

Floral biology of *Senecio macrophyllus* M. BIEB. (Asteraceae), a rare Central European steppe plant

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Abstract

Knowledge of the flowering phase and plant pollination ecology is very important for understanding the life history of long-lived perennials. In the case of rare species, the information may have implications for conservation practices. Our studies on flower morphology and blooming biology of the vulnerable plant *Senecio macrophyllus* M. BIEB. were conducted in situ (flowering, activity of insect visitors) in the largest population in SE Poland and in laboratory (light and scanning electron microscopy). The disc florets open diurnally with most intensive anthesis in the early afternoon and attract insect visitors with nectar and pollen. In highly protandrous disc florets, pollen viability decreases in time, whereas stigma receptivity increases. The upper part of the pistil forms a brush-like pollen presenter, covered with unicellular trichomes with chromoplasts. Pollen presentation lasts 4–11 hours per floret, and 8 days in a single head, the main function of which is to extend the availability of male gametes for reproduction. The number of florets per head, the head size, and the number of pollen grains produced per anther, and the pollen grain viability differed significantly between microsites. The finding indicates that, apart from the biotic factors, abiotic conditions may considerably influence generative reproduction of the species. In the study area, the principal visitors of *S. macrophyllus* flowers were Hymenoptera, with predominance of *Apis mellifera* (53.4% of visits). The remarkable share of butterflies (13.9%) recognized as the long-distance moving insects may improve the genetic variability of individuals within a fragmented population. A more detailed study is necessary to explain the role of insect visitors in effective pollination and in gene transfer between patches. The assessment of optimal conditions for the generative reproduction is fundamental for the in situ conservation of this rare species.

Keywords: *Senecio macrophyllus*; flower life-span; insect visitor spectrum; female phase; male phase; pollen production; pollen viability; stigma receptivity

Introduction

Conservation of plant species biodiversity is one of the most important challenges, as most of them, including rare and endangered species, exert an impact on the different interactions linking organisms within biocoenoses, e.g. ensure stability of reproduction [1,2]. Asteraceae is a large family with 900 genera and 13 000 species [3] and shows an extreme diversity of breeding systems with many species having a pronounced flexibility in the breeding strategy. Self-incompatibility being the most common mode of outcrossing has been recorded in 40 genera [4], including some species from the genus *Senecio* [5]. However, self-compatible and strongly self-pollinating species are reported among the genus *Senecio*, e.g. *S. vulgaris* L. [6]. Regardless the breeding system, outcrossing is always an advantage

because it counteracts the effects of inbreeding depression [4,7]. Allogamy leads to an increase in the proportion of recombinants, allows a cosmopolitan distribution of numerous taxa from the Asteraceae family, and seems to be particularly important for endangered species occurring in fragmented habitats, including the study object, *Senecio macrophyllus* M. BIEB. [8]. To assure the exchange of pollen between different individuals, Asteraceae plants are generalists with flowers adapted to pollination by several insect groups – Hymenoptera, Coleoptera, Diptera, and Lepidoptera [7,9–11].

S. macrophyllus, a large-leaved ragwort, is a rare element of the Central European vascular flora (south-eastern Poland, western Ukraine). With fewer than 10 localities in the recent EU borders, the species has been categorized as VU (vulnerable) and included in the Polish red data book [12] and Polish red list [13]. The ragwort is an iteroparous (polycarpic), rhizomatous plant with dominance of generative reproduction, a very slow rate of individual development, and a long life span of genets lasting over 30 years.

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The species is known to be insect-pollinated [14–17], but it is among the red list plants for which the literature data on pollination and/or the breeding system is unavailable [18].

Many papers show that floral morphology and biology may be useful for a full understanding of the plant pollination ecology [19–26]. The flowering biology represents adaptations to various modes of pollination and it is associated with the reproductive systems [27]. The phenology and the diurnal or nocturnal pattern of blooming are among these features [28–30]. A majority of studies also show that plant reproduction depends on the quality and quantity of pollen. In small, fragmented populations, the amount of pollen produced is particularly important since it may act as a strong barrier for gene flow. Also, with its dual function in biocoenoses, pollen is very important as food for insects. One of the theories suggests that the plant's pollen dosing strategy should evolve to match the abundance and pollen transfer efficiency of its pollinators [31,32]. Prolonged pollen presentation should be favored when pollinators are infrequent or inefficient at delivering the pollen they remove, whereas gradual dosing should optimize delivery by frequent pollinators [24,33–35]. In recent years, investigations underline the impact of spatiotemporal changes in plant communities, population size, and density of individuals on the interaction between plant species and flower visitors [11,36–39].

The study was conducted to determine the floral traits of *S. macrophyllus*. In particular, we monitored (i) flowering biology, (ii) the morphology of the reproductive apparatus and pollen features, and (iii) the microsite effect on selected reproductive traits of the species. We also tried to assess if floral reward attracts insect visitors, therefore we observed both their activity and spectrum. Our observations are preliminary but they may have an applied goal: to estimate the chances of rare, vulnerable species to preserve 'genetic health' understood, among others, as the possibility of gene exchange by cross-pollination in the changing plant population and its environment.

