

Comprehensive analysis of reproductive ecology in *Jasione supina*: A multi-subspecies perspective

Volkan.Eroglu Eroğlu

Volkan.eroglu@ege.edu.tr

Ege University: Ege Üniversitesi <https://orcid.org/0000-0003-4868-5988>

Serdar Gökhan Şenol

Ege University: Ege Üniversitesi

Research Article

Keywords: *Jasione*, conservation, reproductive ecology, pollination

Posted Date: July 28th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6961963/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Comprehensive analysis of reproductive ecology in *Jasione supina*: A multi-subspecies perspective

Volkan Eroğlu¹ & Serdar Gökhan Şenol¹

¹ Ege University, Science Faculty, Department of Biology, Bornova, İzmir, Turkey

Volkan Eroğlu (volkan.eroglu@ege.edu.tr), Web of Science ResearcherID: AAG-7704-202,
<https://orcid.org/0000-0003-4868-5988>

Serdar Gökhan Şenol (serdar.gokhan.senol@ege.edu.tr), Web of Science ResearcherID: AAG-7051-2021,
<https://orcid.org/0000-0003-4564-2608>

Correspondence: Volkan Eroğlu (volkan.eroglu@ege.edu.tr)

Abstract

This study examines the variation in reproductive traits among four subspecies of *Jasione supina* (subsp. *akmanii*, subsp. *pontica*, subsp. *supina*, and subsp. *tmolea*) endemic to Turkey. Key parameters such as flowering phenology, floral morphology, ovule and pollen characteristics, pollen viability, stigma receptivity, self-incompatibility, and pollinator visitation were comparatively analyzed under field conditions. *J. supina* subsp. *supina* exhibited the greatest reproductive success, with the highest number of corymbs per plant (25.93 ± 16.09), flowers per corymb (36 ± 11.66), ovules per ovary (24.1 ± 3.07), and fertilised ovules (19.9 ± 4.36). It also exhibited the highest pre-anthesis pollen viability ($86.5 \pm 6.26\%$). Despite having a lower floral output, *J. s. tmolea* achieved high fertilisation rates (12.2 ± 2.35 ovules) and had the highest post-anthesis pollen viability ($67.5 \pm 16.87\%$). Subsp. *akmanii* produced the highest pollen count (2110 ± 435.12) but had the lowest pre-anthesis pollen viability ($56 \pm 14.3\%$). Self-pollination resulted in zero fertilisation in all subspecies, confirming complete self-incompatibility. Under cross-pollination, fertilization rates ranged from 41.83% in subsp. *pontica* to 82.75% in subsp. *supina*. Physical stimulation of pollen-collecting stylar hairs significantly reduced the time to female-phase onset, from 22–26 hours to 6–6.75 hours after anthesis. Pollinator observations over a period of 216 hours revealed that Hymenoptera accounted for 63.33% of visits, followed by Diptera (34.6%), Lepidoptera (1.88%), and Coleoptera (0.03%). Subsp. *pontica* was the only subspecies in which Diptera exceeded Hymenoptera in visitation frequency. These results indicate that floral traits, pollinator composition, and reproductive efficiency vary significantly among subspecies, which supports the hypothesis that divergent evolutionary pressures and ecological heterogeneity have shaped distinct reproductive strategies within the *Jasione supina* complex.

Keywords – *Jasione*, conservation, reproductive ecology, pollination

1 Introduction

Understanding the mechanisms that underlying pollination and reproductive systems is essential for explaining gene flow, genetic structure, population dynamics, and the processes of speciation in angiosperms (Barrett and Harder 2006, 2017). These systems regulate the success of sexual reproduction and thereby shaping the evolutionary trajectories of plant lineages. Key aspects such as the temporal separation of sexual phases, the spatial configuration of reproductive organs, and pollinator interactions during anthesis play a crucial role in promoting reproductive efficiency and avoiding sexual interference (Barrett 2002). Among these mechanisms, secondary pollen presentation (a feature exhibited by several members of the Campanulaceae) is particularly notable. This trait extends the male phase and spatially or temporally separates pollen release from stigma receptivity, enhancing cross-pollination potential and facilitating effective reproductive outcomes (Erbar and Leins 2006).

The reproductive success of plant species with narrow geographic ranges or fragmented habitats can be particularly affected by ecological constraints. Limited population sizes, reduced pollinator diversity, and habitat isolation can compromise sexual reproduction and thus decreasing genetic diversity and increasing inbreeding, which ultimately elevates extinction risk (Morgan and Wilson 2005). Therefore the integration of reproductive biology into conservation biology is therefore essential, as the structure and function of reproductive traits offer critical insights into population viability and resilience (Murren 2002). Furthermore, comparative studies of reproductive systems are valuable for understanding not only ecological functioning but also for detecting fine-scale evolutionary divergence, particularly in morphologically similar but geographically isolated taxa (Vamوسي et al. 2007).

Microevolutionary divergence among narrowly distributed taxa provides unique opportunities to identify the ecological and evolutionary forces that drive reproductive differentiation. Local adaptation, even among

ecologically similar lineages, may lead to variation in traits such as floral morphology, phenology, pollen viability, and mating system attributes (Strauss and Irwin 2004; Ludwig et al. 2013; Hamston et al. 2017). These traits not only determine reproductive success but also mediate species interactions, particularly with pollinators. As primary agents of gene transfer, pollinators significantly influence reproductive outcomes through their abundance, diversity, and behavior. Evaluating plant–pollinator interactions within and between populations thus enhances our understanding of mutualistic networks and co-evolutionary dynamics (Myroud and Glover 2017). In addition, antagonistic floral visitors such as nectar robbers or floral predators may exert selective pressures that shape

