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.

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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 km^2 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 km^2.

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 km^2 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 Nízký 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-

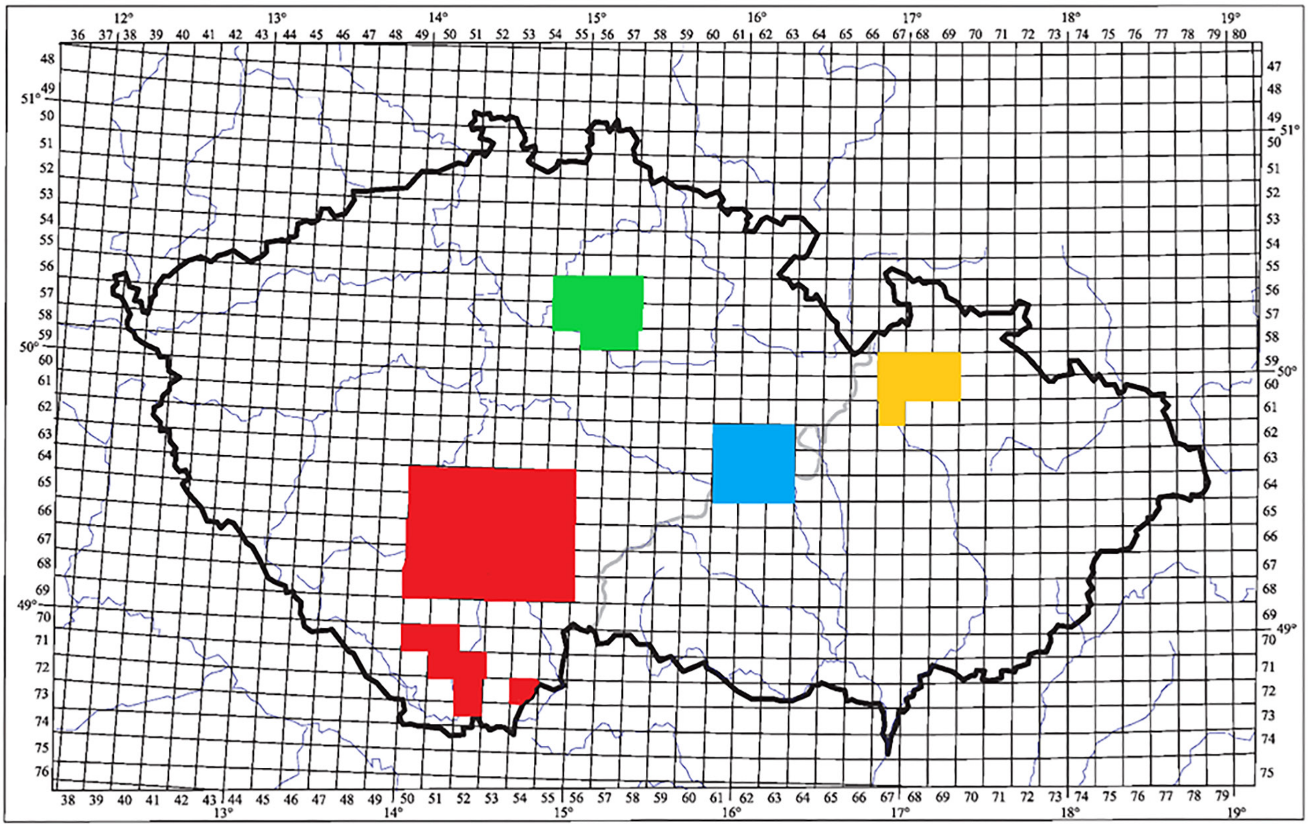


Fig. 1 Map of the Czech Republic divided into vegetation squares showing the areas studied: Polabí (green), South Bohemia (red), Bohemian-Moravian Highlands (blue) and Jeseníky (yellow). Source: https://www.entospol.cz/sit-mapovych-ctvercu/.

