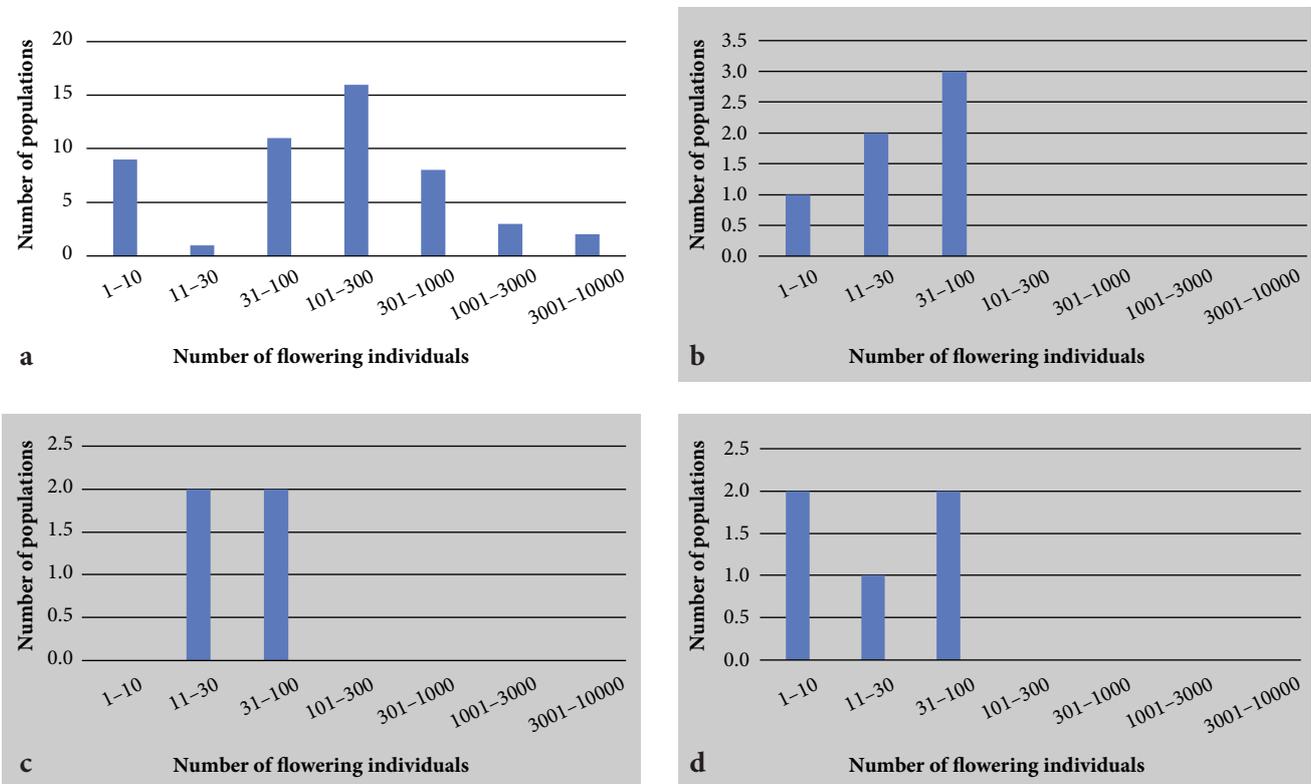


Fig. 6 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in the Bohemian-Moravian Highlands. For grey images, the number of populations for all categories of number of individuals is less than ten.

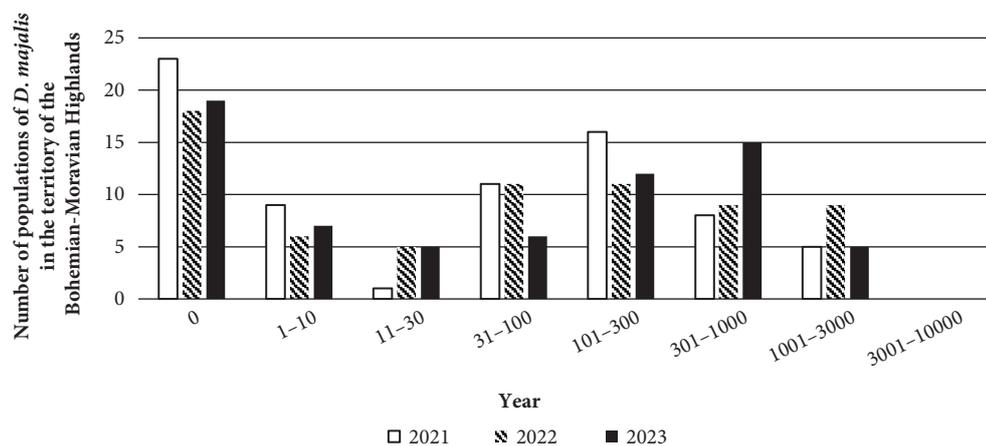


Fig. 7 Associations between the numbers of populations and the numbers of flowering individuals of *D. majalis* in the territory of the Bohemian-Moravian Highlands in 2021 (white), 2022 (hatched) and 2023 (black).

