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Competing interests

No competing interests have been declared.

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ORIGINAL RESEARCH PAPER

Demographic responses of boreal-montane orchid *Malaxis monophyllos* (L.) Sw. populations to contrasting environmental conditions

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* Corresponding author. Email: edytabot@uwb.edu.pl**Abstract**

In an age of changes in species' geographical ranges, compounded by climatic and anthropogenic impacts, it becomes important to know which processes and factors influence plant populations and their persistence in the long term.

Here we investigated dynamic and fitness components in twelve populations of *Malaxis monophyllos* (L.) Sw., situated in different geographical (regions) and ecological (type of habitat) units. Although *M. monophyllos* is a rare species, characterized by highly fragmented, boreal-montane distribution range, in last few decades it successfully colonized secondary habitats in Polish uplands. Our results indicate that *M. monophyllos* is represented mainly by small populations, which annual spatial and temporal changes might be very high, what affects the ephemeral character of these populations, regardless of the region and type of habitat. This dynamic structure, in turn, is caused by intensive exchange of individuals in populations, as well as by their short above-ground life span. Despite the large range of variation in size and reproductive traits, we can distinguish some regional patterns, which indicate boreal region as the most optimal for *M. monophyllos* growth and persistence in the long term, and with montane and upland/anthropogenic populations, due to lower reproductive parameters, as the most threatened. Although it should be considered that anthropogenic populations, despite their lower reproductive parameters and instability in the long term, present an intermediate, geographical and ecological character, therefore they may be valuable in shaping, both *M. monophyllos*' future range, as well as its potential for response on ongoing and future changes. In general, reproduction is the main factor differentiating of *M. monophyllos* populations in regions, and we can suspect that it may become the cause of the future differentiation and isolation of these populations, occurring with progressive range fragmentation.

Keywords

anthropogenic habitats; population dynamics; red listed species; reproduction

Introduction

Acquiring knowledge regarding within plant population processes and factors that influence population dynamics underpins reliable scientific knowledge based on modern conservation biology, and enables the prediction of species survival under changing environmental conditions [1]. Simultaneously, demographic changes in populations arise from the life histories of individuals which respond to selection caused by environmental changes [2–4], thus the investigation of population dynamics is also important from the evolutionary point of view.

The main globally important factors influencing plant species survival are human activity and climate changes [5–8], which are reflected at the individual and population levels [3,9–11]. The models concerning future climate changes predict huge modifications that will take place in Europe in the coming century [12,13]. The most important changes will be an increase in temperatures and the magnitude of their annual fluctuations, as well as changes in the level of precipitation [14–16]. Some habitats and some species might be more sensitive to these changes than others. Southern and mountainous species, for example, seem to be the most endangered, although northern species will also be forced to respond [17,18]. In general, plants can react to changes in their habitats by persisting in new conditions through ecological plasticity and adaptations, or they can avoid new circumstances by shifting their habitats and ranges [19–24]. As a consequence, the reactions to ongoing changes will be largely dependent on species and population properties [25,26]. The theory predicts that long life span and iterated reproduction is favored in stochastic environments [27], thus habitat-specialists with a short life span, low reproductive rates and huge demographic fluctuations are more endangered [10,28–30].

The model group of plants which, because of strong connections with other components of their habitats play a vital role in sensitivity to global and local changes and provide a warning of impending damages (acting as bioindicators), are orchids [31–33]. They very often exhibit fast responses to environmental changes, which is reflected mainly in decreases in abundances of local populations, as well in lowering their reproductive potential (e.g., [34–37]), which makes most orchid species relatively highly threatened. Moreover, high variations in demographic parameters over time and space are a property of orchids populations [38–43], and this is usually connected with differences in environmental and climatic conditions through geographical range of species, as well in differences in management [39,44–46]. Intrinsic factors, however, like genetic variability, or population age and history have also a great importance, and are highlighted as factors significantly influencing within-population processes [47]. Thus, the populations of the same species in distinct habitats may differ in structure, stages transition and in reproduction, and therefore in persistence in the short and long term.

To enrich knowledge about population dynamics in distinct geographical and ecological units, in the context of declining range, we investigated populations of the orchid *Malaxis monophyllos* (L.) Sw. This rare, boreal-montane species is represented mostly by small populations that naturally occupy calcareous fens, wet meadows, spruce and alder forests [48,49]. Despite its rarity and progressive range fragmentation in the last few decades, *M. monophyllos* has successfully colonized anthropogenic habitats in the uplands in Poland [50,51], as well as in other parts of its geographical range [48]. In general, the decrease in the abundance and reproduction of orchids as a consequence of human activity is a commonly described issue [52], although the opposite reactions to disturbance, when they colonize anthropogenic habitats, is also identified in different parts of the globe [50,53–57]. The demographic processes operating in these secondary populations are still relatively unknown, and they may be of particular value in terms of preserving the rarest species of plants [58]. Our earlier investigations revealed the significance of upland populations in shaping *M. monophyllos*' large-scale genetic structure [59]. Moreover, the extinction of *M. monophyllos* natural populations, their ephemeral character, and instability in the long term [51], with poor information regarding their life history highlight the need for detailed studies on the mechanisms and processes shaping population viability. Although the demography of orchid populations are widely discussed in many different contexts, data concerning northern species from this group are sparse [60–62] and require a deep insight, as they are particularly important and valuable in the context of the decrease of global biodiversity and its conservation.

Thus, the main aim of this study was to answer the following questions: (i) what is the range of the demographic variation in *M. monophyllos* populations in different parts of its geographic range in Poland and under different habitat conditions, and (ii) what is the potential of populations from anthropogenic habitats to persistence in the longer time scale? Thus, we hypothesize that (i) habitat and climatic differentiation can modify life strategies at the individual level, which is manifested in regional patterns of population structure and dynamics; (ii) *Malaxis monophyllos* individuals from the

boreal part of the geographical range are characterized by higher levels of fitness than those from mountains, which exist under more severe environmental conditions, or than populations from uplands, which exist in anthropogenic habitats.