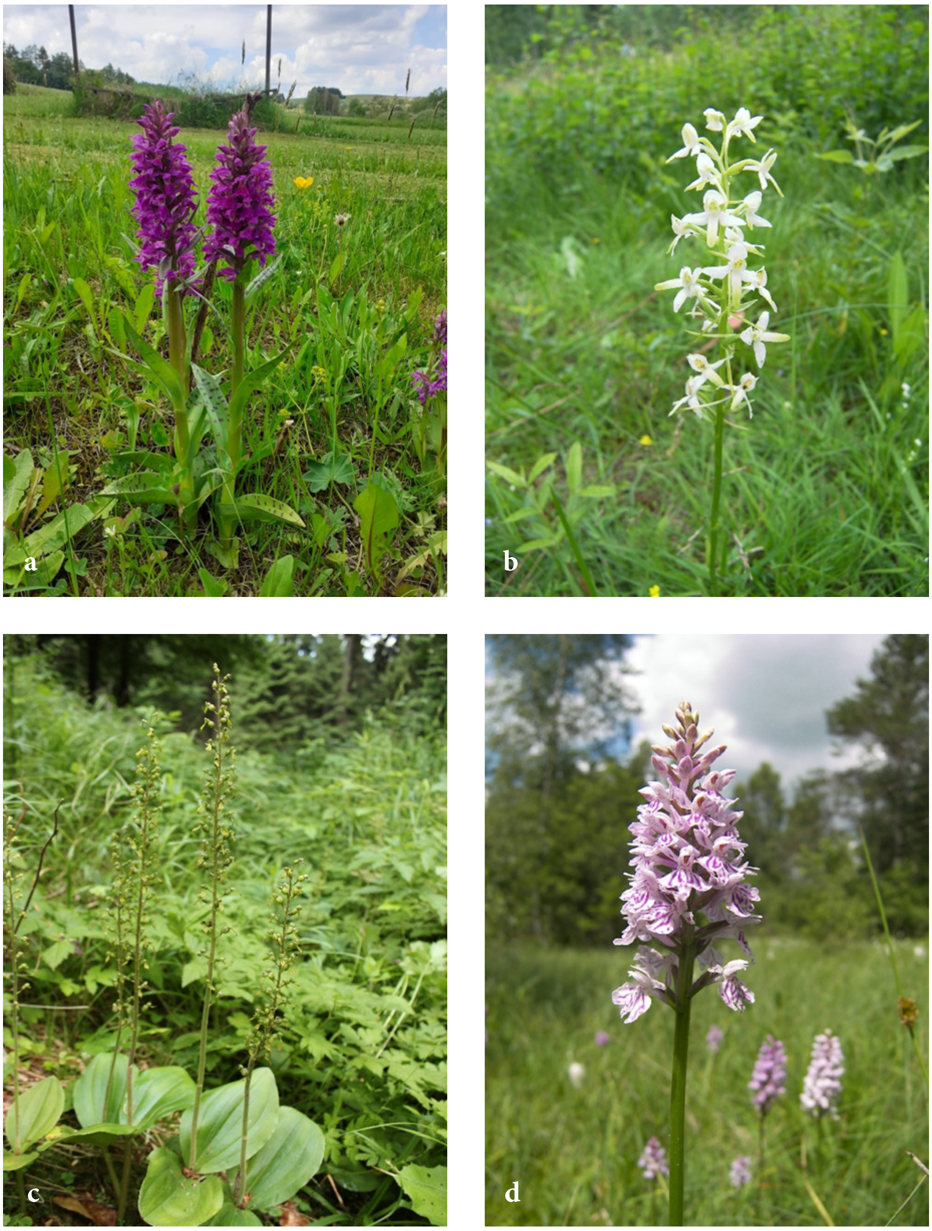


Fig. 2 Species of orchids studied:
(a) *Dactylorhiza majalis* (Čech et al. 2017b), (b) *Platanthera bifolia* (Čech et al. 2017d),
(c) *Neottia ovata* (Čech et al. 2017c), (d) *Dactylorhiza fuchsii* (Čech et al. 2017a).

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 Protection (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).