Material and methods

Study site and material collection

In situ research was performed in 2008, 2009 and 2012 in the largest natural population of *Senecio macrophyllus* in south-eastern Poland. The population is located in the hilly landscape of the Central Roztocze Highland (Biała Góra – White Mountain; 50°28'N, 23°29'E). The current size of the population is several thousand of genets [17]. The population is fragmented into patches of various size inhabiting microsites differing in terms of the vegetation structure, light intensity, soil wetness, and fertility, etc. [14–17]. Plant material was collected from three microsites (M1, M2, M3), separated from one another with a distance of 150–350 m in a straight line. Microsite M1 was a high and dense grassland dominated by *Brachypodium pinnatum* (L.) P. BEAUV. and *Peucedanum cervaria* (L.) LAPEYR., developed on medium-deep calcareous rendzina, moderately shaded by a loose pine stand, with a relatively small share of *S. macrophyllus*. Microsite M3 was a patch of low, loose, and sun-exposed grassland with the dominance of ragwort accompanied by

Carex flacca SCHREB., *Salvia verticillata* L., *Aster amellus* L., and *Teucrium chamaedrys* L., growing on very shallow rendzina (merely rubble at places). In turn, microsite M2 was a small clump of ragwort established between sites M1 and M3 situated in a sun-lit area overgrown with low grassland. Individual plants ($n = 10–15$) from each microsite were randomly marked. The number of disc florets per head was established ($n = 20$ heads per each microsite, randomly chosen from different individuals). The length of buds, the total diameter of flower heads, and the measurements of the diameter of disc florets were then performed, using a digital caliper with an accuracy of 0.02 mm.

Flowering dynamics and flower life-span

The diurnal pattern of blooming of ligulate and disc florets was observed ($n = 20$ heads, $n = 10–15$ individuals). The preliminary observations excluded night anthesis, thus detailed observations were conducted from 6.00 h (GMT+2 h) until 19.00 h. In one-hour intervals, all opened flowers were counted and marked in order to exclude repeated counting. Duration of the life-span was recorded for inflorescences and for individual disc florets ($n = 7$ heads, $n = 21$ disc florets). The flowers were checked every 1 h, beginning at the time when the flower was in the bud and ending when the corolla was shed. We defined the total floret lifetime as extending from the flower opening to corolla shedding. The male phase proceeded from the beginning of pollen presentation to the beginning of opening of stigma lobes. The female phase was defined as a period between opening of stigma lobes and corolla wilting. The inflorescence life-span was defined as the time from the first disc floret blooming until the end of last flower blooming.

Insect visitors

Preliminary studies of insect visitors focused on (i) the diurnal behavior of forage and (ii) the visitor composition was conducted in the first decade of August 2009, i.e. at the height of the *Senecio macrophyllus* flowering season. The weather conditions were as follows: maximum daily temperature 24–28°C, wind speed <10 km h⁻¹, no precipitation. The observations were performed at three randomly chosen 1-m² study plots. Plot No. 1 was located in the middle part of an unshaded slope overgrown with loose xerothermic grassland. Its main components were as follows (species names after Mirek et al. [40]): *Aster amellus*, *Teucrium chamaedrys*, *Carex flacca*, *Melampyrum arvense* L., and *Salvia verticillata* with an admixture of *Agrimonia eupatoria* L., *Leontodon autumnalis* L., *Galium verum* L., *Trifolium pratense* L., and locally *Brachypodium pinnatum*, and *Calamagrostis epigejos* (L.) ROTH. Plot No. 2 was established on the outskirts of shrubs dominated by *Crataegus monogyna* JACQ. and *Viburnum opulus* L., with addition of *Prunus spinosa* L., *Cornus sanguinea* L., *Rhamnus cathartica* L., *Pyrus communis* L. and *Malus sylvestris* MILL. The shrubs were accompanied by tall herbs characteristic for scrub outskirts, such as *Peucedanum cervaria*, *Medicago falcata* L., *Trifolium alpestre* L. and *Origanum vulgare* L. Plot No. 3 was located at the footstep of the slope within a patch of the grassland of the above-mentioned species composition, and was surrounded by bushes, which, in turn, surrounded the grasslands. The bushes were made

up of *Prunus spinosa*, *Rhamnus cathartica*, *Corylus avellana* L., and *Rosa canina* L. Insect foragers were noted at one-hour intervals (6.00–19.00 GMT+2 h) during 10-minute observations in three plots simultaneously; there were 14 censuses for each plot, 52 in total. We counted all visitors grouped into several categories (nomenclature according to Bogdanowicz et al. [41] and “Fauna Europaea” [42]), with special attention paid to honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.) and solitary bees (other Apoidea); remaining insects were classified to proper orders.

Stigma receptivity

Stigma receptivity was determined using H₂O₂ (30%) in three basic stages, i.e. the bud stage (A), male stage (B – during pollen presentation), and female stage (C – after pollen presentation). Flowers ($n = 10$ for each stage) were randomly collected from the heads of different plants. The stigma was excised and placed in a drop of hydrogen peroxide. Receptive stigmas had bubbling on their surface when placed in this solution. Vigorous bubbling was counted as receptive, whereas weak bubbling was assumed as non-receptive [39]. These observations were made under a stereoscopic microscope.

Pollen production and viability

The number of pollen grains per anther and flower, pollen viability, and pollen grain size were determined in three different microsites (M1–M3). The anthers were harvested from closed flowers ($n = 12$) and placed on a microscopic glass, covered with a drop of water on top and the pollen sacs were squashed with a cover slip and pollen grains were counted. Pollen viability was estimated using the standard acetocarmine test [43]. Fresh pollen was collected from randomly chosen flowers in the bud, male, and female stages. In this test, pollen grains with cytoplasm stained red were considered as viable and with cytoplasm remaining transparent as nonviable. The viability of the pollen grains was calculated in 3 repeats ($n = 3 \times 100$ for each stage) and expressed as percentage of stained grains in the total analyzed. Pollen grain dimensions were determined in glycerol-gelatine slides [44]. The length of the polar axis (P) and equatorial axis (E) was determined ($n = 300$). These observations were conducted using Nikon Eclipse 200 LM.

Pistil morphology

Pistils from closed and opened floret flowers were examined by means of light (LM) and scanning electron microscopy (SEM). For SEM, flower bases were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH = 7.4 at a temperature of 4°C for 12 hours. Then, the material was washed in phosphate buffer, dehydrated in graded ethanol series, and transferred into acetone. Subsequently, the tissues were critical-point dried using liquid CO₂, sputter coated with gold, and examined at an accelerating voltage of 30 kV under a TESCAN/VEGA LMU scanning electron microscope.