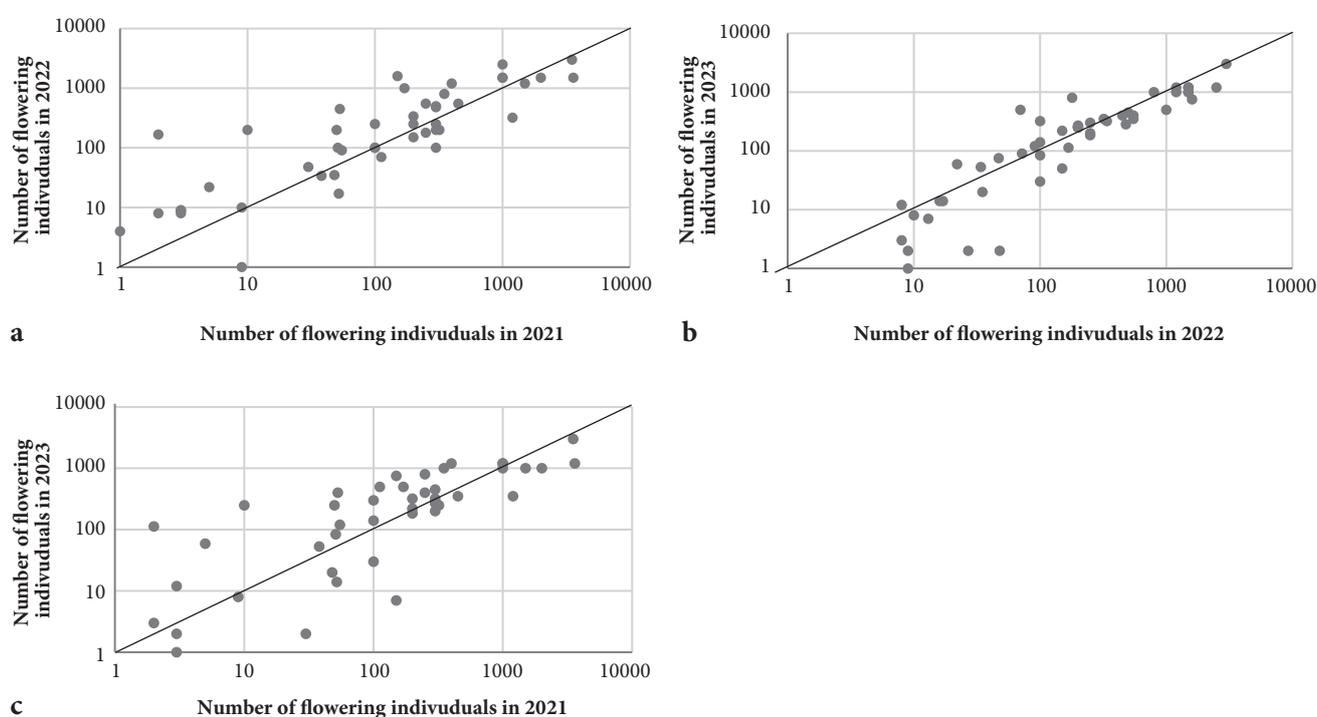


Fig. 8 Numbers of flowering individuals of *D. majalis* at individual locations in: (a) the Czech-Moravian Highlands in 2021 and 2022, (b) 2022 and 2023 and (c) in 2021 and 2023.

ing individuals of *D. fuchsii* at ten of the fifteen localities at which it was recorded. Sixteen localities were visited for *P. bifolia* (Fig. 6b).

Analysis of *Dactylorhiza majalis* in the territory of the Bohemian-Moravian Highlands

In the years 2021–2023, a total of 69 locations of *D. majalis* were monitored in the territory of the Bohemian-Moravian Highlands (Fig. 7). In 2021 (marked in white in the figure), a lower number of flowering individuals was found overall at locations in the Czech-Moravian Highlands than in the two following years, which is mainly due to the large numbers of flowering individuals

(300 and more). In 202 the most zero findings were recorded. In contrast, in 2022 (hatched) the largest number of flowering individuals was recorded.

Fig. 8 shows the correlation between the frequencies of flowering individuals of *D. majalis* at individual locations in the Bohemian-Moravian Highlands in two consecutive years. The points that lie on the straight line ($y = x$) in Figs 8a–c or are minimally distant from this straight line mean that the frequency of flowering individuals of *D. majalis* did not differ much at each locality in the two years monitored. On the contrary, points far from the line marked in the figure ($y = x$) indicate higher differences in the frequency of flowering individuals of

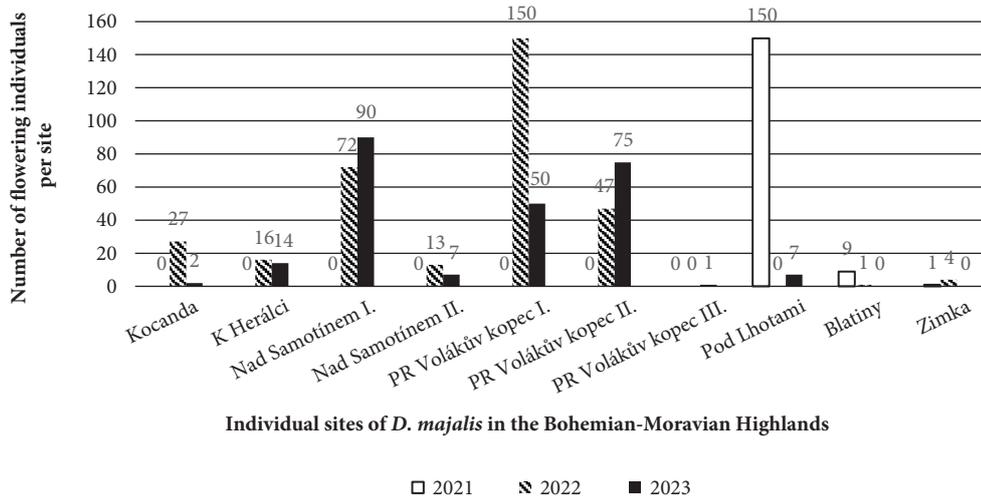
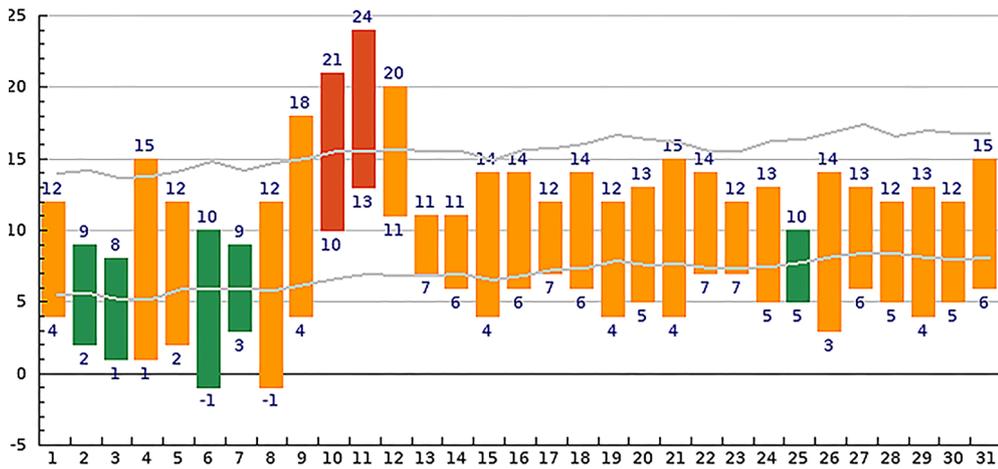
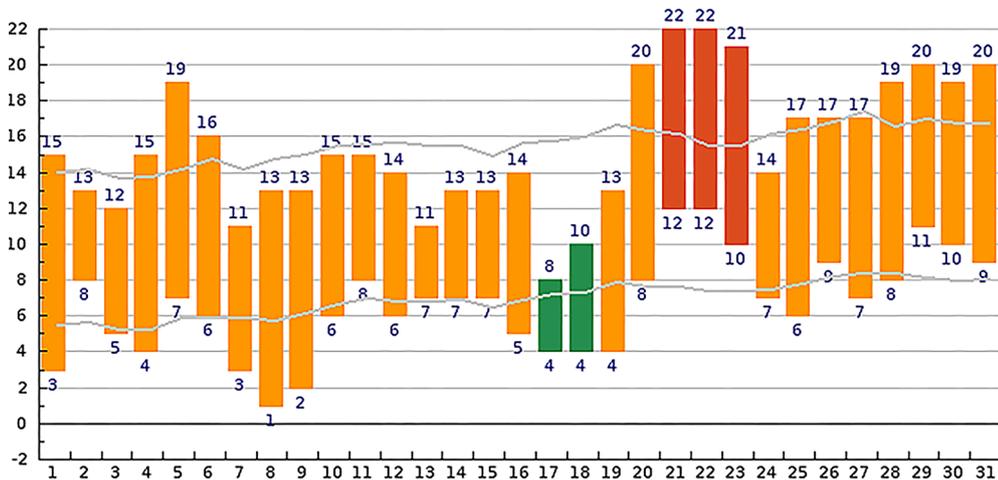