Jeseníky 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-

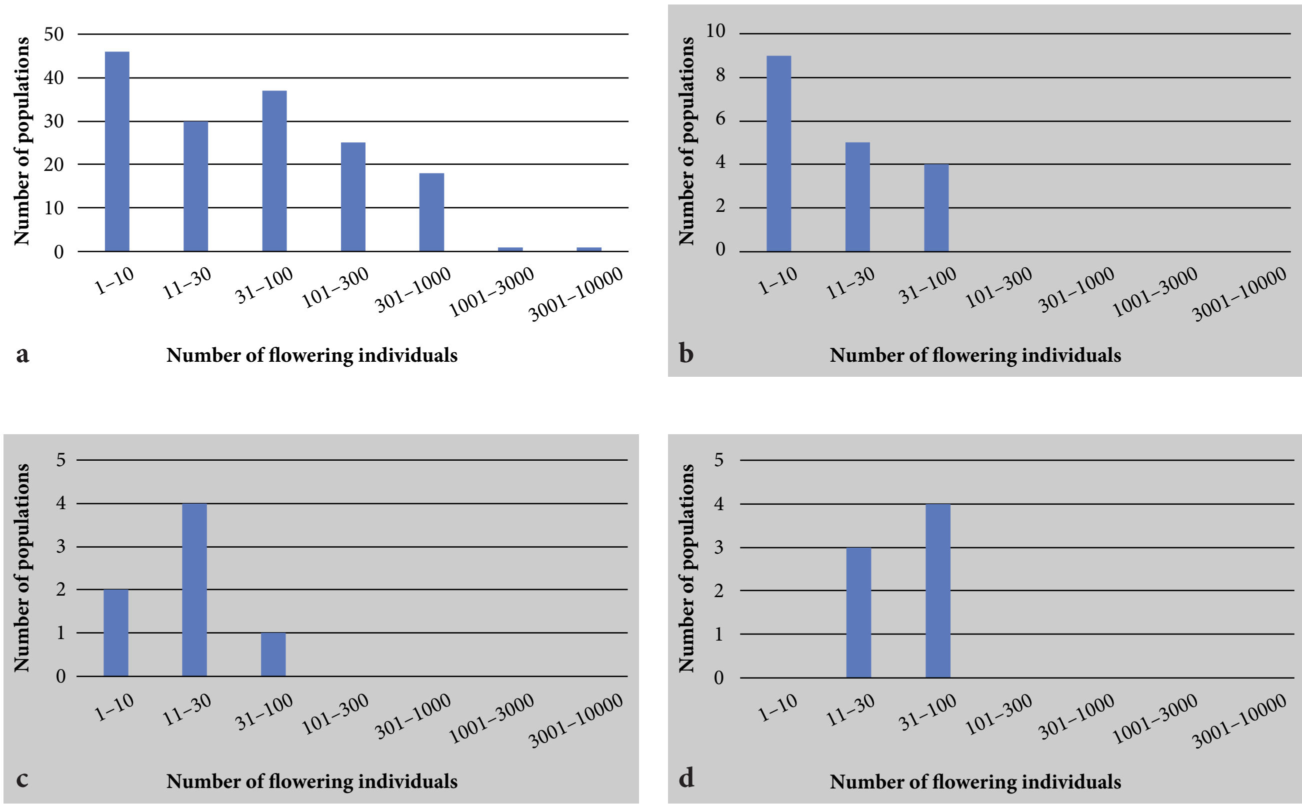


Fig. 3 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in South Bohemia. For grey images, the populations in all categories consisted of less than ten individuals.