Material and methods

Study species

Malaxis monophyllos (L.) Sw. has a fragmented distribution, including boreal Eurasia, the northwest region of North America, and Central European mountain ranges [48,63,64] Almost everywhere it is a red-listed species [49,65,66], which despite its wide phytocenotic scale, prefers moderately humid, calcareous soils with at least moderate moss cover. In Poland it occurs both in boreal parts of the country and in the mountains [50,67,68], where it occupies different types of habitat (Tab. 1).

Malaxis monophyllos is a short-lived, inconspicuous, green orchid that reproduces exclusively by seeds and forms one basal pseudobulb. The average height of flowering stalks is approximately 20 cm ([48] and authors' observation). Flowering takes place from June to August; whereas in the south of Poland flowering starts about two weeks later (authors' observation). Fruiting occurs from July to August. *Malaxis monophyllos*

Tab. 1 Population coding, geographical origin and habitat characteristics of twelve investigated *Malaxis monophyllos* populations.

Population code	Population locality	Altitude (m a.s.l.)	N (min-max.)	Type of plant community
B1	Rospuda River Valley (East Baltic Lake District)	120	44–156	Alder–willow thickets (<i>Alnetea glutinosae</i>)
B2	Czarna Hańcza River Valley (East Baltic Lake District)	138	37–68	Boreal spruce bog (<i>Vaccinio-Piceenion</i>)
B3	Kunis Lake (East Baltic Lake District)	124	47–189	Alder–willow thickets on the peat layer (<i>Alnetea glutinosae</i>)
B4	Kirsznickie Lake (Masurian Lake District)	119	9–70	<i>Carex elata</i> community with the peat layer (<i>Magnocaricion</i>)
B5	Łażnica Lake (Masurian Lake District)	117	0–83	<i>Picea abies</i> and <i>Betula pendula</i> forest in organic soil (<i>Vaccinio-Piceenion</i>)
S1	Bukowno Walcownia (Silesian-Krakow Upland)	284	40–46	<i>Pinus sylvestris</i> cultivation with succession of coniferous forests species in undergrowth (<i>Vaccinio-Piceetea</i>)
S2	Sławków (Silesian-Krakow Upland)	289	33–127	<i>Prunus spinosa</i> thickets on railway embankment (<i>Rhamno-Prunetea</i>)
S3	Olkusz (Silesian-Krakow Upland)	319	646–795#	<i>Pinus sylvestris</i> and <i>Betula pendula</i> cultivation on reclaimed heap with succession of coniferous forests species in undergrowth (<i>Vaccinio-Piceetea</i>)
S4	Miechów (Małopolska Upland)	345	152–275	<i>Fagus sylvatica</i> forest on railway embankment (<i>Fagion sylvaticae</i>)
C1	Babia Góra Massif (West Beskids Mts)	1021	19–38	<i>Picea abies</i> renewal on roadside slope (<i>Vaccinio-Piceenion</i>)
C2	Chochołowska Valley (Western Tatra Mts)	1003	22–34#	<i>Picea abies</i> forest along the montane stream (<i>Vaccinio-Piceenion</i>)
C3	Jaworzynka Valley (Eastern Tatra Mts)	1113	81–94#	Montane meadow (<i>Molinio-Arrhenatheretea</i>)

N – number of individuals in population (minimal and maximal observed number); # – number of individuals in permanent plots.

is presumably an obligatory outcrossing plant, with minute, green flowers that are adapted to pollination by small insects, i.e., fungus gnats from the Mycetophilidae family [48,69]. The life cycle of *M. monophyllos* is poorly known, and according to Vekhrameeva [48] takes approximately 20 years, including a subterranean phase between germination and the first appearance above-ground (it lasts approximately 8 years). The first flowering occurs an average of 10 years after germination, and can be repeated for up to 4 seasons. In *M. monophyllos* simultaneously to other orchids, phenomenon of dormancy (not show up of above-ground shoots) can occur. In different species of orchids dormancy can last from 1 to even 5 subsequent years, depending on environmental factors and weather [39]. Furthermore, in *M. monophyllos*, like in other species of orchids, both flowering can be disrupted by non-flowering stage, as well non-flowering one can be extended if the environmental conditions are not optimal. In case of *M. monophyllos* after flowering period, the plant dies without experiencing a senescent phase [48].

Study populations

We studied twelve populations of *M. monophyllos* situated in three regions of Poland, which differ in terms of climate conditions, for example in the level of precipitation (Tab. 1, Fig. 1). Boreal populations are located in the East Baltic Lake District (population B1, B2, B3) and in the South Baltic Lake District (Iława Lake District, B4, B5). The montane populations are situated in Beskid Żywiecki (C1), in the West (C2) and East (C3) Tatra Mountains. Populations from boreal and montane regions exist mainly in natural habitats, and only some of them in seminatural habitats. The other four populations studied are located in the Małopolska and Silesian-Krakow Uplands, all in anthropogenic habitats, but with different levels of disturbance (S1, S2, S3, S4; Tab. 1). In further analyses upland populations will be described in the ecological context as anthropogenic habitat populations, with the rest of the analyzed populations as natural ones.