Statistical analysis

Standard one-way ANOVA procedures were applied to assess the difference in the mean values of the analysed criteria between the three microsites. To detect differences among the means, post hoc comparison was made by Tukey’s HSD

test. The level of statistical significance for the differences between the means for all the analyses was established at $P = 0.05$. Statistica software package version 6 developed by StatSoft (Cracow, Poland) was applied for these analyses.

Results

Floral morphology

The flowers of *Senecio macrophyllus* are arranged in head inflorescences (Fig. 1a,b). The total diameter ranged from 17.1 mm to 31.9 mm and the inner disc diameter 8.9–11.7 mm. The microsite effect was found for the number of disc florets per head, the bud length of disc florets, and for the size of the head diameter (Tab. 1). Each head was surrounded by green involucre bracts that accumulate anthocyanins in the apical part (Fig. 1c). The tubular perfect ray florets (mean = 35.0 per head) opened first on the periphery of the inflorescences. The size of the disc florets in the bud stage was 16.9 mm, on average. Each tubular floret had numerous hairs of the calyx (pappus). The fused corolla formed a cylindrical tube and campanulate 5-toothed limb.

Floral biology

The individuals of *Senecio macrophyllus* flowered during July–August with the peak in mid and late July (2012 and 2010, respectively) or in the first decade of August (2008, 2009). The heads comprised 6–8 ligulate female or sterile florets that developed gradually. Anthesis of the ligulate flowers occurred early in the morning (6.00–8.00 GMT+2 h). The disc florets of the ragwort exhibited diurnal opening and the process was most intensive in the early afternoon (15.00–18.00 GMT+2 h; Fig. 2). The inflorescence life-span ranged from 7 to 9 days (mean = 7.8 days). The male phase with pollen presentation outside the florets, available for insects, lasted from 4 to 11 hours (mean = 6.6 hours). The female phase lasted for 25–47 hours (mean = 37.1 hours) per individual disc floret. The mean life-span of the disc florets was 43.8 hours (Tab. 2). Simultaneous pollen presentation was observed in 1–12 disc florets per head (Fig. 2d,e). Pollen presentation per single head persisted for 5–8 days (mean = 6.3 ± 1.1).

The reproductive apparatus of the species

The androecium consists of five syngenesious stamens. The anthers are dithecous and are fused to form a tube around the style. The gynoecium is bicarpellary syncarpous with an inferior unilocular one-ovule ovary. The stigma is two-lobed and dry. In mature flowers, the upper part of the pistil forms a pollen presenter. Initially, the style presenter is compact and becomes dichotomously branched in older flowers (Fig. 1e,g–i). Numerous densely distributed papillae of different length are present on the surface of the style presenter. The papillae are basally distended with a narrower neck in the apical part (Fig. 1f–h). Numerous small chromoplasts are present in the papillae cells; hence, the style is yellowish-orange in colour (Fig. 1f). The nectar is present only in disc florets, and it is secreted by glands arranged as a disc present at the base of corolla tube above the lower ovary and partly surrounding the style.