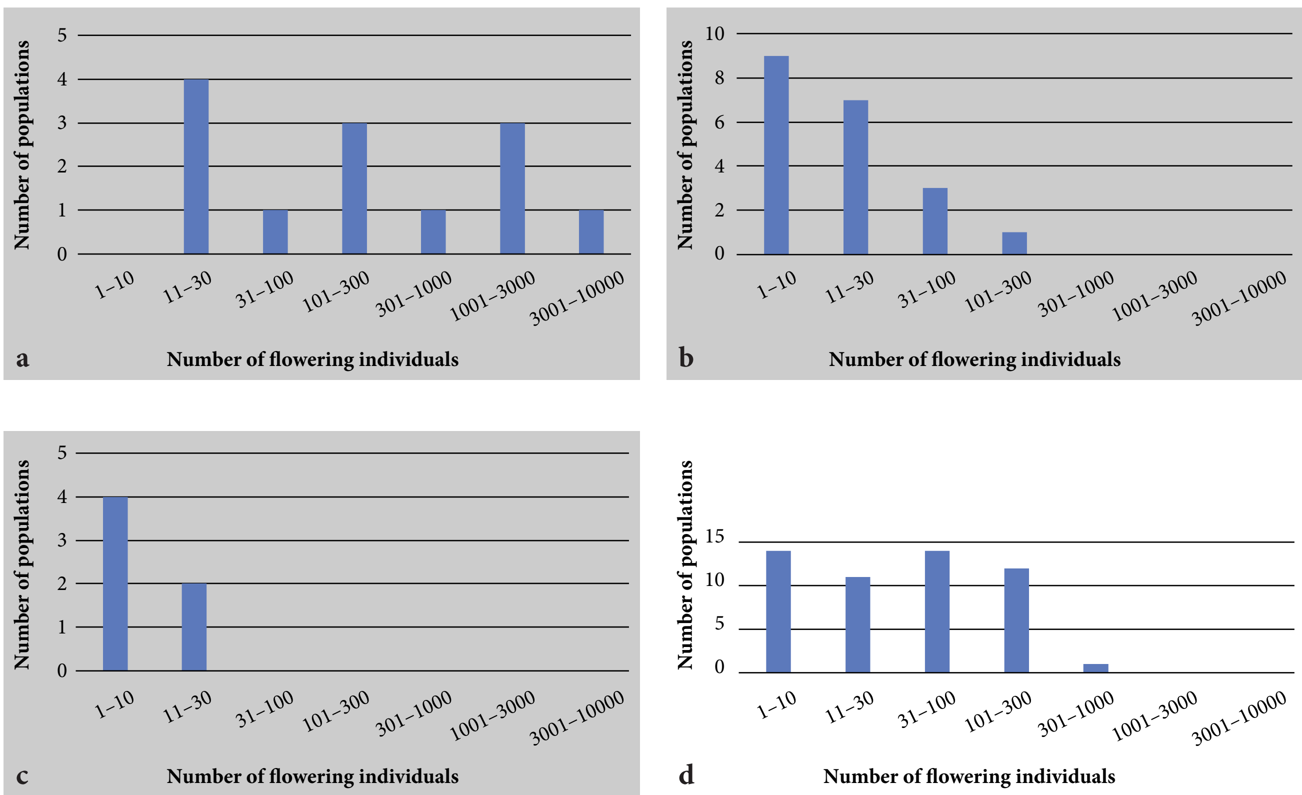


Fig. 4 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in Jeseníky Mountains. For grey images, the number of populations for all categories of number of individuals is less than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

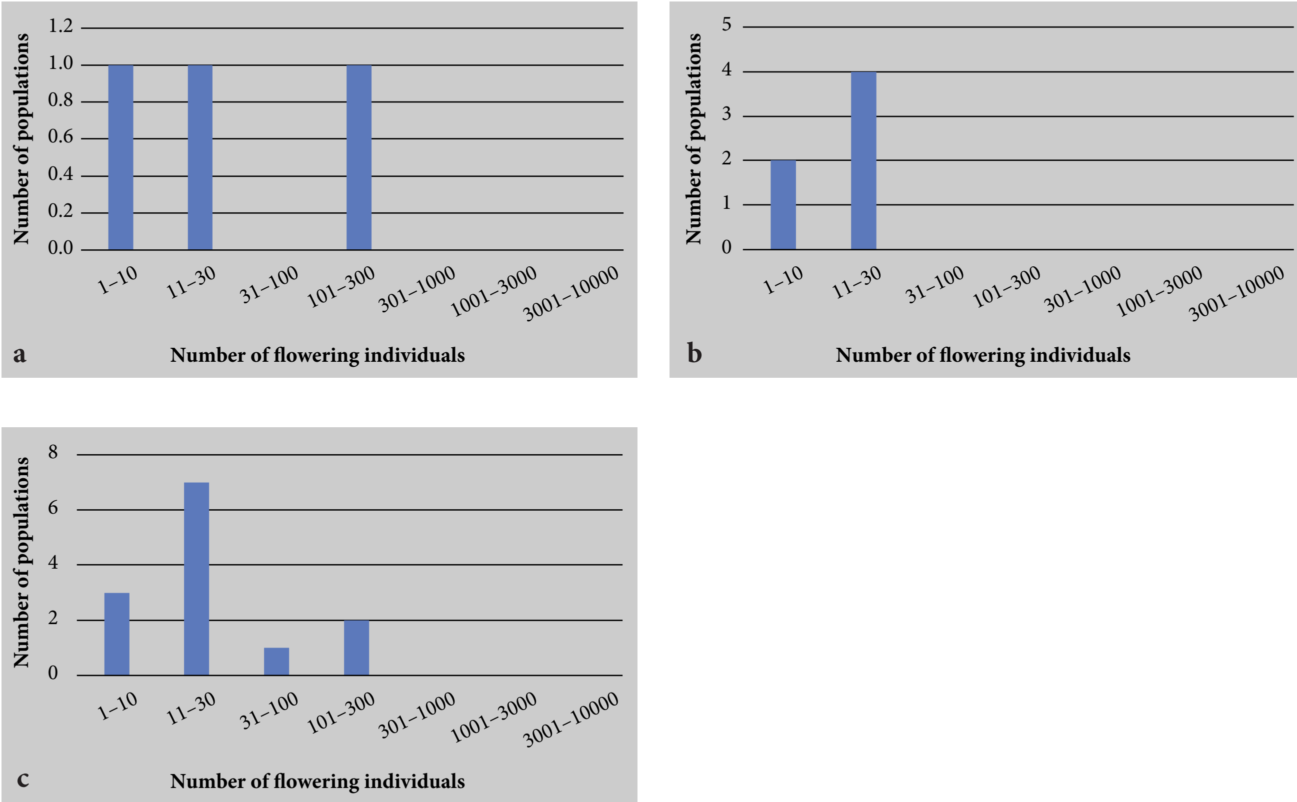


Fig. 5 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b) and *N. ovata* (c) in the Polabí region. For grey images, the number of populations for all categories of number of individuals is less than ten.