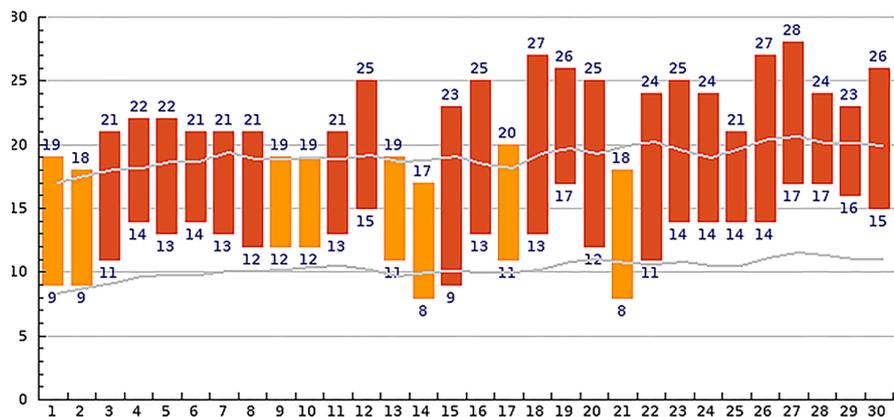
Fig. 9 Dependence of the number of flowering plants per site on years and individual sites of *D. majalis* in the Bohemian-Moravian Highlands in 2021 (white), 2022 (hatched) and 2023 (black). The image contains localities where no flowering individual was found in any of the monitored years.



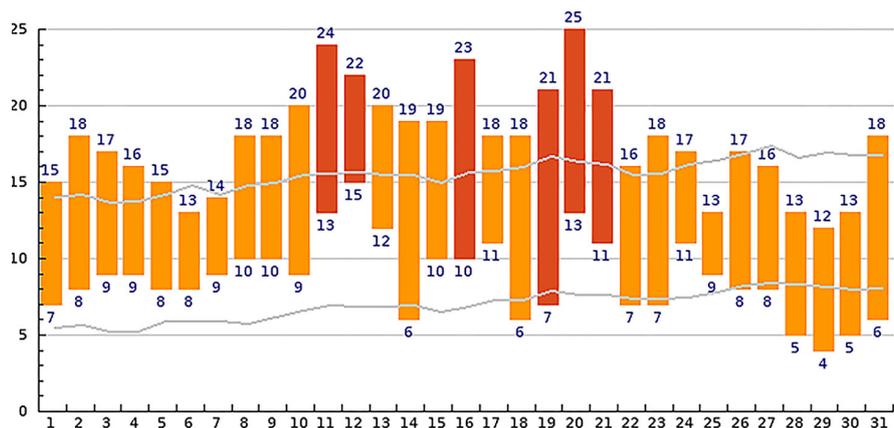
a



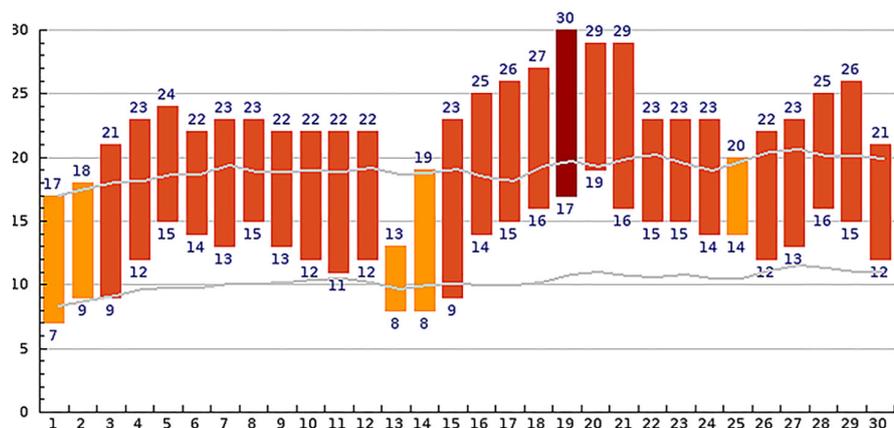
b



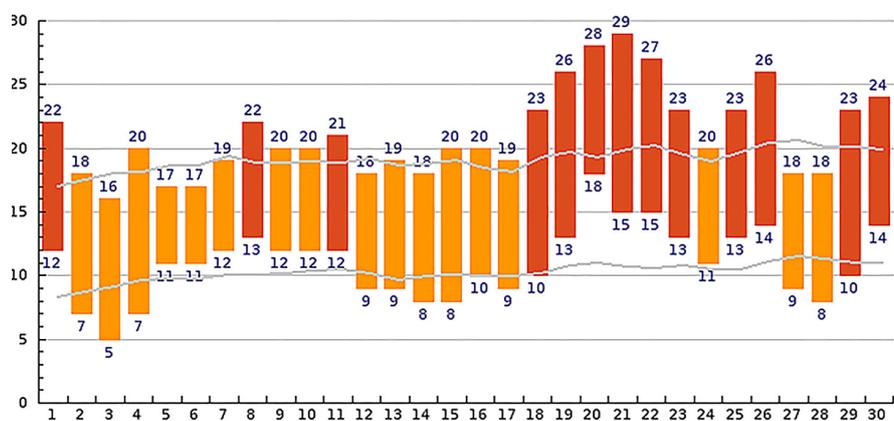
c



d



e



f

Fig. 10 (pp. 103-104) Highest and lowest daily temperatures during the months of May 2021 (a), 2022 (b) and 2023 (c) and June 2021 (d), 2022 (e) and 2023 (f). Long-term temperature averages are shown by grey lines (https://www.inpocasi.cz/archiv/svratouch/?&typ=snih&historie_bar_mesic=2&historie_bar_rok=2023).