One of the most striking microevolutionary mechanisms in Campanulaceae flowers, to which the genus *Jasione* L. belongs, is secondary pollen presentation (Carolin 1960; Erbar and Leins 1989; Leins and Erbar 1990). Secondary pollen presentation has evolved in 20 plant families (Howell et al. 1993; Yeo, 2012). In *Jasione*, this mechanism involves a specialised style for pollen presentation, known as a 'pseudo-staubblatt' or, by some authors, a 'piston mechanism' (Yeo 2012). Jost (1918) described how the pollen-collecting hairs located on the style play a crucial role in this mechanism by gathering pollen during the male phase of the flower, and noted that these hairs are shed quickly when pollinators are present. Subsequently, Shetler (1979) demonstrated through experimentation that, in the absence of pollinators, the shedding of these hairs occurs significantly more slowly than when pollinators are present. Nyman (1993) reported that, in addition to their role in pollen collection, these hairs also act as a regulatory mechanism that controls style maturation and thereby promotes cross-pollination. While this mechanism has been well documented within the genus, its evolutionary implications and variations among closely related subspecies, such as *Jasione supina*, remain largely unexplored.

The genus *Jasione* comprises 14 species and 30 infraspecific taxa, most of which are distributed across the Mediterranean Basin (Lammers 2007; POWO 2025). Phylogeographic and paleoclimatic studies suggest that the diversification of the genus was influenced by glacial–interglacial cycles, particularly during the last glacial period (Paus et al. 2023). *Jasione montana* is considered to have colonised alpine and subalpine zones, whereas *J. heldreichii* is associated with lowland diversification, reflecting two major Plio-Holocene lineage expansions (Rocha et al. 2022). In Turkey, the genus is represented by four species: *J. montana*, *J. idaea* and *J. supina* in subalpine regions and *J. heldreichii* at lower elevations.

The focal taxon of this study is *Jasione supina*, which comprises four endemic subspecies (subsp. *supina*, subsp. *pontica*, subsp. *akmanii*, and subsp. *tmolea*). These subspecies are geographically isolated across distinct mountain ranges in western, central, and North-eastern Turkey. Previous morphometric analyses have revealed a high degree of similarity between subsp. *pontica*, subsp. *akmanii*, and subsp. *supina*, whereas subsp. *tmolea* exhibits significant morphological divergence (Sezer and Ardiç 2022; Serrano et al. 2023). This pattern suggests that, despite shared ancestry, local adaptation and geographical isolation may have driven reproductive differentiation among the subspecies. Mountain systems, promote ecological heterogeneity and restrict gene flow, serving as natural laboratories for studying divergence and speciation (Armbruster et al. 2009; Arnold et al. 2001).

Despite their ecological and evolutionary significance, the reproductive traits of the *J. supina* subspecies are poorly understood. The extent to which morphological similarity conceals functional divergence in reproductive strategies is currently unknown, which limits both evolutionary inference and conservation planning. A detailed comparative study of their reproductive biology is therefore necessary to uncover potential differences in traits that directly influence reproductive success.

This study aims to assess whether the *J. supina* subspecies differ in key reproductive traits, such as flowering phenology, floral morphology, pollen viability, stigma receptivity, self-incompatibility rates, seed-set success, and pollinator diversity. Specifically, we test the following hypotheses: (H1) Reproductive traits differ significantly among subspecies; (H2) Pollinator assemblages and visitation behaviour vary and influence seed-set outcomes; (H3) Variation in self-incompatibility rates is associated with floral structure and pollinator accessibility; and (H4) Observed differences reflect subspecies-specific reproductive strategies shaped by divergent evolutionary pressures.

2 Material and methods

2.1 Plant species and study site

Jasione supina Sieber ex Spreng. is a perennial taxon with four endemic subspecies, that grow in the subalpine zone of western and northern Anatolia (Asia Minor) at an altitude of approximately 1800–2400 m, bloom between July and September. *J. supina* subsp. *supina*, subsp. *akmanii* and subsp. *tmolea* are locally endemic, whereas subsp. *pontica* can be found in multiple localities in northern Anatolia. Table 1 provides information on

the areas where the study was carried out, their altitudes, GPS coordinates and voucher specimen details (Figure 1).

Table 1. Plant species and study area

Species	Study area	Altitude	Rock type	GPS	Voucher specimen ID
<i>J. supina</i> subsp. <i>akmanii</i>	Bolu, Kartalkaya around ski center	2020 m	andesite	40°19'25.34"N, 31°48'20.58"E	EGE 42048
<i>J. supina</i> subsp. <i>pontica</i>	Kastamonu, Ilgaz Mountain from the back slopes climb to the top	2060 m	calcist	41°04'34.39"N, 33°50'42.84"E	EGE 43589
<i>J. supina</i> subsp. <i>supina</i>	Bursa, Uludağ, around ski center	1815 m	granite	40°06'16.08"N, 29°08'17.01"E	EGE 42049
<i>J. supina</i> subsp. <i>tmolea</i>	İzmir, Bozdağ around ski center	1850 m	schist	38°19'27.14"N, 28°06'46.23"E	EGE 31528