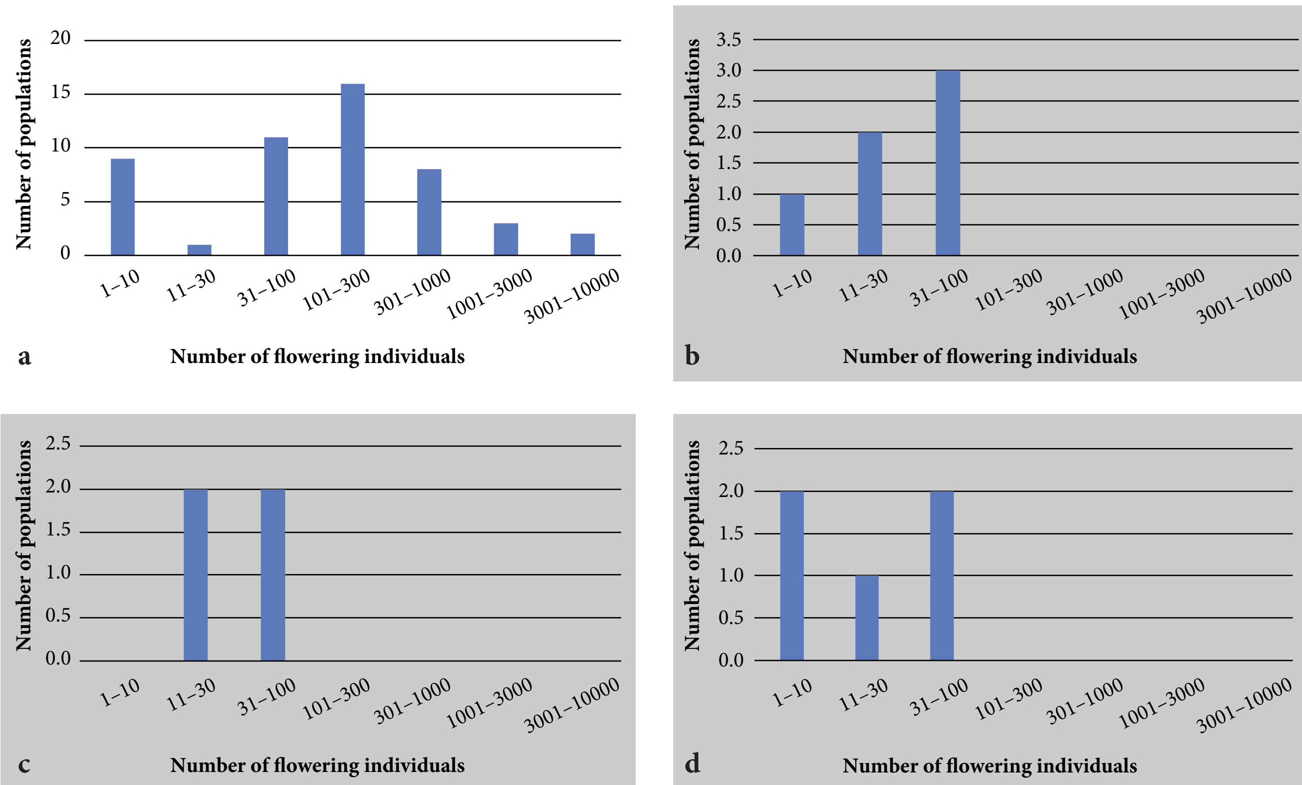


Fig. 6 Associations between population size and the number of flowering individuals for four orchid species: *D. majalis* (a), *P. bifolia* (b), *N. ovata* (c) and *D. fuchsii* (d) in the Bohemian-Moravian Highlands. For grey images, the number of populations for all categories of number of individuals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

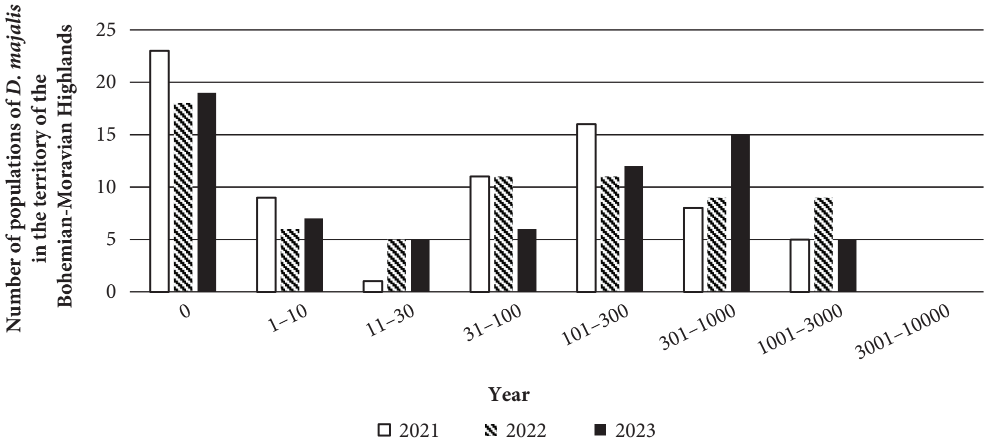


Fig. 7 Associations between the numbers of populations and the numbers of flowering individuals of *D. majalis* in the territory of the Bohemian-Moravian Highlands in 2021 (white), 2022 (hatched) and 2023 (black).