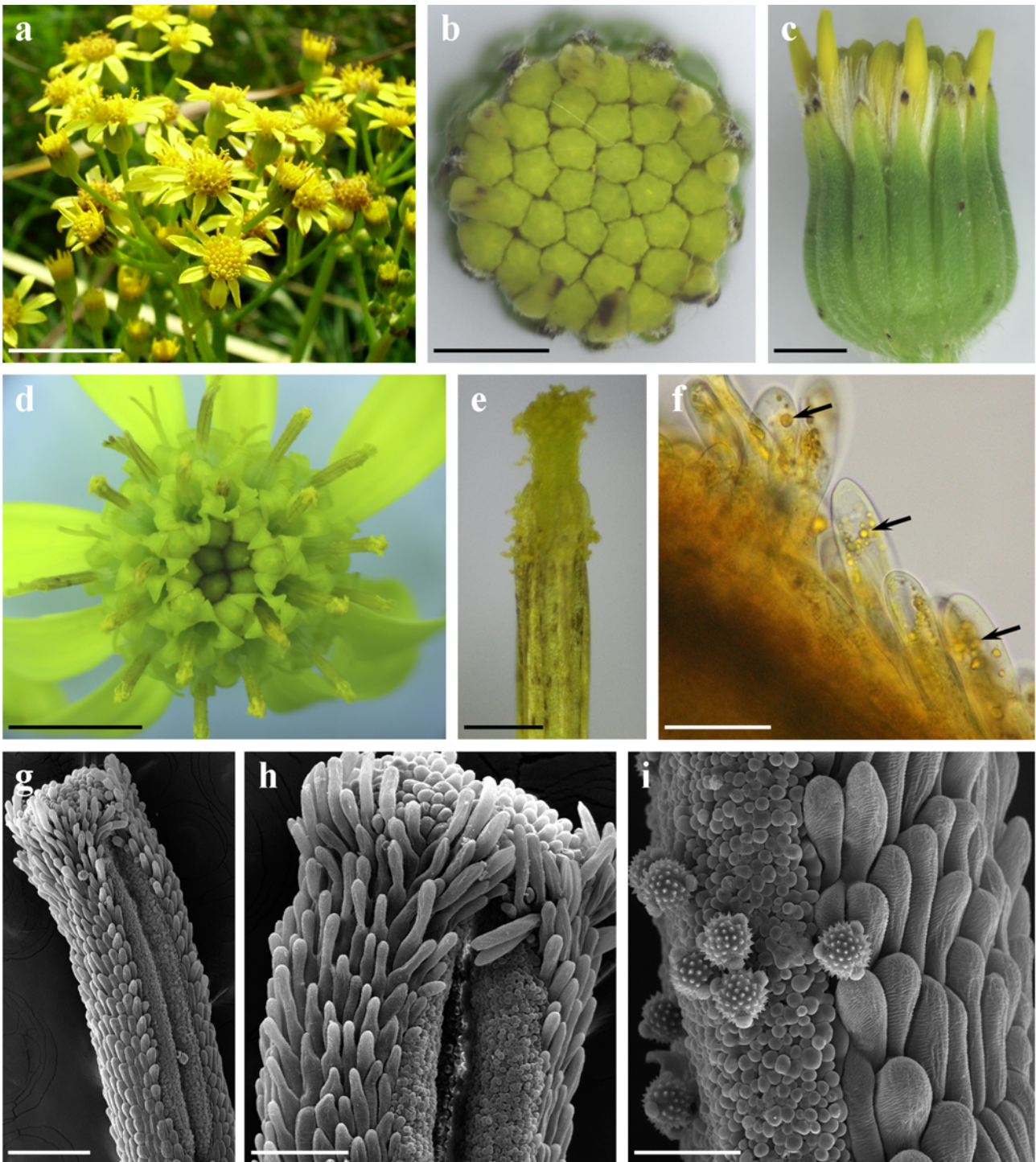


Fig. 1 Habit of the flower and pistils of *Senecio macrophyllus*, macro images and SEM. **a** Overall view of stem with numerous flower heads; scale bar: 2 cm. **b** Flower head with disc florets in bud stage; scale bar: 2 mm. **c** Green involucler bracts with anthocyanins in the apical part; scale bar: 2 mm. **d** Pollen presentation in disc florets; scale bar: 5 mm. **e** Pollen presenter with pollen; scale bar: 600 μm . **f** Numerous small chromoplasts (arrow) in the papillae cells on the stylar presenter; scale bar: 50 μm . **g** Compact style presenter in bud stage; scale bar: 200 μm . **h** Details of basally distended style papillae with narrower neck shape in apical part; scale bar: 100 μm . **i** Lobe of the dichotomously branched style at anthesis with pollen grains on the stigma; scale bar: 50 μm .

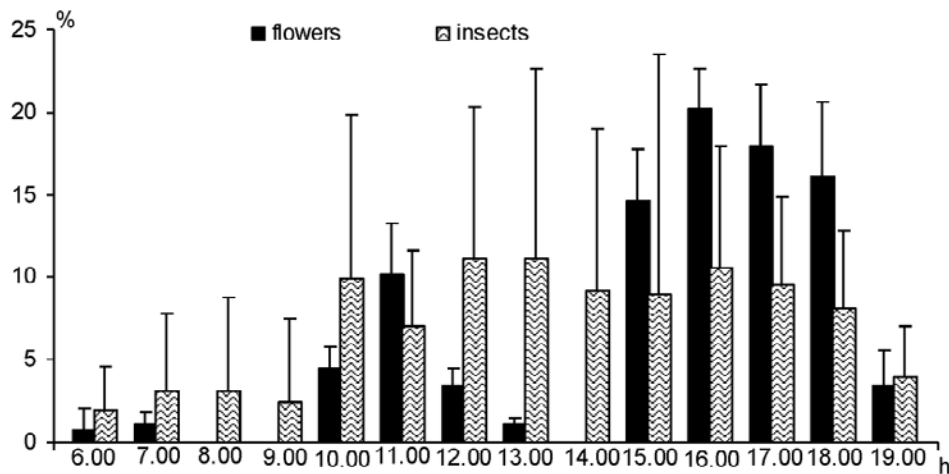
The disc florets are highly protandrous. The anthers opened by longitudinal slits. The dehiscence of anthers started just before opening of the floret. The pollen shed inside the floral tube was transferred to the highest part of the flower by the papillae of the style presenter. At the time when the anthers developed longitudinal slits, the style was

short with the stigma tightly closed. The pollen shed inside the floral tube was carried out slowly and the style elongation lasted 1–2 days (33 h on average). During the style elongation, the pistil acted as an apparatus that pressed through the anther tube and swept the pollen out of the tube. Then entire pollen load was presented as a cluster trapped in

Tab. 1 The microsite effect on the number of disc florets per inflorescence, the bud length, inflorescences diameter and the number of pollen grains produced in anthers of *Senecio macrophyllus* in the White Mountain population, SE Poland.

Feature	Microsite									ANOVA <i>P</i>			
	M1			M2			M3						
	Mean	$\pm SD$	CV%	Mean	$\pm SD$	CV%	Mean	$\pm SD$	CV%				
Number of disc florets per head	35.5 ^a	± 3.9	10.9	25.5 ^b	± 1.7	6.7	30.2 ^c	± 2.9	9.6	30.4	± 6.1	20.1	0.038
Bud length of disc florets (mm)	17.9 ^a	± 1.7	9.5	16.1 ^b	± 1.1	6.8	16.8 ^b	± 0.7	4.2	16.9	± 0.7	4.1	0.000
Total head diameter (mm)	27.8 ^a	± 5.7	20.5	24.2 ^b	± 0.8	3.3	25.3 ^b	± 0.4	1.6	25.8	± 5.6	21.7	0.000
Number of pollen grains per anther	95.4 ^a	± 33.6	35.2	509.6 ^b	± 195.2	38.3	222.6 ^a	± 58.4	26.2	275.4	± 224.4	81.5	0.001
Viability (%)	67.3 ^{ab}	± 1.2	1.8	63.7 ^a	± 2.5	3.9	73.3 ^b	± 3.9	5.3	68.1	± 5.1	7.5	0.033

Means $\pm SD$ and CV (%) are given. ANOVAs were performed separately for each of analyzed feature. Means in rows indicated with the same small letters are not significantly different with *P*-level <0.05 according to Tukey's HSD test.

**Fig. 2** Diurnal dynamics of disc florets opening ($n = 20$ heads) and the daily pattern of activity of insect visitors ($n = 3$ microsites) on *Senecio macrophyllus* in the White Mountain population, SE Poland. Means are given. Whiskers indicate $\pm SD$.**Tab. 2** Duration of male and female phases, life-spans of disc florets and inflorescences and the pollen traits of *Senecio macrophyllus* in the White Mountain population, SE Poland.