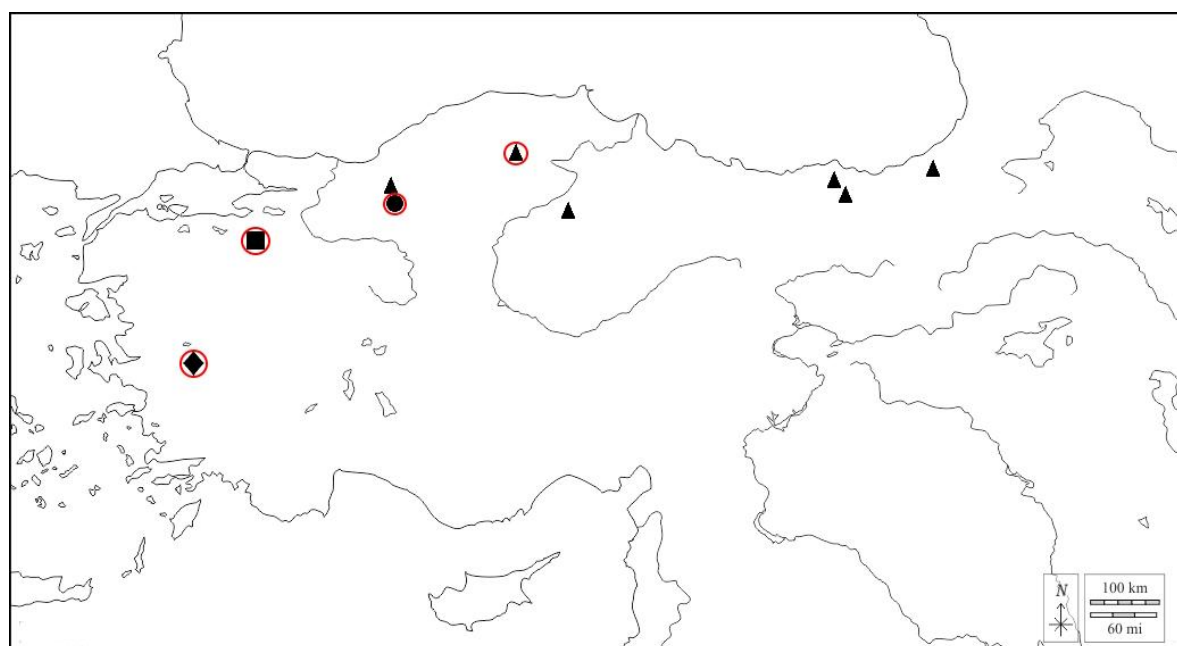


Figure 1. Distribution area of *Jasione supina* subspecies (●) subsp. *akmanii*, (▲) subsp. *pontica*, (■) subsp. *supina*, (◆) subsp. *tmolea*. The study areas are shown in red circles.

Jasione supina is a perennial, $\pm$ caespitose species with numerous procumbent or ascending stems; basal leaves are oblong, spatulate, $\pm$ obtuse, ciliated at the base; stem leaves are ovate-lanceolate, sessile; and the capitulum is 10 (-20) mm in diameter. The four subspecies of *J. supina* are distinguished from each other by differences seen in calyx and bracts. In subsp. *akmanii* and subsp. *pontica*, calyx lobes are 1-1.5 mm, triangular-linear and violet. In subsp. *akmanii*, involucre bracts are dentate, while involucre bracts are complete in subsp. *pontica*. In subsp. *supina*, calyx lobes are 2-3 mm and outer involucre bracts are generally broad, dentate and glabrous on the inside; while in subsp. *tmolea*, calyx lobes are 1.5-2 mm, outer involucre bracts are narrow, ovate-elliptic, sub-entire and have short hairs on the inside (Damboldt 1978).

The acropetally opening flowers of *J. supina* are protandric and have a special secondary pollen presentation mechanism involving pollen-collecting hairs located on the distal part of the style, also known as the 'piston mechanism'. Once the hairs on the style have been shed and the pollen removed, the plant enters the female phase, characterised by the opening of the stigma lobes (Faegri and Van Der Pijl 1979; Yeo 2012).

2.2 Quantitative and functional analysis of floral and reproductive traits

Measurements were taken of 20 flowers from each subspecies at the pre-anthesis and anthesis (secondary pollen presentation) stages. The samples were transferred to a solution of formalin, acetic acid and ethanol (FAA: 5% formaldehyde, 5% acetic acid and 90% ethanol) in the laboratory. To examine the flowers' structural features, the samples were observed using an Olympus SZ61 stereo microscope, and the style and stamen measurements were taken using a Dino-Eye AM7025X camera.

To determine the effect of flower visitors on the lifespan of flowers, 20 flowers from each subspecies at the bud stage were marked, and changes to the flowers were noted at 15-minute intervals. At the beginning of the anthesis stage, ten of the twenty selected flowers were touched with a cotton swab on their stigmas, while ten were left untouched. Then, all flowers were covered with fine netting to prevent pollinators from reaching them. Observations continued until the end of the female phase.

The functional characteristics of the flowers of each subspecies were observed in their natural environment as they changed from bud to full bloom. This process was captured using a 12-hour time-lapse shot taken every five minutes with a Nikon D500 camera. The data obtained from these observations were evaluated in four stages: bud, pre-anthesis, anthesis (secondary pollen presentation) and female. Flower phenology observations were conducted between July and September. During these observations, the times of the flower axis, flower bud, anthesis, capsule and seed were determined.

Pollen viability was measured using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma M-2128, 10 mg) test on pollen samples taken from ten pre-anthesis and ten post-anthesis flowers during the male phase of each subspecies. To detect the presence of dehydrogenase, MTT was dissolved in a 5% sucrose solution, stored as a stock solution at 2–8 °C in the dark, and 5–10 µl of the reactive solution was placed on a slide with pollen samples. The slide was left to dry, and this process was repeated three times. After 10 minutes, pollen grains that stained dark purple were considered to have dehydrogenase activity (Rodríguez-Riano and Dafni 2000). Stigma receptivity was measured using the DAB (3,3'-Diaminobenzidine) test on stigma samples taken during the female phase of each subspecies. To detect peroxidase activity, DAB tablets (Sigma, D4293) were dissolved in 1 ml of distilled water in an Eppendorf tube to prepare the reactive solution. The stigma samples were then immersed in 1 ml of this solution. After five minutes, pollen grains that stained brown were considered to have peroxidase activity (Dafni 1992).