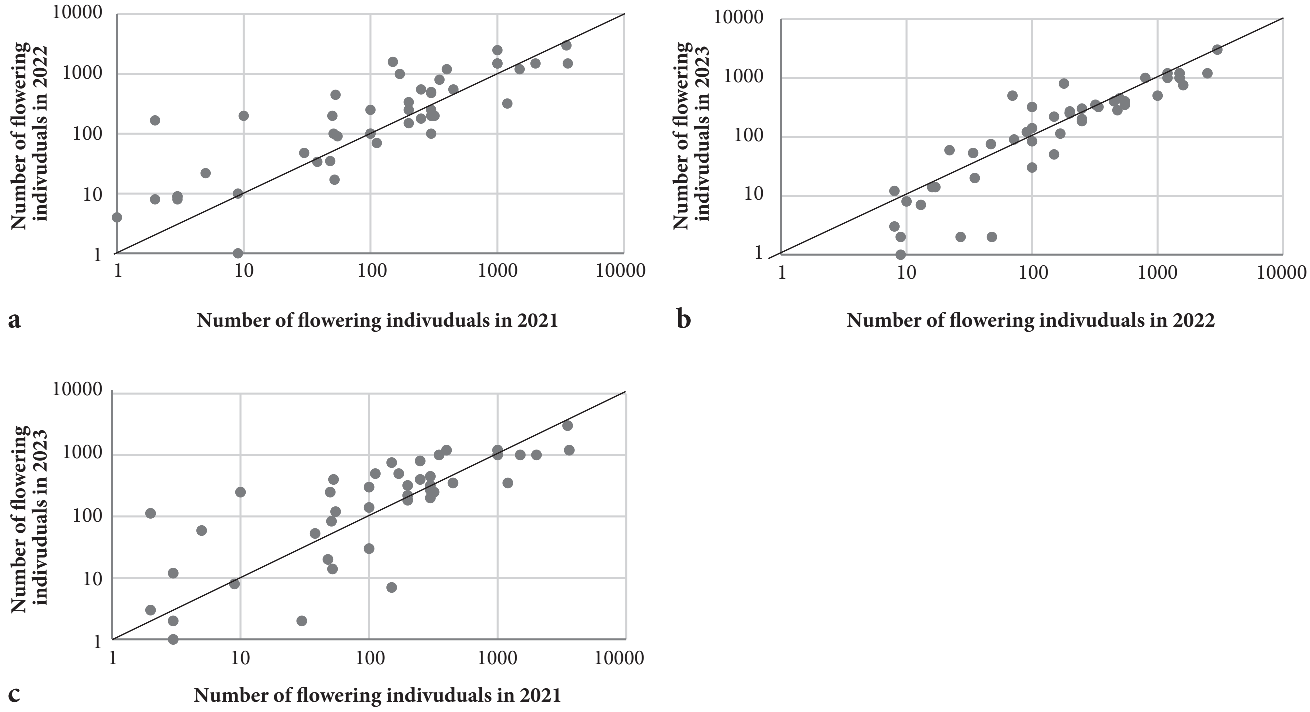


Fig. 8 Numbers of flowering individuals of *D. majalis* at individual locations in: (a) the Czech-Moravian Highlands in 2021 and 2022, (b) 2022 and 2023 and (c) in 2021 and 2023.