Feature	Min-Max	Mean $\pm SD$	CV%
Male phase (hours)	4–11	6.6 ± 2.3	34.8
Female phase (hours)	25–47	37.1 ± 6.7	18.1
Disc floret life-span (hours)	29–52	43.8 ± 8.4	19.2
Head life-span (days)	7–9	7.8 ± 0.7	8.9
Number of pollen grains/anther	53–816	276.0 ± 224.0	81.2
Number of pollen grains/flower	246–4080	1463.0 ± 1217.0	83.2
Length of polar (<i>P</i>) axis (μm)	25–29.3	27.4 ± 1.8	6.6
Length of equatorial (<i>E</i>) axis (μm)	27–32.5	28.5 ± 1.0	3.5

Minimum, maximum, means $\pm SD$ and CV (%) are given. For definitions of phases see the text.

pollen holders formed on the top of the anthers (Fig. 1d,e). Numerous tryphine droplets present on the surface of pollen grains protected the pollen load against the wind agent. The microsites effect was found for the number of pollen grains per anther and for the pollen viability (Tab. 1). The mean number of pollen grains produced was 276 per anther, 1463 per flower, and 51 219 per head; therefore, the pollen : ovule ratio (P/O) was high and amounted to 1463. Anthers deprived of pollen changed colour to brown. The stigma was not receptive until it protruded from of the floral tube. The two-lobed shape of the stigma indicates the onset of stigma receptivity. The stigma receptivity lasted usually for 2–3 days (from 12 to 47 hours). Pollen viability decreased in time and averaged 82.1% in the bud stage, 49.5% during the male phase, and 18.7% in the female phase (Fig. 3). The pollen grain of *Senecio macrophyllus*, roundish in outline, tricolporate, with echinate ornamentation is characterized by

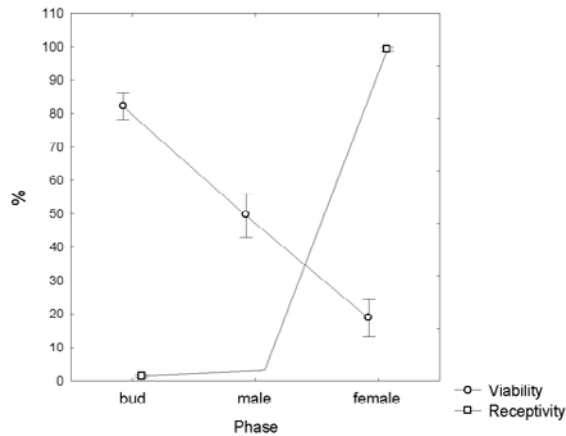


Fig. 3 The viability of pollen grains and stigma receptivity in bud, male and female floral phases of *Senecio macrophyllus* in the White Mountain population, SE Poland. For the description of floral sex phases see text. Whiskers show 95% confidence intervals.

2.2–3.1- μm long spine exine sculpturing (Fig. 1i). The mean value of the P/E ratio is 0.96 (shape: oblatto-spheroides). The polar axis (P) ranged 25.0–29.3 μm and the equatorial axis (E) ranged 27.0–32.5 μm (Tab. 2).

Insect visitation

Senecio macrophyllus attracted numerous insect visitors. Under good weather conditions, they visited flowers throughout the day (Fig. 2, Fig. 4, Fig. 5). The diurnal activity of insect visitors began early in the morning and the insects foraged evenly until 19.00 h. In the study area, the principal visitors were Hymenoptera, with dominance of *Apis mellifera* (Apoidea), which visited intensively a large number of flowers (53.4% of visits). The second group of visitors were dipterans (22.1%). Moreover, the flowers were visited by bumblebees; among them, the most frequent were *Bombus terrestris* (Linnaeus 1758), *B. lapidarius* (Linnaeus 1758), and *B. pascuorum* (Scopoli 1763). Insects collecting both nectar and pollen were observed. Additionally, a broad spectrum of lepidopterans was noted with the remarkable share of 13.9%. The most numerous were two species: *Aglais io* (Linnaeus 1758) and *Lasiomnata megera* (Linnaeus 1767). Less frequent were other representatives of the Nymphalidae family – *Vanessa atalanta* (Linnaeus 1758), *V. cardui* (Linnaeus 1758), *Argynnis paphia* (Linnaeus 1758) and *Polygonia c-album* (Linnaeus 1758), as well as Zygaenidae – *Zygaena filipendulae* (Linnaeus 1758), Pieridae – *Gonepteryx rhamni* (Linnaeus 1758), *Aporia crataegi* (Linnaeus 1758), *Pieris* spp., and Lycaenidae – *Lycaena virgaureae* (Linnaeus 1758) and *Polyommatus* spp.

The groups of insect visitors varied between the individuals located in the three different plots (Fig. 6). Throughout the observation day, the only visitors to plot No. 1 (i.e. in open xerothermic grassland) were honeybee workers and dipterans. Five different groups of visitors were present on plot No. 2 (on the shrub outskirts), and the most diverse were foragers to the individuals in plot No. 3 (in the grassland patch surrounded by bushes).

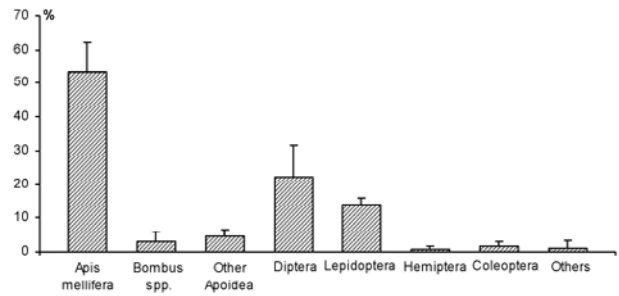


Fig. 4 The relative abundance of different categories of insect visitors ($n = 3$ microsites) to *Senecio macrophyllus* in the White Mountain population, SE Poland. Means are given. Whiskers indicate \pm SD.