2.3 Self-incompatibility rate (ISI)

To investigate self-incompatibility, ten flowers were pollinated with their own pollen, while ten others were pollinated with pollen from different individuals and then enclosed in a bag. The pollinated flowers were collected within two weeks, just before they shed their seeds, and the fertilised ovules were counted under a stereo microscope. The self-incompatibility rate was calculated using the following equation, as described by Zapata and Arroyo (1978): $ISI = \text{fruit set from self-pollination} / \text{fruit set from cross-pollination}$. The values of the ISI reflect the following possibilities: >1 = self-incompatible; $>0.2 < 1$ = partially self-incompatible; <0.2 = mostly self-incompatible; 0 = completely self-incompatible. The rates of fruit set following artificial self-pollination were ranked as follows: Self-incompatible: 0–3% (class 0); slightly self-compatible: 3–30% (class 1); highly self-compatible: $>30\%$ (class 2).

2.4 Floral visitors and their behaviours

Data on the floral visitors of the *Jasione supina* subspecies were collected through direct observations conducted in July, during the peak flowering period, on sunny days with low to moderate wind conditions. For each *J. supina* subspecies, observations were carried out in three 4 m² quadrats (2 m × 2 m) distributed at random within their distribution areas. Each quadrat contained ten mature individuals. Observers remained 1 metre away from the quadrats to avoid disturbing pollinator activity. Each quadrat was observed for nine hours per day over two consecutive days, with observations recorded at one-hour intervals between 08:30 and 17:30. Wind speed and temperature were also recorded during each hourly observation. The hourly mean values of wind speed and temperature were then calculated for each subspecies based on data from the three quadrats (each repeated twice) for the preparation of tables (supplementary data table). In total, 54 hours of observations were performed for all subspecies combined. The following data were collected within each quadrat: the identity of floral visitors observed on corymbose; the number of corymbose visited by each visitor; and whether each visitor exhibited behaviour related to nectar foraging, pollen collection, or floral/bud predation.

2.5 Statistical analysis

All statistical analyses were performed using GraphPad Prism, version 8.0. First, data for each morphological and reproductive parameter were tested for normality using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was then conducted to assess differences among the four *Jasione supina* subspecies. When significant differences were detected ($p < 0.05$), the Tukey's HSD post hoc test was used to identify the pairs of subspecies that differed significantly. Mean values and standard deviations (mean ± SD) were reported for each parameter. Different symbols were used in all figures to denote statistically significant differences ($p < 0.05$) between subspecies based on post hoc comparisons. Significance levels were indicated visually in all graphs for clarity, as well as being interpreted by p-values.

3 Results

3.1 Quantitative and functional analysis of floral and reproductive traits

3.1.1 Flower phenology

Floral phenology observations revealed that, in all subspecies, flower bud formation occurred one week after the floral axis stage and that the buds developed and entered the anthesis stage one week after that. Fertilisation was found to occur in the same week as anthesis, when capsule development also began. The capsule was observed to complete its development within two weeks, entering the seed dispersal stage.

The floral phenology parameters of *J. supina* subsp. *akmanii* and subsp. *pontica* were found to have the same start and end times and stage durations. However, subsp. *tomlea* exhibited a floral phenology that started one week later, with the same stage durations. For all three subspecies, the floral axis, flower bud and anthesis stages were found to last four weeks, while capsule development and seed dispersal were found to last six weeks.

For *J. supina* subsp. *supina*, the start times of the floral phenology parameters were the same as for subsp. *akmanii* and subsp. *pontica*, but each parameter lasted two weeks longer (see Table 2).

Table 2. Flower phenology of *Jasione supina* subspecies.

		July				August				September			
Weeks		1	2	3	4	1	2	3	4	1	2	3	4
subsp. <i>akmanii</i>	Flower axis												
	Flower bud												
	Anthesis												
	Capsule												
	Seed dispersal												
subsp. <i>pontica</i>	Flower axis												
	Flower bud												
	Anthesis												
	Capsule												
	Seed dispersal												
subsp. <i>supina</i>	Flower axis												
	Flower bud												
	Anthesis												
	Capsule												
	Seed dispersal												
subsp. <i>tomlea</i>	Flower axis												
	Flower bud												
	Anthesis												
	Capsule												
	Seed dispersal												

3.1.2 Flower functions

The flowers of *Jasione supina* are protandrous. While still in the bud stage, the introrse anthers form a closed cylinder around the immature style. During this stage, the anthers open and release pollen onto the unicellular collecting hairs at the tip of the style. Before the flowers open, the corolla becomes increasingly narrow and the stamens touch the style (Fig. 2a). (Fig. 2a). A rapid elongation of the lower part of the style causes the collecting hairs at the distal end to sweep up the pollen as the style grows past them (Fig. 2b). The anthers then wither and remain in the lower part of the flower. The style splits the corolla open from the top, presenting the pollen on the style hairs to pollinators (Fig. 2c). The corolla then opens completely, making the nectariums on the top of the ovary accessible to pollinators (Fig. 2d). The hairs on the stilus are shed and the surface that receives pollen is exposed (Fig. 2e). Once the pollen-collecting hairs have been completely shed, the style splits by a tenth, exposing the stigma, after which the male phase ends and the female phase begins (Fig. 2f). The stigma lobes then bend back at a 60–90° angle to accept pollen grains.