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

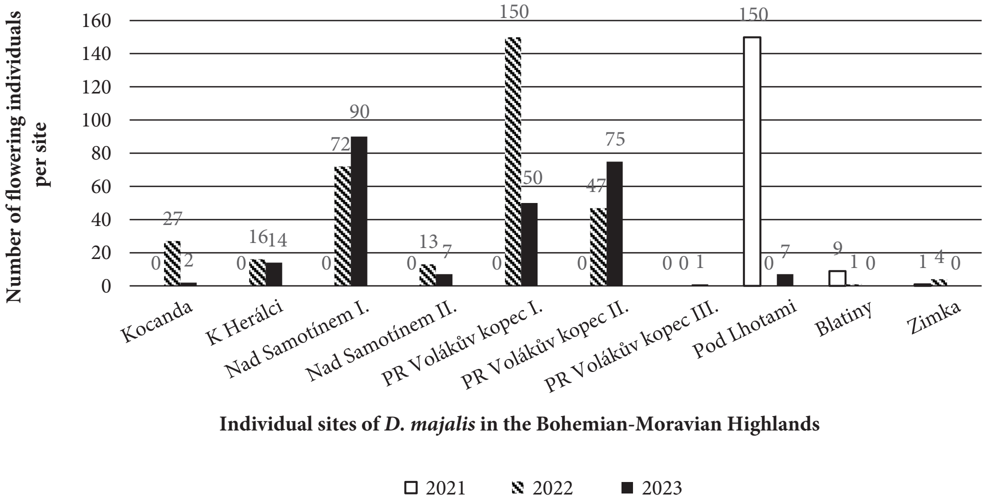
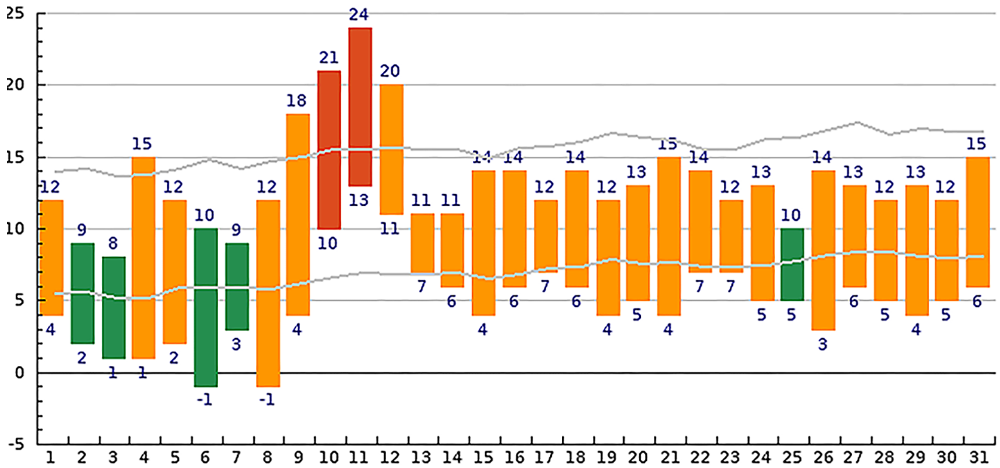


Fig. 9 Dependence of the number of flowering plants per site on years and individual sites of *D. majalis* in the Bohemian-Moravian Highlands in 2021 (white), 2022 (hatched) and 2023 (black). The image contains localities where no flowering individual was found in any of the monitored years.



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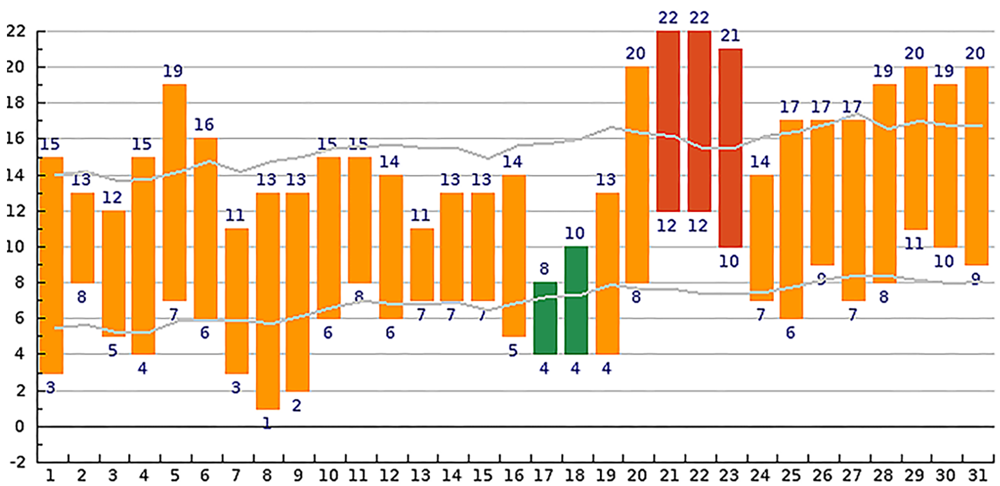