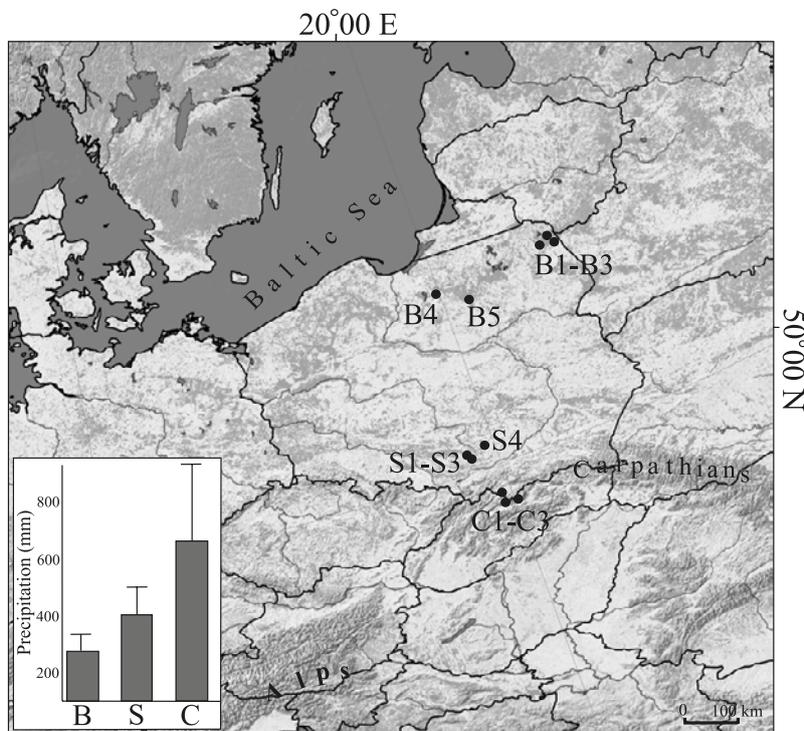


Fig. 1 Distribution of twelve study populations of *M. monophyllos* in Poland. Codes correspond with populations described in Tab. 1. Average monthly precipitation in March-June period in particular regions of Poland: B – boreal; S – upland; C – mountainous (Carpathians).

Data collection

In 2008, we began investigation of two *M. monophyllos* populations from northeast Poland (B1, B2). In subsequent years, ten more populations were included in the studies. In general, populations were investigated for 6 (2 pop.), 3 (7 pop.), or 2 (3 pop.) years. To measure population sizes (N) and their dynamics, in every year we counted all individuals in the population or, in the case of the biggest and most dispersed populations (S4, C2, C3), individuals on permanent plots.

In four populations from different regions and habitats (B1, B2, S2, C1), for which we were able to collect the most complete data sets (min. 3 years of observations of individual shoots), we performed analyses which allowed the determination of the basis of populations

dynamics. During the first year of observation (t) we individually labeled and mapped every shoot of *M. monophyllos* to follow its history in subsequent years ($t+1$, $t+2$, ...). Additionally, labeled individuals were classified according to their developmental and reproductive status into three life-stage classes: juvenile (J), nonflowering (NF), and flowering (F). This enabled us to describe the history of individuals, the rate of exchange of individuals in populations, as well as to quantify mortality and dormancy in different habitat conditions, as well to enrich knowledge regarding the life cycle of the studied species and to specify the spatio-temporal dynamics of their populations.

To estimate fitness components in all the investigated populations we assessed the reproductive potential, measured by the participation of flowering individuals (F). Additionally, as the size of individuals is one of the most important characteristics of fitness, for all the reproductive individuals we counted the numbers of flowers per inflorescence (F_{inf}), and we measured the height of shoots (H_F). Finally, as additional measures of reproductive potential, we assessed the level of fruiting (FR; ratio of fruit number to flower number). Reproductive success was assessed by the proportion of juveniles (J) in the total number of shoots in the population (as juveniles we took shoots whose leaf size did not exceed 1 cm in length).

Data analysis

We used different approaches to define the spatio-temporal dynamic of *M. monophyllos* populations in different parts of the geographical range and habitat conditions. First of all, on the basis of changes in the number of individuals in populations, we assumed a threshold of 20% change as an indicator of significant changes in population size. Changes in the number of individuals between 20% and 50% were considered moderate, and changes $>50\%$ as high. Additionally, all *M. monophyllos* occurrences mapped in the field were digitized in a vector map. Then the density of individuals in four populations was calculated with a kernel density tool in Spatial Analyst, ArcGIS 10.0 [70] using the kernel function (K_D). Each study plot had different dimensions, and therefore the kernel function parameters were set using the same assumptions with respect to the output raster extent. Mean and standard deviations were calculated for the whole output raster extent; the area varied by season, depending on the spatial distribution of individuals. The higher values of K_D indicate larger aggregations of *M. monophyllos* individuals.

Principal component analyses (PCA) were calculated using all characters describing fitness components in populations: F, F_{inf} , H_F , FR, J. PCA analyses, based on the correlation matrix, were performed in order to get insight into the overall variation on species and regional levels. The relationship between the original parameters (means per region) and the (varimax rotated) PCA scores was examined by means of rotated component loading.

Subsequently, standard analyses using the Kruskal–Wallis H test were performed to assess differences in the mean values of the most important fitness components for *M. monophyllos* populations in regions and types of habitats (analyses of the differences between populations were also made, but for clarity of results are not shown in this paper). The normality of the data was evaluated prior to the analyses, using the Shapiro–Wilk test. Spearman range correlations (R) were applied to measure the strength of the relationship between (*i*) individual fitness components and (*ii*) between fitness components and regions. Finally, we assessed the influence of climatic factors on populations' properties (N, F, F_{inf} , H_F , FR, J). For this purpose we used populations from which we had at least three years of observations. The data from the nearest meteorological stations were used in analyses (IMGW annual reports 2008–2013 for Suwałki, Stary Folwark, Sejny, Prabuty, Szczytno, Katowice, Jabłonka, and Polana Chochołowska). In our investigations we used precipitations in periods of year important in *M. monophyllos* ontogenesis [71]: March–June and September–December periods in the year preceding year t ($t-1$), and for March–June period of year t (year for which we analyzed demographic parameters). The effects were evaluated using Spearman's rank correlation coefficient in STATISTICA PL. ver. 10 [72] software packages.

The life stage transition, as well as rates of mortality and dormancy were assessed in the B1 and B2 populations, due to the largest data sets. We defined *M. monophyllos* as a dead when it did not appear above ground for two subsequent years. This assumption was made after preliminary analyses of dormancy, which revealed that only one individual among the 64 included in the analyses was dormant for two consecutive years.