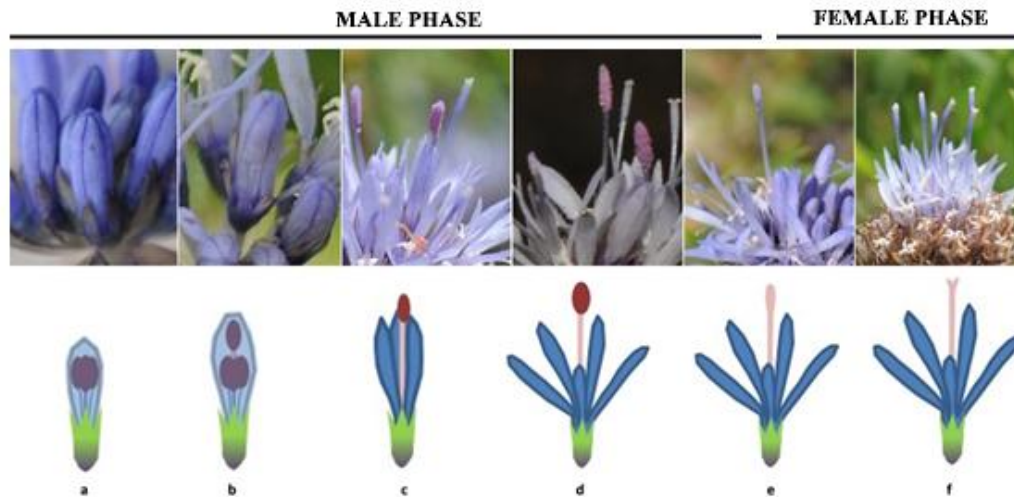


Figure 2. Flower developmental stages of *Jasione supina*. **a)** bud phase **b)** pre-anthesis phase **c)** beginning of anthesis phase **d)** pollen presentation phase at full anthesis **e)** shading phase of pollen collecting style hairs **f)** stigma lobe opening phase

There are temporal differences among the subspecies in the developmental stages of the flowers. During the bud phase, when the colour has just transitioned from green to purple prior to anthesis, the process begins within two hours in *J. supina* subsp. *akmanii* and subsp. *pontica*, and within one and a half hours in subsp. *supina* and subsp. *tmolea*. The time from the start of anthesis to full corolla opening is 45 minutes in subsp. *pontica* and subsp. *supina*, 1 hour in subsp. *tmolea* and up to 3 hours and 15 minutes in subsp. *akmanii*.

After anthesis, when the style is touched at the pollen-collecting hairs, subsp. *akmanii*, *pontica* and *tmolea* open their stigmas and enter the female phase within six hours. In contrast, subsp. *supina* transitions to the female phase within six hours and 45 minutes. In the absence of contact with the pollen-collecting hairs on the style after anthesis, the transition to the female phase occurs after 26 hours in subsp. *akmanii*, 22 hours in subsp. *pontica*, 24 hours in subsp. *supina* and 25 hours in subsp. *tmolea* (see Table 3).

Table 3 Temporal differences in flower opening stages and effects of touched stigmas on flowering duration in *Jasione supina* subspecies.

Subspecies	Bud-pre-anthesis	Beginning of anthesis- full anthesis	Beginning of anthesis - stigmatic lobe opening (touched)	Beginning of anthesis - stigmatic lobe opening (untouched)	Bud - stigmatic lobe opening (untouched)
subsp. <i>akmanii</i>	2 h	3 h 15 min	6 h	26 h	37 h 15 min
subsp. <i>pontica</i>	2 h	45 min	6 h 45 min	22 h	31 h 30 min
subsp. <i>supina</i>	1 h 30 min	45 min	6 h	24 h	32 h 15 min
subsp. <i>tmolea</i>	1 h 30 min	1 h	6 h	25 h	33 h 30 min

3.1.3 Flower traits

The differences in morpho-physiological structures of individuals belonging to four subspecies were evaluated comparatively. According to the one-way ANOVA test results ($p < 0.05$), significant differences were detected among the subspecies in all tests except the number of pollen in the anther and the post-anthesis pollen viability test (Table 4). Each parameter was subjected to Tukey's post-hoc test and the results obtained are given in Figure 3. The number of corymbose inflorescences per individual was highest in subsp. *supina* (25.93 ± 16.09), followed by subsp. *pontica* (20.03 ± 17.8), subsp. *akmanii* (19 ± 13.4), and subsp. *tmolea* (11.5 ± 4.38). Significant differences were detected in the number of corymbose per plant between subsp. *pontica* and *tmolea* and between subsp. *supina* and *tmolea*. Similarly, the number of flowers per corymbose was significantly greater in subsp. *supina* (36 ± 11.66) than in subsp. *pontica* (26 ± 4.08), subsp. *tmolea* (19 ± 2.94), and subsp. *pontica* (14.3 ± 4.85). In terms of flower numbers in corymbose, no significant differences were found between subsp. *akmanii* and *tmolea* and between subsp. *pontica* and *tmolea*, but differences were detected among other subspecies.

Table 4. Comparison of reproductive parts among subspecies.

Characters	subsp. <i>akmanii</i>	subsp. <i>pontica</i>	subsp. <i>supina</i>	subsp. <i>tmolea</i>	F value	P value	Significant (P < 0,05)
------------	-----------------------	-----------------------	----------------------	----------------------	---------	---------	------------------------