Discussion

The largest but fragmented population of *Senecio macrophyllus* on the White Mountain hill is known to have still a heterogeneous genetic structure with values of heterozygosity amounting to 0.853 [45]. Although the breeding system in *S. macrophyllus* was not experimentally studied, we found an array of floral morphology and biology features that may counteract the inbreeding depression by assurance of cross-pollination.

The first strategy relies on the morphology of showy, yellow ligulate flowers, peripherally situated in the heads. In contrast, the reduction of ligulate flowers in the heads of autogamous *Senecio vulgaris* was described by [6]. The growth of ligulate flowers in *S. macrophyllus* begins early in the morning (6.00–8.00), before the gradual progress of disc florets, which was observed fundamentally from 10.00 h with peaks between 15.00 and 18.00. According to many authors, a major function of ligulate flowers is visual attraction of pollinators [7,10,46]. Another visual attractant for insect visitors to *S. macrophyllus* is the changeable color of anthers in disc florets, which acts as an indicator of flower reward availability. The visual signaling of flower reward shortage is widespread and the mechanism could involve different parts of flowers [28,47]. The anthers of different Asteraceae taxa are known to change color after pollen presentation [26,29].

We also found a broad array of factors that may have an impact on the activity of insect visitors to *S. macrophyllus* flowers and individuals. The frequency of flower visitors changed over the course of the day and the pattern of their diurnal activity was associated with opening of new flowers and with floral reward availability. A similar correlation was previously described in many other taxa [22,27,28,30].

The flowers of entomophilous taxa from the genus *Senecio* attract visitors by primary attractants – nectar and pollen [21]; these floral rewards were also present in *S. macrophyllus*, although the amount of nectar was not measured in the present study. Single pollen grains were released from pollen sacs in the bud stage when the stigma lobes were joined and the stigma was not receptive. Then, the pollen was pushed out of the anther tube by the growing style and was attached to the sweeping hairs of the style. This is one of the important mechanisms in presenting pollen

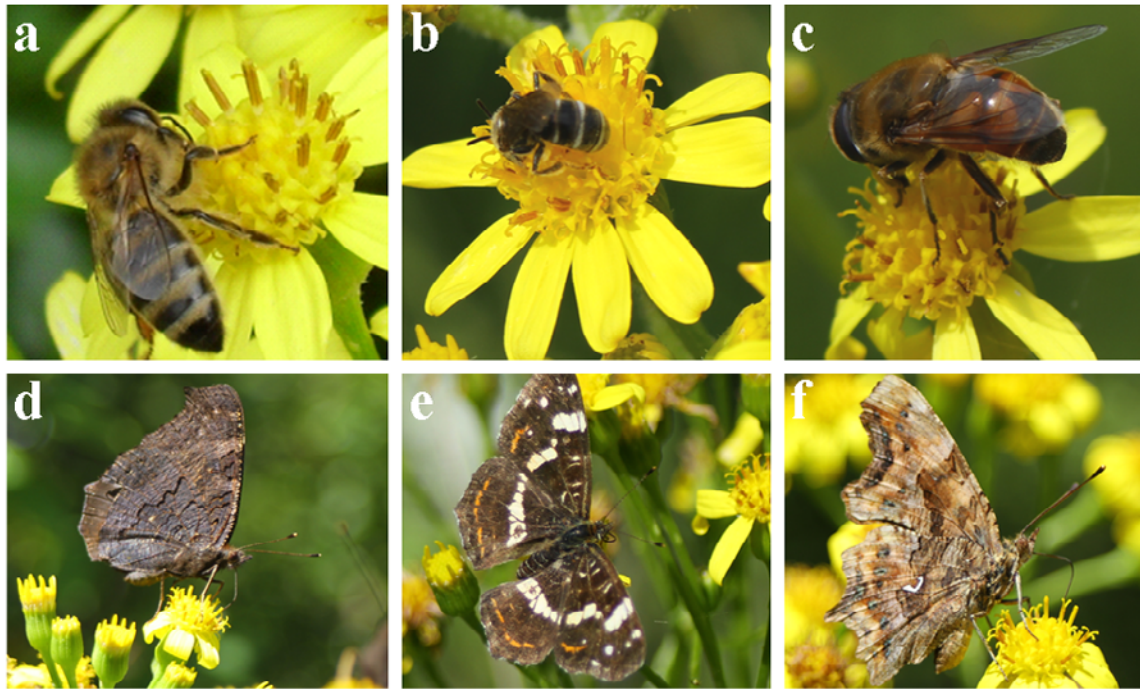


Fig. 5 Insect visitors on *Senecio macrophyllus* flowers: *Apis mellifera* (a), *Andrena* sp. (b), *Eristalis tenax* (c), *Aglais io* (d), *Araschnia levana* (e), *Polygonia c-album* (f).

to pollinators known as a secondary pollen presentation system. Secondary pollen presentation has evolved in several groups of angiosperms including Asteraceae [24,31,33], and *S. macrophyllus* is no exception. The pollen presenters in the Asteraceae family have different forms, one of which is a brush-like presenter covered with papillae on its surface [24,31]. Such a type of pollen presentation occurs in hermaphrodite flowers of *S. macrophyllus*. The trichomes of the pollen presenter are unicellular with chromoplasts, similar to those in *Petasites* [26].