Results

Population sizes and dynamics

Twelve populations of *M. monophyllos* differed considerably in size (Fig. 2a), ranging from 19 (C1 population in 2009) to almost 800 individuals (S3 population in 2010). However, our results suggest that *M. monophyllos* is mainly represented by small populations, which even at the peak of abundance rarely exceed one hundred individuals. In most cases population sizes changed significantly during the observation period (Fig. 2b). The percentage differences in the number of individuals between subsequent years exhibited moderate (B2, S4, C1, C2) or large changes, whose absolute values approached or exceeded 100% (B1, B4, B5, S2) or even 300% (B3). The highest values refer to increasing population abundance. Declines usually occurred at the 30–50% level, and only one time reached 80%. In 7 out of the 27 cases (one case is one year in one population) inter-annual differences were small (<20%; Fig. 2b).

All four populations, investigated in details, were characterized by an aggregative spatial structure, but the sizes and densities of groups of shoots in populations, described by the mean values of the kernel function (K_D), varied between populations, and fluctuated from year to year (Fig. 3). As a consequence, B2 and C1 represented a dispersed (mean $K_D = 0.012$ and $K_D = 0.082$, respectively) pattern, and populations B1 and S1 exhibited a more concentrated pattern of spatial structure (mean $K_D = 0.63$ and $K_D = 0.66$, respectively). Additionally, values of K_D can change radically from year to year, which indicates that the spatial structure of *M. monophyllos* populations, regardless of the region or type of habitat, can also be very dynamic (Fig. 3).

Fitness components

PCA. PCA reduced the total set of partly intercorrelated variables to two uncorrelated principal components including size and reproductive traits (Tab. 2). Together the two principal components explained 76.48% of total variation between regions. The first axis explained 58.24% of the variation and the second axis 18.24%. The three groups of populations represented regions were not clearly separated on the scatterplot (Fig. 4), and the particular populations represented a partly overlapping range of size and reproductive traits. The first PCA axis was most highly influenced by size parameters (H_F and F_{inf}), and less by frequency of flowering individuals (F), while the frequency of juveniles (J) and fruit set (FR) were most strongly related with the second PCA axis.

Size structure. Among the traits analyzed, study regions differ with respect to fitness components, measured by the size of individuals, particularly in the mean height of flowering shoots (H_F : $H = 14.40$, $df = 2$, $p = 0.001$). We noted the lowest individuals in mountainous populations where they ranged from 7.4 to 17.7 cm, with median values 14.7 cm, moderate in uplands/anthropogenic populations, ranging from 11.2 to 27.6 cm, with median values 17.6 cm, and the highest in the boreal region, from 14.7 to 27.3 cm, with a median of 21.4 cm (Fig. 5a).

Reproduction. We found no significant differences in the frequency of flowering individuals between regions ($H = 0.90$, $df = 2$, $p = 0.64$). In general, the highest frequency of flowering individuals was found in boreal populations ($F = 65.3\%$ and 62.2%), which ranged between 12.6–65.3%, while in uplands/anthropogenic and in

mountains it appeared at the lower level, between 15.7–38.5% and 12.5–45.4%, respectively. Despite differences in the number of flowers per inflorescence between regions (means = 44.5 ± 10.9 ; 44.7 ± 12.9 , 34.2 ± 10.3 for boreal, upland and mountane region respectively), we found no statistically significant differences between them (F_{inf} : $H = 4.81$, $df = 2$, $p = 0.09$; Fig. 5b).

The second parameter which reflects reproductive potential in a given environmental conditions is the level of fruiting (FR), which strongly varied between regions ($H = 15.49$, $df = 2$, $p = 0.000$; Fig. 5c). The fruit set was significantly higher in the