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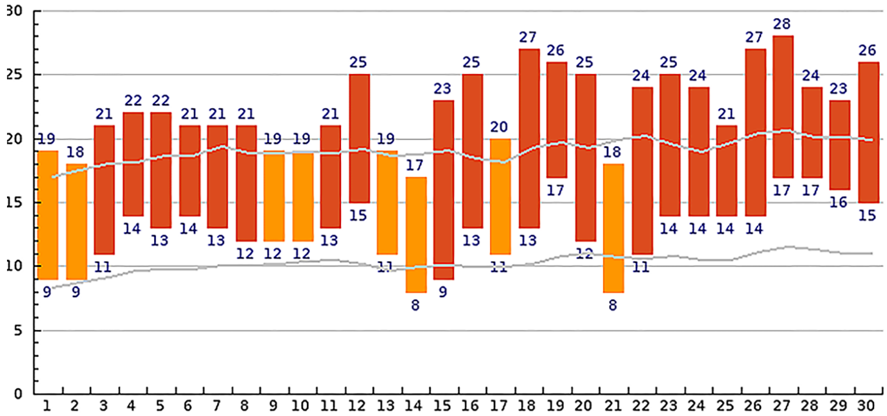

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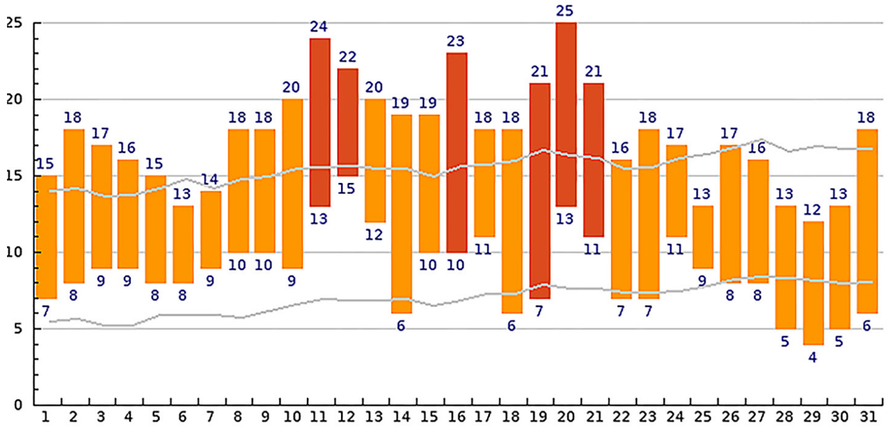

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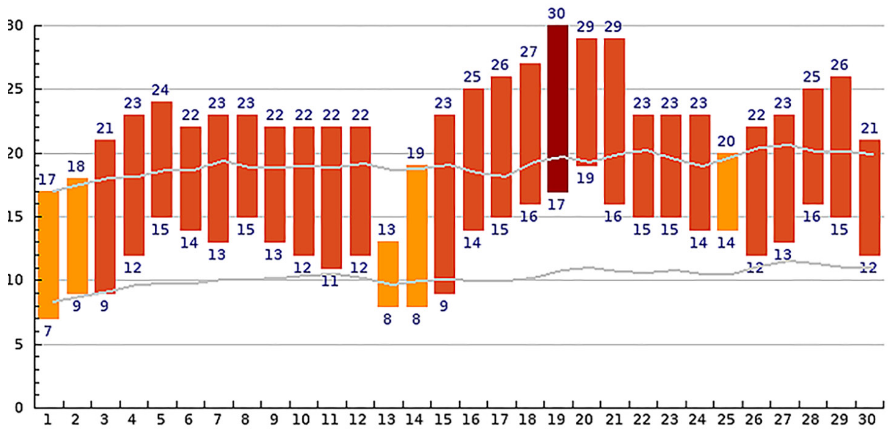

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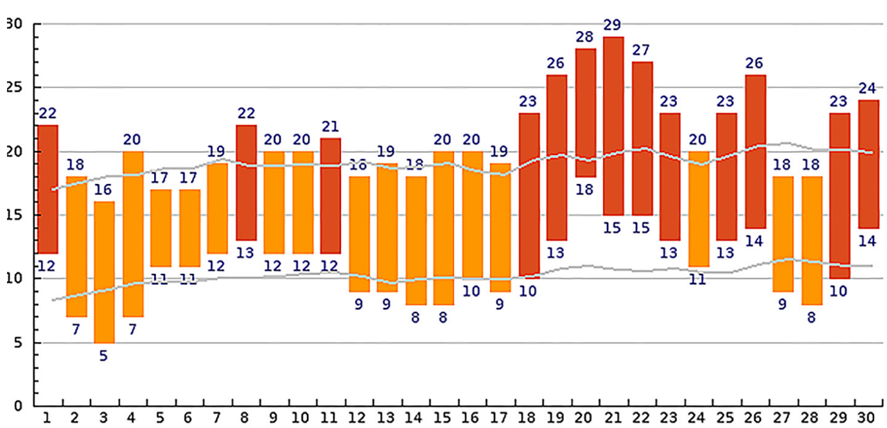

f

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 Svratouch. 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 contrary, 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 D*actylorhiza 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. Wotavová 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 Voláků̊v 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 m^2. 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 localities 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.

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