The tubular flowers of *S. macrophyllus* produced a copious amount of pollen (276 pollen grains per anther and 1463 per flower), but this is almost three times less than in the anemophilous *Artemisia maritima* L. [25]. Pollen production in plants varies greatly from one species to another and it is directly related to the size of the anther and depends on environmental factors and pollination modes [30–32]. The quantity of pollen grains produced in entomophilous taxa is usually lower, compared to that in anemophilous species. Furthermore, the high pollen : ovule ratio (P/O) determined has proved that *S. macrophyllus* is an outcrossing plant. According to Cruden [19,20], the P/O value is a good estimator of the breeding system of a plant; the higher the ratio of pollen grains to ovules within an individual flower, the higher the rate of outcrossing in a population or species.

The pollen presentation per single disc floret was relatively long and lasted 4–11 hours (mean = 6.6 hours). The average duration of pollen presentation in other Asteraceae species disc florets has been reported to last 2–6 hours [48]. The pollen presentation for a single *S. macrophyllus* head was prolonged to 8 days, on average. The duration of pollen presentation is subjected to different abiotic factors; therefore, it may exhibit large discrepancies [24,32]. Regardless the

impact of the environment on pollen release and exposition, the main function of prolonged pollen presentation is to increase the mating opportunities by extension of availability of male gametes for reproduction [49]. An increase in the duration of male-function may be an effect of the selective pressure of changes in the pollinator visitation rate during the flowering season. In addition, the increased proportion of female flowers during the flowering season and therefore the increased availability of ovules create conditions for a rapid increase in male fitness. Likewise, insect visitor activity and frequency may play a significant role in duration of the male phase in dichogamous flowers. Insects may operate by pollen-thieving and therefore limit its availability for pollination [50].

S. macrophyllus is a strongly protandrous species. Protandry characterizes the Asteraceae family [31,35] and is considered to be an effective mechanism preventing self-pollination. We also found that protandry was supported by the correlation of the lowest pollen viability with the highest stigma receptivity. A similar relationship was described for other Asteraceae taxa [24], thus the dependence may act as an alternative, evolutionarily conserved mechanism against self-pollination. However, synchronous occurrence of flowers in male and female phases within the head creates conditions for geitonogamous self-pollination and inbreeding.

Pollen grains of *S. macrophyllus*, which represent the Aster type, are marked by the presence of conical spines. According to Lowe and Abbot [9], the average size of *Senecio* pollen grains is 20–35 μm . Our study shows that pollen grains of *S. macrophyllus* from the White Mountain population are similar (25–32.5 μm). Thick triline on pollen grains was observed. It is characteristically associated with entomophilous plants [23].

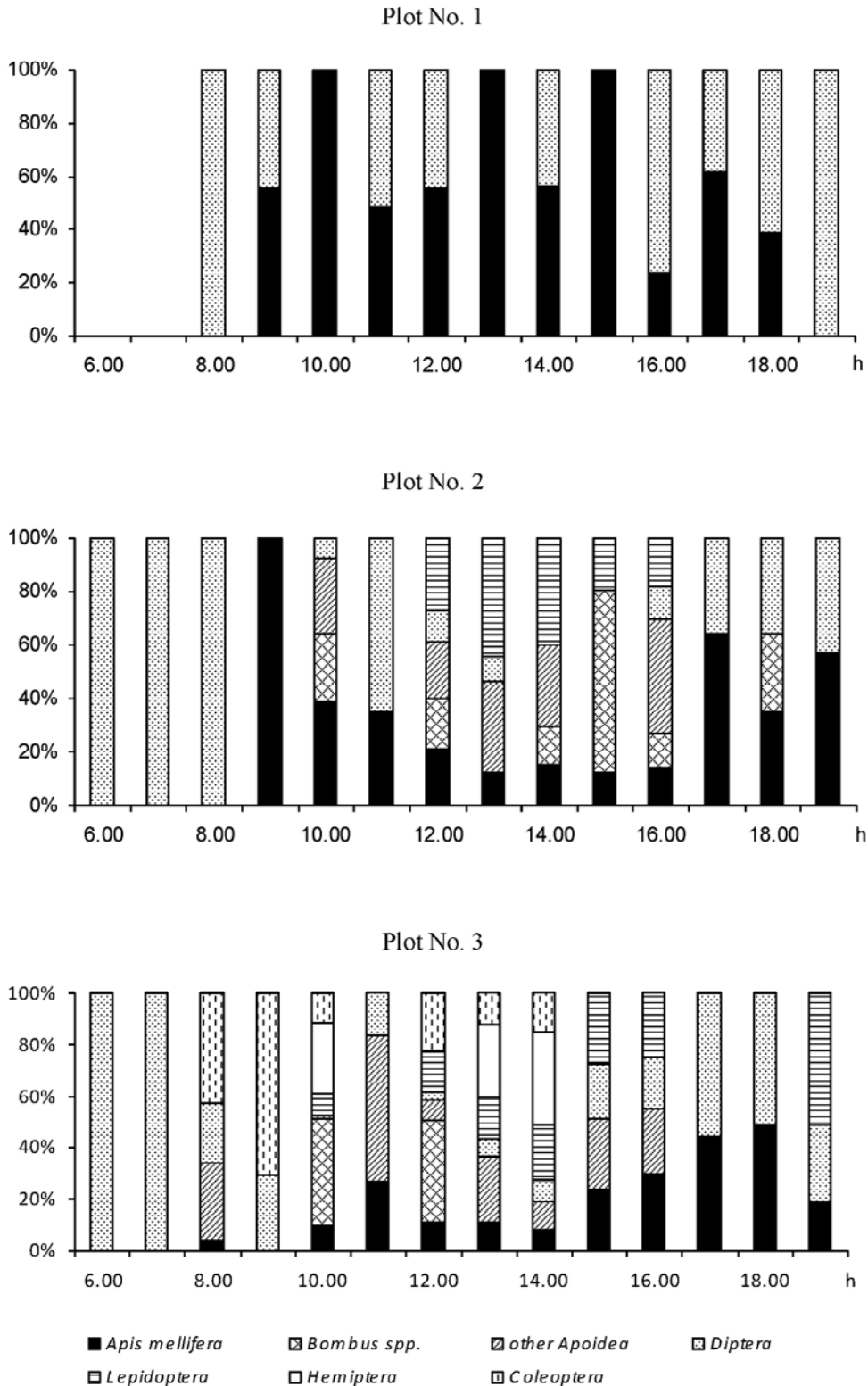


Fig. 6 The diurnal spectrum of insect visitors to *Senecio macrophyllus* in three study plots of the White Mountain population, SE Poland.