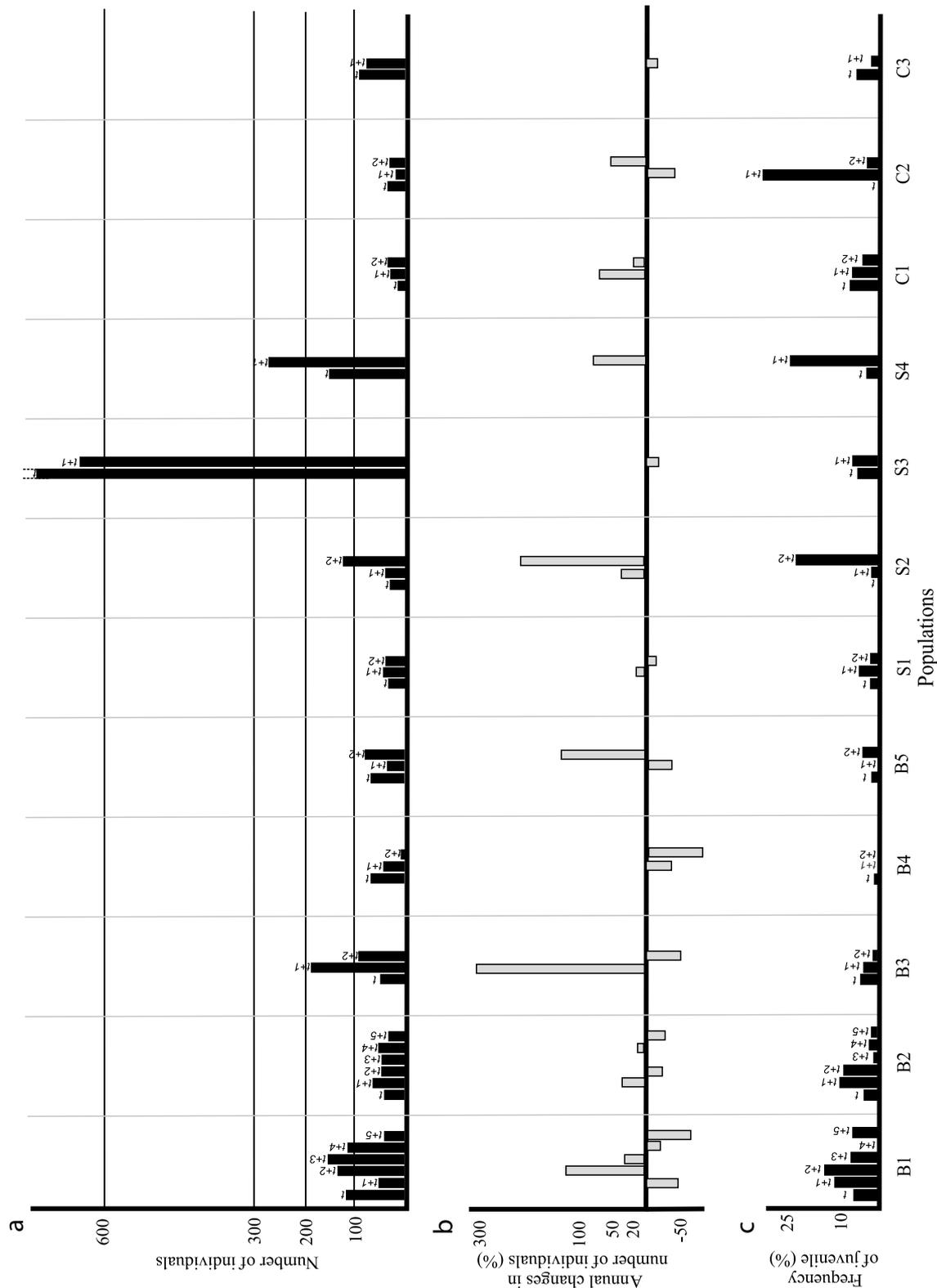


Fig. 2 Populations size changes (a), percentage difference in the number of individuals between two subsequent years (b), and frequency of juveniles (c) in twelve investigated populations (t – first year of observation; t+1, t+2, ... – subsequent years of observation).

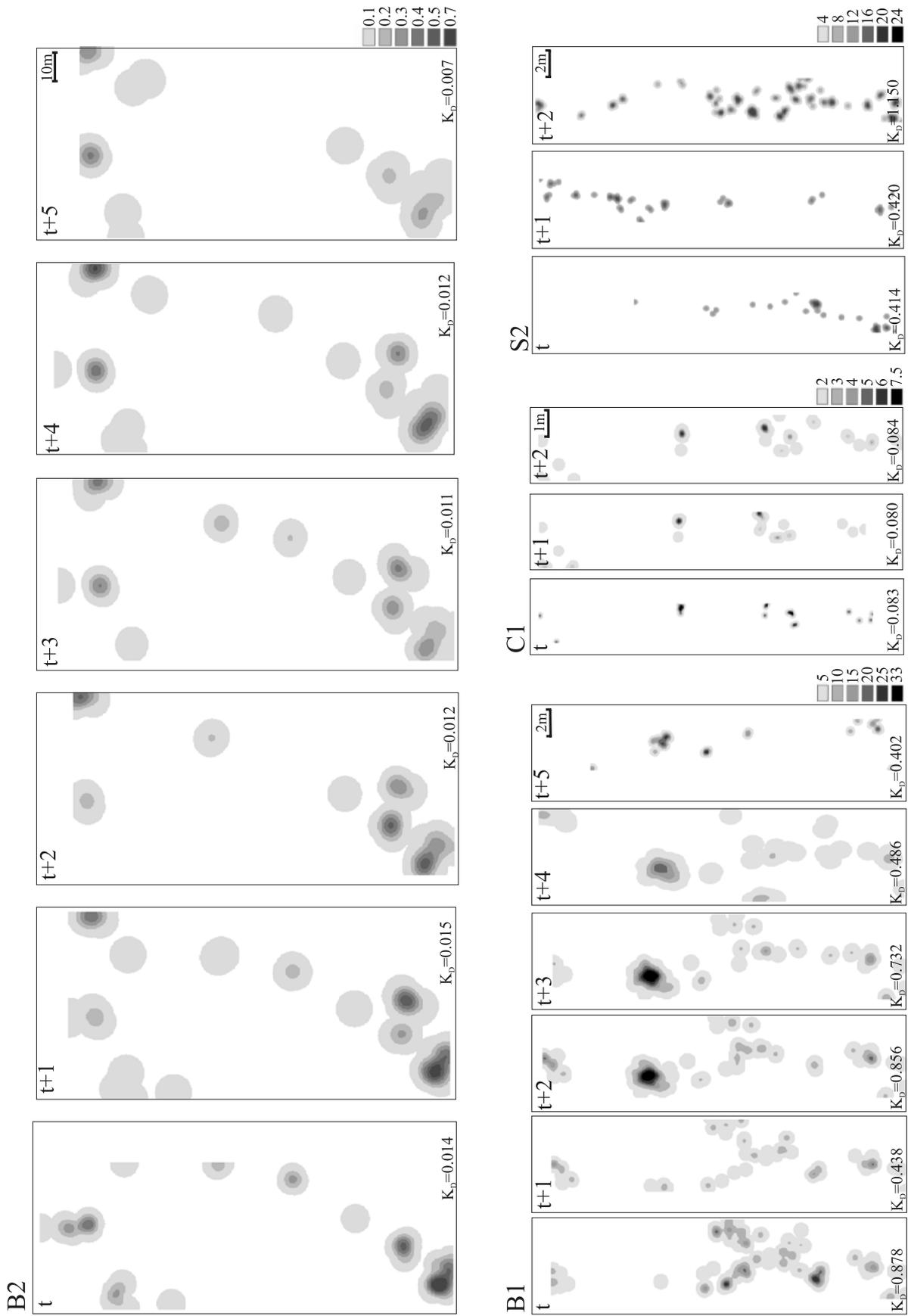


Fig. 3 Changes in spatial structure of *M. monophyllos* populations during subsequent years of observation (legend: average values of the kernel density estimator K_p ; gradients show densities of individuals estimated by kernel density function).