Table 1 The average amount of precipitation in May and June in the Vysočina region in the years 2021, 2022 and 2023 (<https://www.chmi.cz/historicka-data/pocasi/uzemni-srazky>).

Month	Long-term precipitation normal (mm)	Total rainfall (mm) in 2021	Total rainfall (mm) in 2022	Total rainfall (mm) in 2023
May	71	94	65	41
June	80	90	85	40

D. majalis at the locality. Comparing the abundances of *D. majalis* at sites in 2021 and 2022 (Fig. 8a), it is apparent that more flowering plants were found in 2022 than in 2021, and a significant number of outliers were also found. However, this was more the case for sites with lower numbers of individuals. In locations containing more flowering individuals, the year-on-year differences are no longer so striking. The results show that the findings in 2022 and 2023 (Fig. 8b) have a linear relationship. When comparing the frequencies in 2021 and 2023 (Fig. 8c), we can state that more outlying observations were detected especially in localities with a smaller number of individuals in the population. In Figs 8a–c, localities where no individual was found were not recorded.

A total of 15 locations were found in the Bohemian-Moravian Highlands, where not a single flowering individual was found in any of the monitored years. Such localities were not recorded in Figs 8a–c due to the logarithmic scale since the logarithm of zero is not defined. However, ten localities were found where significant variability in the abundance of *D. majalis* was observed in individual years, when no flowering individual was found for one or two years (Fig. 9). At seven locations, no flowering individual was found in 2021, but in subsequent years there was an increase in the frequency of flowering individuals at the monitored locations. In the 2022 season, there was a significant decrease in the number of flowering individuals at the Pod Lhotami and Blatiny sites, where 150 and 9 were counted in 2021 respectively, but in 2022 there were none, or only one flowering individual recorded at the location. No flowering individuals were found at the Zimka and Blatiny locations in 2023.

Meteorological data

Temperature data were obtained from publicly available data of the Meteorological Station in the village Svatouch. This village is in the Bohemian-Moravian Highlands on the border of the Vysočina and Pardubice regions. However, this area well represents the character of the study area. Data regarding the total amount of precipitation in the Vysočina region were obtained from the Czech Hydrometeorological Institute. When comparing year-to-year differences in population sizes, records of temperatures and total precipitation in the Bohemian-Moravian Highlands were monitored.

Interannual temperature variability

In 2021, May temperatures were significantly lower than in 2022, when May temperatures were, on the con-

trary, above average (Figs 10a–b). In 2023, May temperatures hovered around the long-term average (Fig. 10c). June was above average warm in 2021 (Fig. 10d).

Interannual variability in precipitation

In 2021, the total amount of precipitation in the observed area during the month of May was 94 mm (Table 1), also in June of this year, the amount of precipitation was rather above average. In 2022, the total of May and June precipitation was close to the long-term precipitation normal. May and June 2023 were very dry months, the total precipitation in both months was around 40 mm.

Discussion

There are not many studies dealing with the issue of plant metapopulations, especially in orchids. This is probably because it is difficult for plants to map accurately the migration of seeds, the colonization of new territories, or to clearly mark the monitored population as extinct. Therefore, when studying plant metapopulations, we should consider other factors, such as plant dormancy and the occurrence of sterile individuals (Jersáková and Kindlmann 2004), the existence of a seed bank, adaptation to changing conditions and limits in seed dispersal (Husband and Barrett 1996).

Due to the complicated symbiotic relationships of orchids with other organisms, monitoring and searching for new orchid locations could correspond to the presence of pollinators and mycotrophic fungi (Gaskett and Gallagher 2018). Therefore, orchids can be referred to as indicators of the state of vegetation (Newman et al. 2007).

This study provides the basis for a new model of orchid population dynamics.

Population sizes of individual orchid species

The typical size of an orchid subpopulation within a metapopulation is among the data that could be essential for species conservation, as it could facilitate decisions about priority protection or the application of appropriate management. The abundance of individual plant species tends to be monitored at individual locations. Orchid coverage is most often studied, i.e., the number of individuals per unit area (Bhattarai et al. 2014), or transects of exact area are measured, where the rate of their occupancy is monitored (most often 10 × 10 km (Kull and Hutchings 2006; Tsiftsis and Tsiripidis 2020), 5 × 5 km,

or 4×4 km (Jacquemyn et al. 2005)). Therefore, it can be quite complicated to compare such values. In addition, scientific works focusing on the population dynamics of orchids often study only one orchid population whose future fate they try to model (Compagnoni et al. 2016). However, such measurements may lead to different conclusions. If we were about to measure the frequency of orchids in this way as part of field research in the Bohemian-Moravian Highlands, we would get distorted results, because the monitored area is not so extensive. In addition, there are relatively large areas covered with spruce forests where orchids have not been found. Therefore, in this work we analyse the frequencies of flowering individuals at individual locations within metapopulations of different orchid species, across regions, as a basis for a new metapopulation modelling approach.

Since there was not enough data available in the NDOP database for all four species of orchids in the monitored regions, we only have relevant data on the typical population size for *Dactylorhiza majalis* in South Bohemia (Fig. 3a) and the Bohemian-Moravian Highlands (Fig. 6a) and for *Dactylorhiza fuchsii* in Jeseníky (Fig. 4d). The data on *Platanthera bifolia* and *Neottia ovata* in individual regions were not considered sufficient, as a maximum of nine individuals were found in the group, and for this work we chose that the minimum is ten localities in the group.

The above-mentioned lack of data was because the data from the NDOP was greatly reduced to be as relevant as possible. Only flowering individuals were analysed, at sites that were verified in 2010 at the latest. If records from one site were available more than once (= e.g., counted in different years), only their arithmetic mean was used. This filtered out the most recent data, but also reduced the total amount of data. The results of this work indicate that the distribution of population sizes within different metapopulations of orchids should be studied rather over a large area, in this case preferably over the entire territory of the Czech Republic.

We can observe considerable variability in the number of individuals in individual populations of *D. majalis*. In 2021 and 2022, populations with 31–300 individuals prevailed in the territory of the Czech-Moravian Highlands (Fig. 7), which can be characterized as a relatively stable population. However, it is possible that a large proportion of small sites with only population units are not reported in the NDOP because larger populations attract more attention from both the public and researchers. It can be seen from Fig. 8b that the seasons of 2022 and 2023 were very similar in terms of the number of flowering individuals of *D. majalis* at the locations in the Bohemian-Moravian Highlands. It is true that the interannual variability in the number of flowering *D. majalis* orchids was observed rather in small populations (Figs 8a–c).