The combined flower traits (e.g. bright ligulate flowers, accessible nectar, and pollen rewards) lured an array of insect visitors, i.e. bees, butterflies, flies, and beetles. The key visitor in the study White Mountain population of *Senecio macrophyllus* was *Apis mellifera*, responsible for 53.4% of the visits. Very similar results, or an even higher

proportion of honeybees, were recorded for other Asteraceae taxa, e.g. 51% for *Tussilago farfara* [46] or even 62–85% for different *Centaurea* species [29]. The presence of large numbers of Apoidea, bumblebees, wild bees (Andrenidae, Megachilidae, Halictidae), beetles, and flies were reported on different Asteraceae taxa [7,9,21,24,46] with 178 species

listed as visitors to *Senecio jacobaea* L. [51]. We observed a particularly diverse composition of lepidopterans, with their participation reaching 13.9% of all visitors. Butterflies are considered as important foragers of Asteraceae [3,10], although this group of insect visitors was not recorded by Knuth [52], who apart from *Apis mellifera* observed only Coleoptera, Diptera, and Hymenoptera foraging on *S. macrophyllus*. Some authors suggest that the foraging behavior of butterflies maintains the gene flow between populations, as they move over longer distances than other flower visitors do [27,37]. The long-distance moving insects may be especially important for fragmented populations and may improve the genetic variability of individuals within the population and between different populations within the metapopulation system. Despite the significant decline in the size of the fragmented study *S. macrophyllus* population, as well as variation in flowering frequency between subsequent years [14–16], the White Mountain population does not present any genetic drift or bottleneck effect; therefore, we assume that the significance of butterflies cannot be excluded for the self-sustaining effect on the population [1,53,54].

We observed some bumblebee visitors in our study object. Taking into account the results of other authors we may conclude that the diversity of Apoidea visitors, including *Bombus* species, was connected with the presence of components of grassland and bush outskirts accompanying *S. macrophyllus* or plant species occurring abundantly in adjacent abandoned fields [16,17,55]. Abundantly rewarding and/or highly energetic plant species may act as magnet plants attracting visitors to the study ragwort [27,39]. These might be, for example, *Medicago falcata*, *Trifolium pratense*, *Centaurea scabiosa* L., *Stachys recta* L., or *Salvia pratensis* for *Bombus terrestris*, and *B. lapidarius*. Similarly, *Euphorbia cyparissias* L., *Senecio jacobaea*, and *Knautia arvensis* (L.) J. M. COULT. present in the study patches as well as *Cirsium arvense* (L.) SCOP. and *Hypericum perforatum* L. with their mass appearance in abandoned fields are key food plants for small bee species, e.g. *Andrena* or *Halictus* [11,30,56]. Both bumblebees and solitary bees are known as visitors that may change their preference over time in response to different factors [22,27].

Our preliminary study on the behavior of insects foraging flowers of *S. macrophyllus* indicates a strong microsite effect, i.e. the influence of accompanying species and the composition of plant community on the variation in the spectrum of visitors to particular individuals. During the last decade, the area of open xerothermic grasslands on the White Mountain hill decreased significantly due to bush encroachment. The share of *S. macrophyllus* and other species

with nectar and pollen rewarding flowers (e.g. *Aster amellus*, *Teucrium chamaedrys*, *Salvia verticillata*, *Stachys recta*, *Galium verum*) diminished noticeably, especially in microsite M1. At the same time, the cover of wind-pollinated grasses, mainly *Brachypodium pinnatum* and *Calamagrostis epigejos*, increased and the *S. macrophyllus* individuals in this patch were foraged by a less diverse assemblage of visitors. On the other hand, the neighborhood of shrubs contributes to the great number and diversity of visiting insects that probably have their nests among bushes. The observation underlines the significance of habitat conditions for the insect behavior and this is in accordance with other authors who stress their importance [21,22,27,36,39].

The interesting finding of our study is that the environmental microsite conditions significantly affected the number of florets per head, the head size, and the quantitative and qualitative pollen traits. The morphological floral features might determine which flowers are visited and which are ignored, and by that means, they may exert an effect on the individual reproductive success. In a natural population of the self-incompatible *Achillea ptarmica* L., the calculated visitation rate per head and the percentage of seed set was a function of the head number and was strongly correlated with patch character [57]. In general, a majority of studies have documented that foragers' behavior is complex, and the size can have a direct and differential effect on reproductive success through its effect on the efficiency on pollen dispersal [27]. Moreover, we revealed a variable nature of pollen resources and pollen viability between the microsites. The opinion that pollen characteristics of a given species may vary between patches and populations is widely accepted [56,58,59]. Particularly, the low amount of pollen produced in flowers, and therefore the reduced pollen availability for pollination may determine negatively the individual reproductive success. An adequate amount of functional pollen that may accomplish its successful delivery to the ovule to complete fertilization is necessary for seed and fruit set [60]. Both the pollen limitation and disorders in pollen biology may have a significant impact on the potential seed set [10,46,56,61] and hence the population structure and population stability.

In conclusion, we need to stress that a more detailed study is necessary to assess the microsite conditions optimal for the generative reproduction of *S. macrophyllus* in relation to the species breeding system and to explain the role of insect visitors for effective reproduction, and for the gene transfer between patches. To explain the relationship between biotic and abiotic factors is fundamental for in situ species conservation and for maintenance of populations within the metapopulation system.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: design of the study: BC, BD; field work: BC; laboratory work: BD; writing the manuscript: BC, BD.

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