Tab. 2 Principal component loadings of the measured size and reproductive characters (after varimax rotation). Loadings given in bold line show the highest correlation between original values and principal components scores.

Variables	Factor loadings	
	1	2
F	-0.44	0.34
H _F	-0.53	-0.29
F _{inf}	-0.51	-0.19
FR	-0.39	-0.42
J	0.34	-0.77
Eigenvalue	2.91	0.91
Cumulative variance (%)	58.24	18.24

F – participation of flowering individuals; H_F – height of shoots; F_{inf} – number of flowers per inflorescence; FR – level of fruiting; J – participation of juveniles.

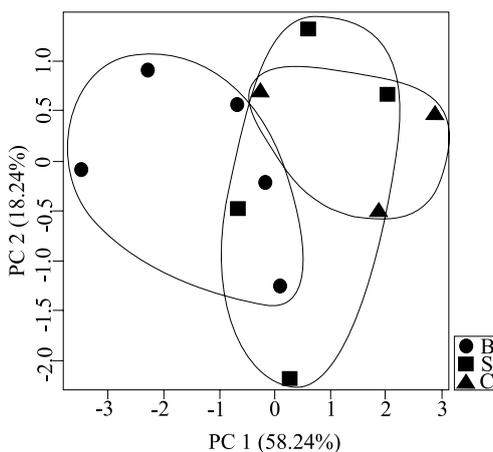


Fig. 4 Scatterplot presenting the result of principal component analysis (PCA) based on five morphological characters for twelve *M. monophyllos* populations from different regions (B – boreal; S – upland; C – mountainous).

boreal group, where the average was 22.53% (± 11.51), it range from 7.5% to 46.6%, with median values 20.8%. In the remaining populations, both from uplands, as well as from mountains, fruiting rarely exceeded 10% (except in one year in population S4). Thus, it ranges from 0% to 27.9% in uplands (median = 2.6%) and from 1.5% to 11.9% (median = 7.1%) in mountains. Additionally, in boreal populations, the majority of flowering individual set fruits, while we found that fruits appeared in less than 50% of the flowering shoots in upland/anthropogenic and montane populations. The number of fruits, in turn, was correlated with the number of flowers per inflorescence in all analyzed cases, although in upland/anthropogenic populations the strength of this relationship was weaker ($R = 0.30$, $p < 0.001$) than in the boreal part and mountains ($R = 0.51$, $p < 0.001$). We also found that fruiting level depended on population size, but only in the case of boreal populations ($R = 0.45$, $p < 0.05$).

In terms of the frequency of juveniles (J), despite the fact that this is one of the traits which distinguished regions from each other in PCA analyses, there were no statistically significant differences for average values between the investigated regions ($H = 1.54$, $df = 2$, $p = 0.46$), and in the majority of populations J rarely exceeded 10% (average J for boreal populations = 5.11%, uplands/anthropogenic = 7.58%, and mountains = 8.82%; Fig. 2c).

The correlations between climatic conditions and fitness components were found only in montane populations, where the number of individuals in a given year, as well average number of flowers per inflorescence, was positively correlated with the mean monthly precipitation during October–December period in year $t-1$ ($R = 0.714$, $p = 0.047$ and $R = 0.857$, $p = 0.007$, respectively). Additionally, in mountains there was a negative relationship with the mean monthly precipitation in March–June period in year $t-1$, and frequency of juveniles in year t ($R = -0.718$, $p = 0.042$).

Dormancy, mortality, and life cycle. The lifespan of above-ground *M. monophyllos* individuals, analyzed in the two populations with the longest data sets, lasted from 1 to 6 years. Individuals in population B2 were characterized by longer life spans (3.5 years) than those from population B1 (2 years). The duration of the non-flowering stage (NF) was the same in both populations (1–4 years), while the duration of the generative stage differed between populations, and took 1–2 years in population B1 and 1–4 years in population B2.

We also found considerable differences between the B1 and B2 populations in regard to life stage transitions (Fig. 6). In population B1, for which we also found the highest annual changes in abundance, 46.9% of non-flowering individuals were in this stage during the observation period, while in population B2 more than 40% of individuals prolonged the generative stage. Populations did not differ in the frequency of individuals which transitioned to the flowering

stage after the non-flowering stage, but almost twice as many individuals in population B1 returned to the non-flowering stage after flowering (Fig. 6).

The observed patterns of the life cycle in the two investigated populations are affected mainly by the rates of dormancy and mortality, which significantly differed between these populations. As a consequence of mortality, 94% of individuals in population B1 and 75% of individuals in population B2 observed during the first year (t) disappeared during the investigation period. In population B2, individuals died more frequently after flowering (60%); in population B1 after the non-flowering stage (67%). Dormancy was noted in 11% and 24% of cases in the B1 and B2 populations, respectively. In the majority of cases dormancy last only one year, and more frequently appeared after the non-flowering stage in B1 and after flowering in the B2 population.