Number of corymbose in an individual	19 ($\pm 13,4$)	20,03 ($\pm 17,8$)	25,93 ($\pm 16,09$)	11,5 ($\pm 4,38$)	6,07	0,0007	Yes
Number of flowers in a corymbose	26 ($\pm 4,08$)	14,3 ($\pm 4,85$)	36 ($\pm 11,66$)	19 ($\pm 2,94$)	19,54	<0,0001	Yes
Total number of ovules in a flower	14,5 ($\pm 1,43$)	15,3 ($\pm 1,42$)	24,1 ($\pm 3,07$)	20 ($\pm 2,49$)	40,46	<0,0001	Yes
Total number of fertilized ovules in a flower	6,4 ($\pm 1,34$)	6,4 ($\pm 1,58$)	19,9 ($\pm 4,36$)	12,2 ($\pm 2,35$)	57,33	<0,0001	Yes
Total pollen grains in an anther	2110 ($\pm 435,12$)	2055 ($\pm 390,48$)	2030 ($\pm 452,89$)	1690 ($\pm 357,3$)	2,15	0,1109	No
Pollen viability in pre-anthesis (%)	56 ($\pm 14,3$)	67,5 ($\pm 12,08$)	86,5 ($\pm 6,26$)	79 ($\pm 8,43$)	15,50	<0,0001	Yes
Pollen viability in post-anthesis (%)	58 ($\pm 14,76$)	54 ($\pm 7,75$)	50,4 ($\pm 18,21$)	67,5 ($\pm 16,87$)	2,43	0,0811	No
Pollen collecting hair part length	2,8 ($\pm 0,05$)	2,8 ($\pm 0,07$)	2,8 ($\pm 0,07$)	2,71 ($\pm 0,09$)	4,314	0,0107	Yes
Style length at the beginning of the presentation	5,2 ($\pm 0,07$)	5 ($\pm 0,07$)	5 ($\pm 0,08$)	4,4 ($\pm 0,08$)	216	<0,0001	Yes
Style length at the end of the presentation	10,4 ($\pm 0,09$)	9,8 ($\pm 0,12$)	10,1 ($\pm 0,17$)	9,01 ($\pm 0,16$)	186,5	<0,0001	Yes
Anther length at the beginning of the presentation	3 ($\pm 0,09$)	3 ($\pm 0,09$)	3,2 ($\pm 0,08$)	2,4 ($\pm 0,07$)	166,2	<0,0001	Yes
Anther length at the end of the presentation	3,2 ($\pm 0,07$)	3,2 ($\pm 0,07$)	3,4 ($\pm 0,08$)	2,6 ($\pm 0,08$)	216	<0,0001	Yes
Stigma receptivity (receptive +, nonreceptive -)	+	+	+	+			

The reproductive traits measured at the ovule level also exhibited significant divergence. The number of ovules per ovary was highest in subsp. *supina* (24.1 ± 3.07), followed by subsp. *tmolea* ($20 \pm 2.49.07$), subsp. *pontica* (15.3 ± 1.42), and subsp. *akmanii* (14.5 ± 1.43). In total ovules, no significant differences were found between subsp. *akmanii* and *pontica*, but high differences were detected between other subspecies. Similarly, the number of fertilized ovules per ovary was highest in subsp. *supina* (19.9 ± 4.36) followed by subsp. *tmolea* (12.2 ± 2.35), subsp. *pontica* (6.4 ± 1.34) and subsp. *akmanii* (6.4 ± 1.58). Again, it was found that there was no significant difference between subsp. *akmanii* and *pontica*, but there was a high difference between the other subspecies. Based on ovule parameters, it was determined that the highest reproductive success was in subsp. *supina* and the lowest in subsp. *akmanii*.

Pollen-related traits further revealed clear differentiation among subspecies. The total pollen production was significantly higher in subsp. *akmanii* (2110 ± 435.12), decreasing gradually in subsp. *pontica* (2055 ± 390.48), subsp. *supina* (2030 ± 452.89), and subsp. *tmolea* (1690 ± 357.3). Pre-anthesis pollen viability was greatest in subsp. *supina* (86.5 ± 6.26), followed by subsp. *tmolea* (79 ± 8.43), subsp. *pontica* (67.5 ± 12.08), and subsp. *akmanii* (56 ± 14.3). In post-anthesis pollen viability, a small increase was observed in subsp. *akmanii*, while a decrease was observed in the others, with the greatest decrease being in subsp. *supina*. Pollen viability at post-anthesis is as follows; the highest is subsp. *tmolea* (67.5 ± 16.87), followed by subsp. *akmanii* (58 ± 14.76), subsp. *pontica* (54 ± 7.75) and subsp. *supina* (50.4 ± 18.21). No significant differences were found among the subspecies in terms of total pollen counts in the anther and pollen viability at post-anthesis, but statistical differences were found among *akmanii-supina*, *akmanii-tmolea* and *pontica-supina* at pre-anthesis.

Floral structures related to secondary pollen presentation also varied markedly. The pollen collecting hair parts on style was similar in subsp. *supina* (2.8 ± 0.07 mm), followed by subsp. *pontica* (2.8 ± 0.07 mm) and subsp. *akmanii* (2.8 ± 0.05 mm), while it was shorter in subsp. *tmolea* (2.71 ± 0.09 mm), and a statistically significant difference was detected only between *tmolea* and the other species. Style lengths measured before pollen presentation were comparable among subsp. *akmanii* (5.2 ± 0.07 mm), subsp. *pontica* (5.0 ± 0.07 mm), and subsp. *supina* (5.0 ± 0.08 mm), all of which were significantly longer than subsp. *tmolea* (4.4 ± 0.08 mm). Post-anthesis style lengths increased notably in all subspecies, with subsp. *akmanii* (10.4 ± 0.09 mm) and subsp. *supina* (10.1 ± 0.17 mm) showing the greatest elongation, while subsp. *tmolea* had the shortest post-anthesis style length (9.01 ± 0.16 mm). In the comparison of style lengths before secondary pollen presentation, there was no significant difference between subsp. *pontica* and *supina*, while the differences in style lengths between the others and after presentation were statistically significant.