In southern Bohemia, medium-sized populations of *D. majalis* dominated (Fig. 3a), i.e. those where the population consisted of a hundred or fewer individuals. Wo-

tavová et al. (2004) reported that 24% of *D. majalis* populations in southern Bohemia were described as stable populations (populations in which more than 100 flowering plants occurred), while in this work 28.5% of populations with stable abundance of *D. majalis* in the same region. From the point of view of nature protection, the most serious thing is that between the 1970s and 1997, approximately two thirds of all monitored populations of *D. majalis* in South Bohemia became extinct (Wotavová et al. 2004).

Platanthera bifolia formed medium-sized populations of 200–250 flowering individuals (Gorchakovskii and Igosheva 2003), in contrast, our results from southern Bohemia show that *P. bifolia* occurs in smaller populations of up to a hundred individuals (Fig. 3b).

Neottia ovata is among the most widespread species in Europe (Kull et al. 2016). In a study from the middle Urals, it is stated that the population of *N. ovata* occurs in small populations of around fifty individuals, and rather in lower cover densities (Gorchakovskii and Igosheva 2003). The results of this work show that *N. ovata* is dominated by populations of 11–30 individuals in the Polabí region (4c).

The frequencies of *Dactylorhiza fuchsii* analysed in the Jeseníky region (Fig. 4d) most often range from one to 300 individuals, when no size group significantly dominates. However, larger populations were found only minimally.

The influence of temperature and precipitation on the size of the metapopulation of *Dactylorhiza majalis* in the territory of the Bohemian-Moravian Highlands

During the year-to-year monitoring of the frequency of *D. majalis* at individual locations in the Bohemian-Moravian Highlands, a certain variability was detected. The data were taken in the months of May and June. In the records, the interannual temperature differences in the months when the data were collected were also significantly reflected.

In 2021, when May temperatures were lower than the long-term average, orchids were observed on average 14 to 21 days later at sites in the Bohemian-Moravian Highlands than in other census years. In addition, an overall lower amount of flowering *D. majalis* individuals was found at the sites in 2021, which is apparently directly related to the low May temperatures in 2021 (Fig. 10a). In May 2021, 23 mm more precipitation was measured than the long-term precipitation average in the Vysočina region (Table 1). These data correspond to the results of Wotavová et al. (2004). According to Hornemann et al. (2012), high temperatures during peak flowering can cause plant wilting. But it seems that the above-average high temperatures that were measured in June in 2022 (Fig. 10e) did not have a negative effect on the number of flowering individuals at the locations in the Bohemian-Moravian Highlands, as the highest number of flowering individuals was found overall in this year. However,

the data collection took place at the beginning of June, so the effect of temperature could become apparent later. May and June 2023 were extremely poor in precipitation in the Vysočina region (Table 1), which amounted to only 41 mm, respectively 40 mm, which corresponds to approximately half of the long-term precipitation normal.

Variability in the number of flowering individuals at sites

At ten localities in the Bohemian-Moravian Highlands, significant variability in the frequencies of *D. majalis* was observed, when no flowering individual was found in one or two years (Fig. 9). A particularly significant increase in the number of flowering individuals of *D. majalis* was recorded in the localities at PR Volákův kopec I. and II. and in the Nad Samotínem I location, where up to 150 flowering plants were recorded in 2022 and 2023. This could be related to the fact that in 2021 there was significantly higher grass at the sites than in the following two years, which caused the orchids to be shaded and subsequently sterile. Or it could be that the orchids were hidden in tall grass and not found during field research. On the contrary, 150 flowering *D. majalis* were described at the Pod Lhotami site in 2021, but then, in 2022, there were zero findings. However, in 2023, seven flowering plants were found here. This was due to the fact that the site was cut at an inappropriate time in 2022, i.e., before the *D. majalis* seeds had matured, which probably caused a decrease in the total number of individuals in the population in the following year as well. The results of this work confirm that inappropriate site management can adversely affect the metapopulation dynamics of orchids.

During field research, subpopulations with no flowering individuals were also observed. It is possible that in the year in question the population consisted exclusively of sterile (non-flowering) individuals, but the seasonal variability in the number of individuals found in the population can also be explained by dormancy in some orchids, when only the underground parts of the plant exist in orchids in each season (Jersáková and Kindlmann 2004; Nicolè et al. 2005). Dormancy usually lasts no longer than two years (Shefferson et al. 2001), but there have also been cases where dormancy in plants lasted for more than ten years (Jersáková and Kindlmann 2004). During the observation of the population of *D. majalis* in the Bohemian-Moravian Highlands, fifteen localities were described where not a single flowering individual was found in any of the observed years. Such localities can be described as extinct, because Wotavová et al. (2004) state that a three-year gap in plant flowering indicates a very high probability that a given population at a site is already extinct.

In this work, only flowering plants were included in the analysis, because for them it is possible to determine their exact number, while it is not possible for sterile individuals.

Records on the number of individuals in the metapopulation should be monitored more than once, because the capture of species during one visit to the site is around 80%, therefore long-term monitoring is needed (Vogt-Schilb et al. 2013). For the reasons stated above, for a better study of the metapopulation dynamics, the localities with the occurrence of *D. majalis* were visited repeatedly (in 2021, 2022 and 2023), and for further research it would be appropriate to visit the monitored localities in the following years as well, since long-term field research and information about the environment are very important for the study of plant populations (Štípková and Kindlmann 2021).

Size of localities

Another question remains what the ideal site size is where the orchid populations are located. As part of this work, it was found that in the Bohemian-Moravian Highlands, orchid localities with a rather small area of up to 1 ha predominate. In a study of Wotavová et al. (2004) it was stated that only 18% of all studied orchid sites of *D. majalis* in the south of Bohemia had an area larger than 5,000 m². In addition, almost half of all populations were found in waterlogged meadows, which are habitats that are among the most endangered in the Czech Republic (<https://mokrady.wbs.cz/Mokrady---zakladni-informace.html>). However, as part of the protection of plants of the Orchidaceae family, it is necessary to monitor not only meadow biotopes, but also forest habitats, which are also frequent orchid sites (Štípková et al. 2021).