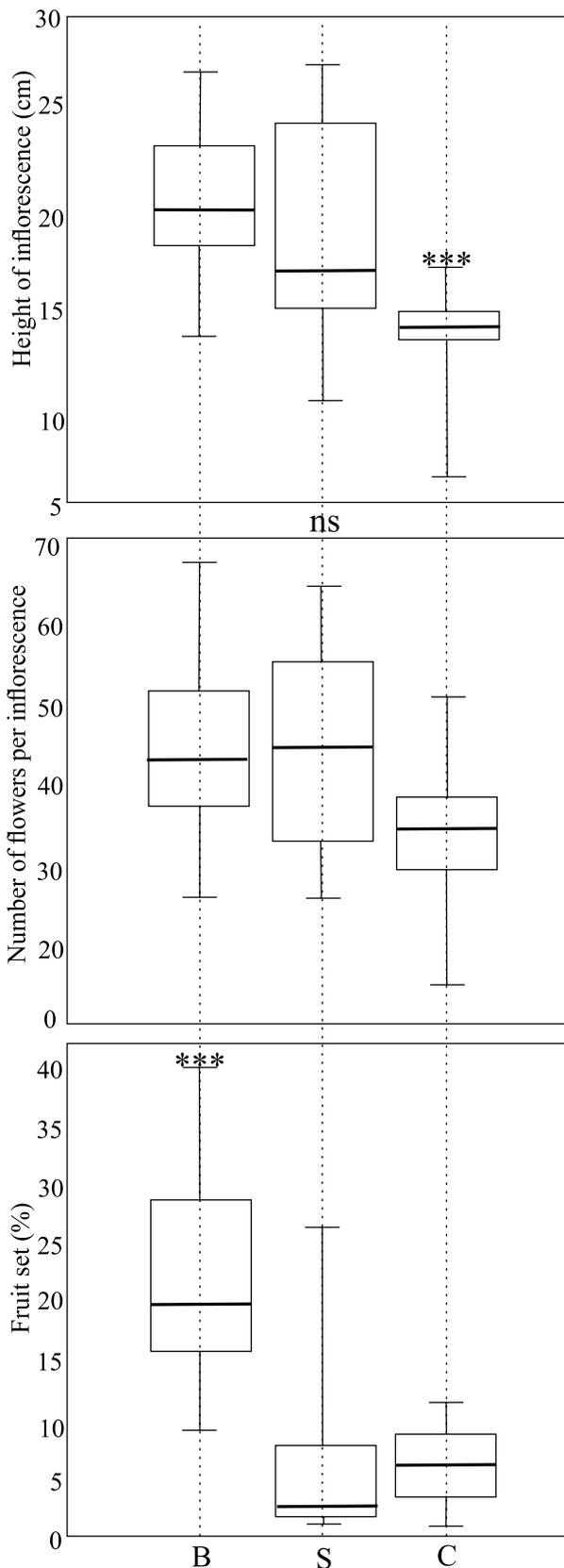


Fig. 5 Median values of the height of flowering shoot, average number of flowers per inflorescence, and the median values of the proportion between the total number of flowers and the flowers that developed into mature fruits in the populations of *M. monophyllos*, grouped according to geographical regions (B – boreal; S – upland; C – mountainous). *** $p < 0.001$; ns – not significant differences (Kruskal–Wallis H test).

Discussion

The literature data point out that *M. monophyllos* is mainly a habitat-specialist that is represented by isolated, small populations, existing in naturally fragmented habitats due to the heterogeneity of suitable environments [48,64]. Recently, natural fragmentation and isolation have intensified due to human activity, which increases the importance of ongoing detrimental processes in isolated populations.

Orchid populations are known for their highly temporal and spatial dynamic structure [39,41,42]. In *M. monophyllos* populations, in every region and type of habitat, year-to-year changes are exceptionally large, both in abundance (up to 300% differences in abundance), as well as in spatial structure ($K_D = 0.414–1.150$). Oostermeijer and Hartman [73], for example, observed large population dynamics for another orchid connected with swampy habitats, *Liparis loeselii*, and concluded that it depends mainly on the colonization of new, suitable habitats. Such an explanation, consistent with the metapopulation model, might be highly probable in the case of *M. monophyllos*, especially when it occurs in areas (i.e., river valleys, along mountain streams) where suitable habitats are patchy in character. This is also in accordance with Hanski [74], who stated that the metapopulation is a kind of network of colonies, which persists as the result of a balance between the extinction of individual colonies and dispersal between available habitats. This is expressed in the highly variable spatial structure of *M. monophyllos* populations, which may also reflect the distribution of sites suitable for germination [41]. The increase in abundance is mainly determined by the increase in the densities of groups of individuals, as well as by the colonization of new fragments of available habitats. The main mechanism causing changes in the abundance of *M. monophyllos* populations is an intensive exchange of individuals (94% in B1 and 75% of individuals in the B2 population observed in the first year were absent in the last year) resulting from its relatively short above-ground life span, lasting presumably from one to six years (on average 2.8 years). Some of the *M. monophyllos* populations seems to be extremely dynamic or even ephemeral in character, which may suggest the influence of local environmental disturbances, that influence process of germination and flowering [42,71].

Population sizes and their dynamics are greatly influenced by the process of reproduction. Fruiting, the common measure of the level of reproduction in orchids, significantly varied between *M. monophyllos* populations in regions and types of occupied habitats. In general, in the case of *M. monophyllos*, the fruit set is relatively low, as with many other orchids that are non-rewarding, self-incompatible and pollinators dependant for the fruit set [75,76]. Exceptionally low levels of fruiting were noted in anthropogenic populations of *Malaxis*, located in the Polish uplands, which is also reflected in studies on other species of orchids in secondary habitats. Pellegrino and Bellusci [77] noted an almost seven

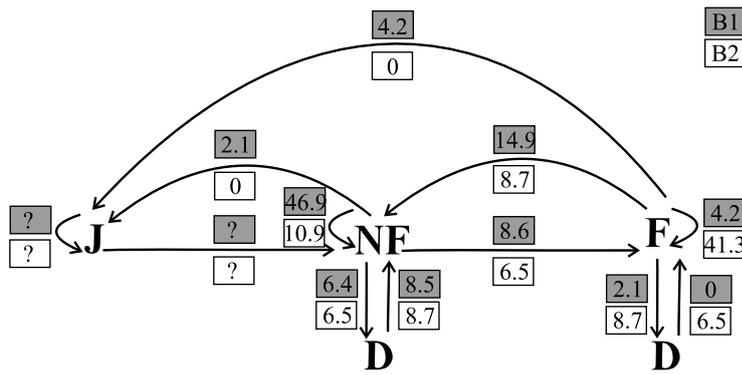


Fig. 6 Life-stage transitions in two *M. monophyllos* populations (B1, B2 – according to Tab. 1). J – juvenile stage; NF – nonflowering stage; F – flowering stage; D – dormancy; the values are given in percentages of all shoots included into analyses in a given population.