In terms of stamen development, pre-anthesis stamen lengths were similar in subsp. *akmanii*, subsp. *pontica*, and subsp. *supina* (all ~ 3 mm), while subsp. *tmolea* exhibited significantly shorter stamens (2.4 ± 0.07 mm). Post-anthesis stamen lengths followed a comparable trend, with the longest stamens recorded in subsp. *supina* (3.4 ± 0.08 mm), followed by subsp. *akmanii* and subsp. *pontica* (3.2 ± 0.07 mm), and the shortest in subsp. *tmolea* (2.6 ± 0.08 mm). While no statistically significant difference was observed in stamen lengths before

presentation in subsp. *akmanii* and *pontica*, the differences between the others were significant. Overall, traits associated with secondary pollen presentation—including style elongation, stamen elongation, and collecting hair length—were generally lower in subsp. *tmolea*, whereas subsp. *akmanii* and *supina* exhibited higher values.

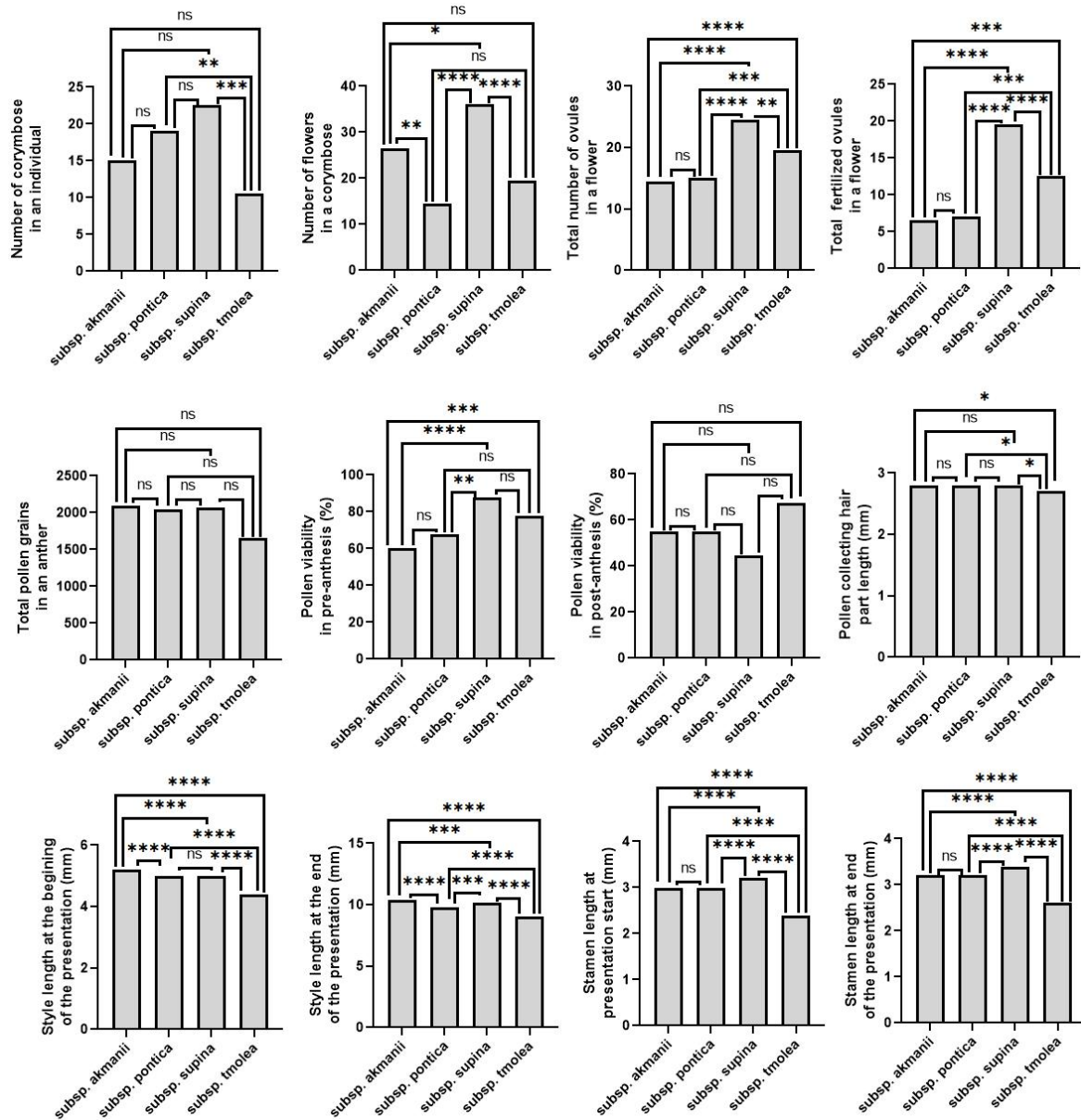


Figure 3. Results of one-way ANOVA significance in reproductive parts among four subspecies

3.2 Self-incompatibility rate (ISI)

None of the ovules were fertilised following self-pollination in all subspecies of *Jasione supina*, indicating complete self-incompatibility. By contrast, cross-pollination resulted in the fertilisation of $6.4 (\pm 1.34)$ ovules in subsp. *akmanii*, $6.4 (\pm 1.58)$ in subsp. *pontica*, $19.9 (\pm 4.36)$ in subsp. *supina* and $12.2 (\pm 2.35)$ in subsp. *tmolea*. Fertilisation occurred in 44.14% of the $14.5 (\pm 1.43)$ ovules produced by subsp. *akmanii*, 41.83% of the $15.3 (\pm 1.42)$ ovules produced by subsp. *pontica*, 82.75% of the $24.1 (\pm 3.07)$ ovules produced by subsp. *supina* and 61% of the $20 (\pm 2.49)$ ovules produced by subsp. *tmolea* under cross-pollination. According to these data, the self-incompatibility ratio (ISI) was found to be zero using the formula of Zapata and Arroyo (1978), indicating that the reproductive system is completely self-incompatible.