Causes of the decline of orchids in the Bohemian-Moravian Highlands

In the case of *Dactylorhiza majalis*, orchid populations in the territory of the Bohemian-Moravian Highlands are threatened primarily by the realization of industrial buildings, but also by the drainage of the landscape, the progressive succession and degradation of meadow biotopes and the absence of mowing or grazing (Čech et al. 2017b). For *Platanthera bifolia*, like the case of *Dactylorhiza fuchsii*, the biggest problem for the growth of these orchids in the territory of the Bohemian-Moravian Highlands is the overgrowth and afforestation of localities, the degradation of non-forest habitats, unsuitable felling times and excessively intensive grazing (Čech et al. 2017a,d). *Neottia ovata* is decreasing in the territory of the Bohemian-Moravian Highlands mainly due to the application of intensive agricultural practices, which lead to eutrophication and often to the disappearance of richer mesophilic lawns and original vegetation. For populations that are found in alder forests or in lit places in deciduous forests, the most threatening is the change in the species composition of the forest to conifers (Čech et al. 2017c).

A large part of the orchid populations in the Bohemian-Moravian Highlands is located especially in protected areas and receives appropriate care. However, small lo-

calities with only plant units that do not attract interest in nature conservation are primarily at significant risk (Čech et al. 2017a).

Orchid restoration

Restoration or creation of new sites is not a very frequent phenomenon. Despite this, there have been efforts to restore orchid populations, to which orchids generally respond very slowly (Jersáková et al. 2002). Restoration of orchid habitats can be complicated due to their complex life cycle. However, it has been proven that introduced orchids can re-establish a symbiotic relationship with fungi (Fay et al. 2018).

Conclusions

The aim here was to find the basis for a new metapopulation model specially adapted for plants from the Orchidaceae family. As part of this goal, it was possible to analyse the population size distribution of some orchid species in three regions, namely: *Dactylorhiza majalis* in South Bohemia and the Czech-Moravian Highlands and *Dactylorhiza fuchsii* in Jeseníky. In Jeseníky, individual populations of *D. fuchsii* reached up to 300 individuals, just like *D. majalis* in South Bohemia. Variability in population size within the metapopulation of *D. majalis* in the Bohemian-Moravian Highlands was monitored at sixty-nine locations over three years (2021, 2022 and 2023) and it was found that the number of individuals in the population is largely influenced by May temperatures and total precipitation in this month. Correct management of the sites, which should correspond to the requirements of the protected orchid species, also has a significant effect on the presence of orchids on the sites. The population size of *D. majalis* in the Bohemian-Moravian Highlands was most often around 31–100 flowering individuals, or 101–300 flowering individuals in the population.

Thus, the variables that should be included in a new metapopulation model for the study of orchids are the following: the typical distribution of population sizes within a single orchid species for a given region, the quality of management, temperature and rainfall, and the possibility of individual sterility or their dormant state.

Acknowledgements

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POLLINATION STRATEGIES OF DECEPTIVE ORCHIDS – A REVIEW

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ABSTRACT

Orchids can be classified in terms of their pollination into rewarding species, which produce nectar in their flowers that serves as a reward for pollinators and deceptive species, whose flowers do not contain nectar and save energy for other purposes. This paper concentrates on the latter. Deceptive orchids attempt to deceive their pollinators by being similar to some non-orchid rewarding species, but not providing a reward. Each of these strategies has its advantages and disadvantages in terms of their effect on future fitness of a plant and/or population and subsequently its survival as a species. In summary, the literature indicates that deceptive strategies may lower reproductive success, but may be compensated for in that they cost less in terms of energy. This should be taken into consideration when developing management strategies for these species, which is often done by non-orchid specialists. This article is intended for such non-specialist audience and includes a description of the main types of deceptive strategies used by orchids, as well as examples of the most typical species.

Keywords: deceptive orchids; Orchidaceae; pollination; pollination strategies; reproductive success

Introduction

Pollination is the transfer of pollen from stamen to stigma. It is one of the reproduction options used by plants (Dressler 1981; Štípková and Kindlmann 2021). There are several ways in which plants can be pollinated: either self-pollination or cross-pollination and eventually geitonogamy. Both cross-pollination and geitonogamy are types of reproduction that require a mediator, such as water, wind or an animal. Orchids usually use animals, especially insects (e.g., wasps, hoverflies or butterflies) as pollinators. Plants must therefore be able to attract a pollinator and potential pollinators are usually mainly foraging for food if they land on a flower (Dressler 1981).

Orchids have evolved highly specialized flowers for attracting pollinators (Dressler 1981), which can be classified into those that produce nectar (rewarding species), those that do not produce nectar (deceptive species), which in this way save energy for other purposes (Gijbels et al. 2015). Orchids with deceptive flowers make up approximately a third of all orchids (van der Pijl and Dodson 1966; Dressler 1981; Ackerman 1986; Tremblay et al. 2005; Renner 2006; Christenhusz and Byng 2016) and are the group of plants with the most species with deceptive flowers (Jersáková et al. 2006).

Pollination strategies of deceptive orchids are very diverse. The characteristics of the flowers have been determined by selection, which was mediated by pollinators (Micheneau et al. 2009). Features attractive to pollinators include scent, length of the spur and colour of the flower, or presence of a reward (Micheneau et al. 2009). This implies that for the plant it is advantageous to offer some form of a reward to the pollinator and to advertise the

fact by developing visually attractive flowers (Roberts 2007).

The more pollinators attracted by a plant, the more successful is the transfer of genes to the next generation (Trapnell and Hamrick 2006). This is important for the survival and evolution of species, and it is why plants produce flowers. Flowers are visually attractive to pollinators foraging for a source of energy (Trapnell and Hamrick 2006).

This review concludes that deceptive strategies may reduce the number of flowers pollinated and consequently plant fitness, but this may be compensated for in terms of energy saved for other uses. This should be taken into consideration when developing management strategies for these species, which is often done by non-orchid specialists. This article is intended for such non-specialists and includes a description of the main types of deceptive strategies used by orchids, as well as examples of the most typical species.