times lower fruit set in anthropogenic populations of *Serapias cordigera* in Italy than in its populations from natural habitats. In population of *Oncidium ascendens* from well-preserved rainforest from Mexico fruit production was likewise almost two times higher than in populations from synanthropic habitats [78]. Among the possible explanations for the variation in fruiting between natural and anthropogenic populations are the differences in pollinators' efficiency [79–81]. These are probably, young anthropogenic populations, that did not establish sufficient relationships with important symbionts (pollinators) which are presumably connected with wet environments [69,82]. We observed a divergence between the high level of

male success (high rate of pollinia removal) and low fruit set in anthropogenic populations of *M. monophyllos*. It might suggest that a high proportion of pollinia are lost, and pollen discounting takes place [83], what may indicate that insects visiting flowers in anthropogenic populations are not pollinators. Despite low reproductive potential (fruiting) in upland populations, reproductive success (frequency of juveniles) was similar to populations from other regions. Juveniles appeared in anthropogenic habitats systematically (similar to boreal and montane populations), at least at the first stages of colonization, which enables the persistence of these populations in time, but also indicates the successional status of populations as the key to interference about population prospects. Although our data are too limited to clarify this issue, we can make some assumptions about the processes that cause instability over longer time, as reported by some authors [51]. Firstly, the emergence through a demographic explosion may suggest many neighboring sources of colonization of anthropogenic sites. Secondly, the decrease in abundance during subsequent years after the first colonization might be a symptom of disappearing external sources of seeds, an insufficiency of internal source (low fruit set). Decline of suitable sites for germination during progressive succession is also very possible. Simultaneously, the lower level of fruiting in the uplands populations may be compensated by bigger fruits [83]. Such a trade-off between the number of fruits and their size (and probably seed count) could be considered an adaptive strategy under resource limitation (pollinators limitation), and could be the confirmation of species potential for modifying its life strategy traits in disturbed habitats. In general, all these issues, in the case of anthropogenic habitats, are crucial for *M. monophyllos* populations' preservation in the long term, and require further investigation, particularly concerning the mode of pollination and breeding system.

As for many terrestrial orchids, the size of plants is an important trait that determines reproductive success [84,85]. This may indicate that the best conditions for the development of *M. monophyllos* populations in Poland are in the boreal region, which comprises the majority of its geographic range. However, individuals in some anthropogenic populations were also large, which suggests that these habitats are also suitable for *M. monophyllos*, due to unused resources or lack of competitors. In mountains (i.e., above 1000 m a.s.l.) environmental conditions are more severe (higher annual temperature amplitudes, shorter growing season) for plant growth and reproduction, which is reflected in, e.g., the smaller sizes of *M. monophyllos* individuals. Other species of plants were also smaller and have lower values of reproductive potential in montane populations, than those from lower altitudes [86,87]. The population size or floral display measured by the size of the inflorescence, are pointed to be crucial for fruiting level [76,88–92]. Although we found positive correlations between fruit set and population size only for natural populations, the relationship between fruiting and the number of flowers per inflorescence was statistically significant for all study regions.

Due to the strong fragmentation of the European part of *M. monophyllos* range, we can consider that it has two main areas of distribution: boreal and montane. Therefore, upland populations could be described as intermediate or marginal ones, especially in the ecological sense, and because of that they may have special evolutionary significance [93,94]. In such populations different traits and/or relationships with other components of the environment can depart from typical situations [41,95]. In consequence, they could be the source of increasing adaptive phenotypic plasticity because they are exposed to harsh conditions, which may influence the selection of individuals who are adapted to such environments [96,97]. Moreover, *M. monophyllos* anthropogenic populations have a special role, because they could be considered as a kind of bridge outside the present geographical distribution, and thus its impact on its future geographical range can be substantial.

In the light of recent and ongoing climatic changes, an important problem is their influence on the properties of rare species' populations and their maintenance in the long run. Despite growing interest in this problem, empirical evidence is relatively scarce. In the case of orchids, the influence of climate has most often been studied in the context of reproductive traits, which both in case of terrestrial and epiphytic orchids suggest precipitation as the main factor [98,99]. Connections of *M. monophyllos* with wet environments, together with an increase in large-scale precipitation in northeast Europe in the coming decades [15] suggest that populations in the boreal part of its range will presumably possess optimal conditions to persist. Although, we found a lack of connections between precipitation and demographic parameters of populations from the boreal region. Additionally, the Europe-wide trends of increasing temperatures might influence the reproduction and morphological traits of plants, especially of cold-adapted species [100]. Moreover, climatic changes may force them to shift their ranges further north and to higher altitudes, which in turn will be limited by the availability of habitats, and by species potential for migration. Most simulations [12,13] suggest that montane populations are especially endangered by the potential lack of reproduction and recruitment due to climatic changes. The presented study also points to montane populations of *M. monophyllos* as those with decreased individual fitness (smaller sizes, lower fruit sets), which is additionally strengthened by negative scenarios for this region, that result from SDM models for *M. monophyllos*' prospects in Europe [59].

However, because all changes on species level are a consequence of accumulated changes within populations [2], we suspect that the wide range of values of many parameters at the population and regional scale reflect *M. monophyllos*' evolutionary potential, which is quite high when we considering diversity on species level. Our results also indicate that reproduction plays a key role in the differentiation of *M. monophyllos* populations in regions, what can determine probability to survival of these populations, and may be also the cause of further progressive differentiations of populations in regions. In consequence, it could presumably lead to the formation of some biological barriers, together with progressive fragmentation and separation of the boreal and montane parts of the range [59].

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