Deceptive orchids and their ability for deceit

The goal of orchids with deceptive flowers is to attract pollinators. This was achieved by evolving flowers that are attractive in terms of their colour, scent or morphology (Dafni 1984). Orchids produce flowers of various shapes that resemble those of plants that are attractive to pollinators or those of a plant that grows close by and produces a reward (Jersáková et al. 2006). A common strategy of deceptive orchids is to produce structures that imitate anthers or pollen (Jersáková et al. 2006). Other species use chemicals, such as pheromones or volatile

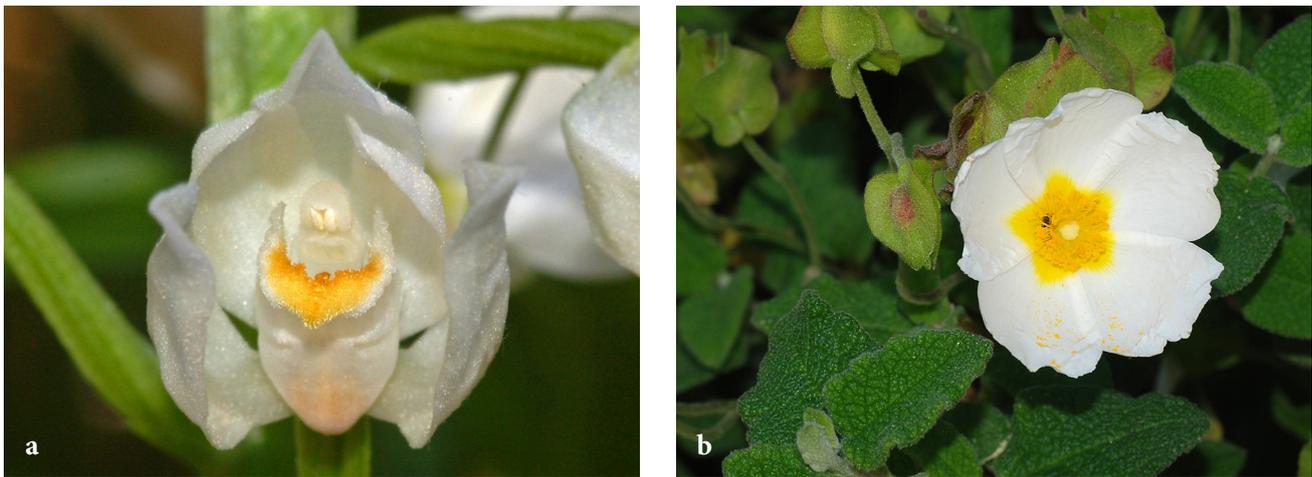


Fig. 1 *Cephalanthera longifolia* (a) and *Cistus salviifolius* (b) (Source: wikimedia.org).

substances, namely essential and ethereal oils, aldehydes or alcohols, to attract pollinators (Brodmann et al. 2008; Stöckl et al. 2011). There are many such signals and some are very specific and it is assumed that this wide range of signals evolved because pollinators are more likely to avoid common deceptions than rare ones (Schiestl 2005).

Two-thirds of orchid species are rewarding, which means that they produce nectar for pollinators. Nectar is the most frequent and common kind of reward offered to pollinators by plants (Roberts 2007). Rewarding species usually produce more seeds (Trapnell and Hamrick 2006) and more fruit than deceptive species (Neiland and Wilcock 1998; Schiestl 2005; Molnár et al. 2015). Several studies involving different species of orchids report that species that provide rewards have higher reproductive success (the ratio of the number of fruits to the number of flowers produced per plant per season) than those that do not (Kindlmann and Jersáková 2006; Hobbhahn et al. 2017). A review of over 100 orchid species concludes that the reproductive success of deceptive orchids is only half of that of rewarding species (Neiland and Wilcock 1998), which clearly indicates that rewarding orchids are more successful at reproducing than deceptive orchids (Hansen and Olesen 1999).

Similarity of deceptive orchids to nectar-producing plants

Some deceptive orchids mimic a nectar-producing (“nectariferous”) plant growing in the same area. This is referred to as Batesian mimicry, which in this case means that the flower of the deceptive orchid mimics the flower of another plant that offers a reward (Dafni 1984; Jersáková et al. 2006). Imitating flower tries to confuse potential pollinators foraging for nectar by being similar in colour or shape to that of a rewarding flower (Johnson et al. 2003). The orchids that are mimicked by other plants (rewarding in this case) are called “magnetic” plants, because they enable other species to mimic them as rewarding plants (Johnson et al. 2003). E.g., the deceptive orchid

Disa pulchra is very similar morphologically to *Watsonia lepida*, a nectar-producing plant belonging to the *Iris* genus (*Iridaceae*) (Fig. 1). Both species are pollinated by the same insects (Duffy and Johnson 2017). The more similar an orchid is to a nectariferous species, the more likely it is that it will attract pollinators (Johnson et al. 2003).

An example that this issue may not be as simple is the deceptive orchid *Cephalanthera longifolia* (Bino et al. 1982; Widmer et al. 2001), whose flowers are supposed to imitate those of *Cistus salviifolius*, a member of the Rock rose family (*Cistaceae*) – see Dafni and Ivri (1981b). Dafni and Ivri (1981b) found that *C. longifolia* produces more seeds in places where *C. salviifolius* also grows. Because both species have the same coloration, they checked the possibility of floral mimicry. By doing that, however, Dafni and Ivri (1981b) observed that orange papillae on the labellum of *C. salviifolius* successfully imitate pollen of *C. longifolia* (= it can be considered as a “pseudopollen”). In addition, they found that the flowers of *C. longifolia* attract pollinators even in the absence of *C. salviifolius*. By putting these two observations together, Dafni and Ivri (1981b) hypothesize that the attractivity of *C. longifolia* to pollinators in the absence of the “magnet species”, *C. salviifolius*, can be explained by the attractive value of the “pseudopollen” alone. The strength of this argument would be increased, however, in the situation, where *C. longifolia* coexists with a differentially coloured *Cistus* species (which also produces pollen). Would this *Cistus* also cause an increase of attractivity of *C. longifolia* for pollinators? This is a matter for future research.

Sexual deception

The strategy of using the flower as a sexual dummy to fool a specific pollinator is less common than the food-deceptive strategy (Tsiftsis and Djordjević 2020). There are about twice as many families that use food-deceptive strategies than those that use sexual deception (Jersáková et al. 2006). Sexually deceptive orchids are highly specialised and typically pollinator-specific (Peakall and Han-

del 1993; Cozzolino and Widmer 2005; Mant et al. 2005; Schiestl and Schlüter 2009; Gaskett 2011).

Flowers of several species of the genus *Ophrys* mimic the form and movement of the female of their specific pollinator (Kullenberg 1950) (Fig. 2 and Schiestl 2005).

The pollinator lands on the flower and tries to copulate with it, during which the pollinium of the flower sticks to it and is subsequently transported to the next flower it visits (Ayasse et al. 2000). Flowers of these orchids do not only use visual signals, but also scent to at-



Fig. 2 *Ophrys insectifera* (Source: wikimedia.org).

tract their pollinators (Schiestl 2005). A flower of *Ophrys sphegodes* attracts males of its pollinator both by looking and smelling like a female. They do this by secreting the same chemical substances as a real female. That the pollination of these orchids is entirely dependent on one particular species of insect would appear to indicate that their reproductive success is very low, but this not the case. The specific pollinator can visit the same plant a second or even a third time, because the chemical composition of scent produced by this orchid differs between plants (Ayasse et al. 2000). Correct positioning of the pollinator is necessary for pollinium removal, which reduces the probability of geitonogamy (Ayasse et al. 2000). The orchid *Caladenia huegelii* is also pollinated by wasps, which emit pheromones when close to the flower of this orchid, which is thought to indicate that the pollinator is responding as if about to copulate with a female and that two thirds of the males that land on a flower will attempt to copulate with it (Phillips et al. 2015). Scopece et al. (2010) report a higher percentage of pollination of orchids using sexual deception than non-rewarding orchids and sometimes even rewarding orchids.

Scent

A relatively newly explored pollination strategy of deceptive orchids is the so-called chemical mimicry, which is to produce a scent that attracts pollinators (Brodmann et al. 2008). For example, the orchid *Epipactis veratrifolia* is pollinated by several species of hoverflies (Stöckl et al. 2011), including *Ischiodon scutellaris* (Kumar and Rawat – see Fig. 3). The flowers of this orchid smell just like aphids, which is attractive for insect predators like hoverflies, the larvae of which feed on aphids. This results in some of the pollen of the flower adhering to the hoverfly and being transported to other plants of this orchid (Ivri and Dafni 1977; Kumar and Rawat 2011; Stöckl et al. 2011; Jin et al. 2014).



Fig. 3 *Epipactis veratrifolia* (a) and one of its pollinators, *Ischiodon scutellaris* (b) (Source: wikimedia.org).



Fig. 4 *Orchis israelitica* (a) and *Bellevalia flexuosa* (b) (Source: wikimedia.org).

Epipactis helleborine, emits volatile aldehydes and alcohols. Brodmann et al. (2008) studied whether the social wasps that pollinate this orchid are attracted by visual signals or scent. Pollinators visited more frequently covered flowers that produced scent than visible flowers, which did not (Brodmann et al. 2008). The scent of *Orchis israelitica* also attracts pollinators, but only over long distances, as when the pollinator is close to the plant, it is attracted by the colour of the flower (Galizia et al. 2005).

Mimicry of anthers and pollen

Another strategy used by orchids is to falsely signal the presence of pollen (Lunau 2000). Flower colour and pattern have a significant role in this pollination strategy. Pollen absorbs ultraviolet radiation and thus protects DNA from damage (Heuschen et al. 2005). Pollinators recognize parts of a flower that absorb UV radiation. Parts of the petals of *Cattleya walkeriana* absorb UV radiation and falsely signal the presence of pollen to pollinators (Aguilar et al. 2020). This was first recorded for a plant, the colour of the centre of the flowers of which is yellow, not the whole flower. *Dactylorhiza sambucina* is an example of such a species (Kropf and Renner 2005). There are few examples of this kind of mimicry in the above species, but it occurs more frequently in other families (Heuschen et al. 2005; Pohl et al. 2008).

Some orchids deceive their pollinators by having flowers with structures that morphologically resemble anthers (Dafni 1984). This visual deception tricks pollinators into visiting the flower. *Orchis israelitica* imitates the rewarding plant *Bellevalia flexuosa*, which is a member of the Liliaceae (Fig. 4). Anthers of the rewarding plant are a dark colour and there are many dark dots in the centre of the flower of the deceptive orchid, which imitate these anthers (Dafni and Ivri 1981a). Both species are pollinated by bees, which are attracted to visual signals, which in this case are in flowers similar in colour and size (Galizia et al. 2005).

Shelter

The flowers of some deceptive orchids appear to provide shelter for their pollinators. This strategy is rare in orchids, with only one documented genus, *Serapias* (Vöth 1980; Dafni et al. 1981; Jersáková et al. 2006; Vereecken et al. 2012). A possible reason is that it is very costly in terms of the investment in resources. The flower must be big enough to provide a refuge for the pollinator. For example, bees, which pollinate the orchid *Serapias vomeracea*, stay overnight in orchid flowers. These bees usually nest in holes in the ground and the flowers of this orchid have structures that simulate such holes (Dafni et al. 1981), Fig. 5.

Bees, which spent the afternoon flying from flower to flower of this orchid, often overnight in a flower. In addition, the flowers are warmed by the morning sun and the



Fig. 5 *Serapias vomeracea* (Source: wikimedia.org).



Fig. 6 *Caladenia nobilis* (Source: chookman.id.au).

bees may also stay in a flower for two nights (Dafni et al. 1981). Compared with *Serapias lingua*, which uses sexual deception to attract pollinators, the percentage fruit set of *Serapias vomeracea* is higher (Pellegrino et al. 2017).

Nectar

Some of the deceiving orchids produce a small amount of nectar, which they supposedly produce to attract insects and pollinators (Phillips et al. 2020). *Caladenia nobilis* (see Fig. 6) is an example of such an orchid. Some of its flowers contain a small amount of nectar, which contains mainly saccharose and also fructose and glucose (Phillips et al. 2020). This orchid is pollinated by only one species of insect and by producing nectar it increases its attractiveness and chance of being pollinated (Phillips et al. 2020).

Jersáková et al. (2008) report how pollinators react to nectar. Adding saccharose to the flower of the deceptive orchid *Dactylorhiza sambucina* increased its production of fruits. In the case of the deceptive orchid *Disa pulchra*, more flowers were pollinated (Jersáková and Johnson 2006).

Nectar is the main reward of orchids because it attracts pollinators, which feed on nectar (Gijbels et al. 2015) and in facilitating the transfer of pollen by pollinators it enhances the reproductive success of orchids.

The nectar produced by orchids is composed predominantly of carbohydrates and amino acids (Gijbels et al. 2015), with the carbohydrate concentration ranging between 3.5% and 71% (Brzosko and Mirski 2021). The composition of nectar differs between genera and even in some deceptive orchids (Phillips et al. 2020; Brzosko and Mirski 2021). The reason for this is unknown. It is likely, however, that either the pollinator is an important factor (Micheneau et al. 2009; Brzosko and Mirski 2021), or the composition of the nectar influences the sexual, social and foraging behaviour of pollinators (Wróblewska et al. 2019).

Conclusion

The most frequently used strategy of deceptive orchids is to closely resemble an orchid that produces nectar (Jersáková et al. 2006). The least used deceptive strategy is providing shelter for pollinators (Jersáková et al. 2006), even though it results in higher reproductive success in terms of higher fruit production, than orchids using insect sexual deception (Pellegrino et al. 2017). Although deceptive strategies may result in fewer flowers being pollinated and indicate a lower plant fitness, this may be compensated for by saving energy.

This should be taken into consideration when developing management strategies for orchids, which is often done by people who are not orchid specialists, which are the main target of this review.

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