3.3 Floral visitors and their behaviours

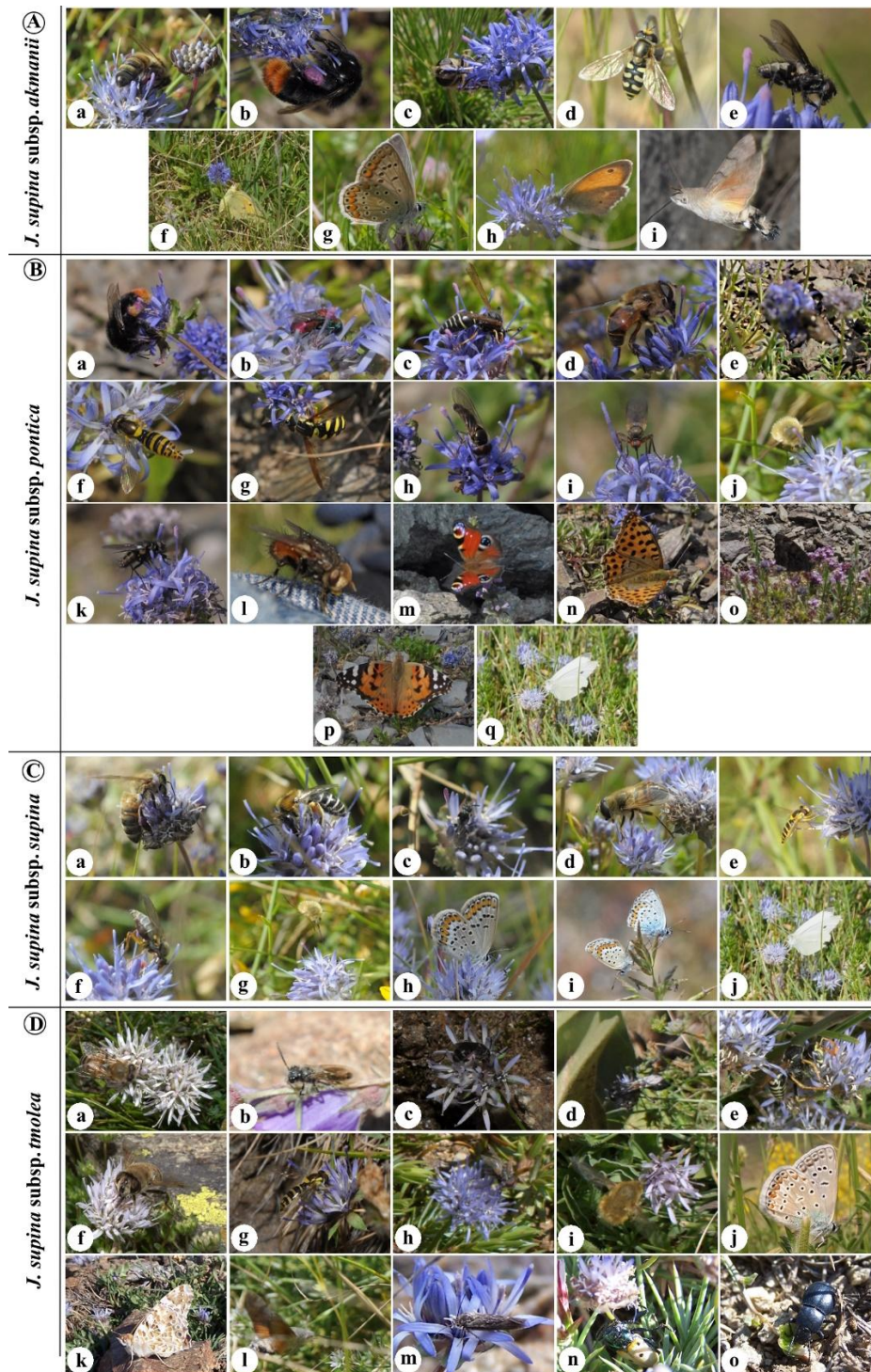


Figure 4. Flower visitors to the subspecies *J. supina*. (A) subsp. *akmanii* a) *Apis mellifera* b) *Bombus (Psithyrus) rupestris* c) *Eristalis tenax* d) *Dasysyrphus albostratus* e) *Anthomyiidae* f) *Colias coroea* g) *Pleibeius idas* h) *Coenonympha pamphilus* i) *Macroglossum stellatarum* (B) subsp. *pontica* a) *Bombus (Psithyrus) rupestris* b) *Chrysura purpureifrons* c) *Dolichovespula (Boreovespula) norvegica* d) *Eristalis tenax* e) *Merodon* sp. f) *Sphaerophoria scripta* g) *Dasysyrphus albostratus* h) *Platycheirus albimanus* i) *Rhamphomyia anomalina* j) *Bombylius venosus* k) *Anthomyiidae* l) *Gonia bimaculata* m) *Inachis io* n) *Issoria lathonia* o) *Aglaia urticae* p) *Vanessa cardui* q) *Pieris rapae* (C) subsp. *supina* a) *Apis mellifera* b) *Megachile (Eutricharaea) pilidens* c) *Chelostoma rapunculi* d) *Eristalis tenax* e) *Sphaerophoria scripta* f) *Rhamphomyia anomalina* g) *Bombylius venosus* h) *Plebeius pylaon* i) *Pleibeius idas* j) *Pieris rapae* (D) subsp. *tmolea* a) *Apis mellifera* b) *Chelostoma campanularum* c) *Halictus* sp. d) *Sphecidae* e) *Vespula* sp. f) *Eristalis tenax* g) *Sphaerophoria scripta* h) *Anthomyiidae* i) *Bombylius venosus* j) *Polyommatus icarus* k) *Vanessa cardui* l) *Macroglossum stellatarum* m) *Micropterigidae* n) *Lachnaia sexpunctata* o) *Dailognatha quadricollis*

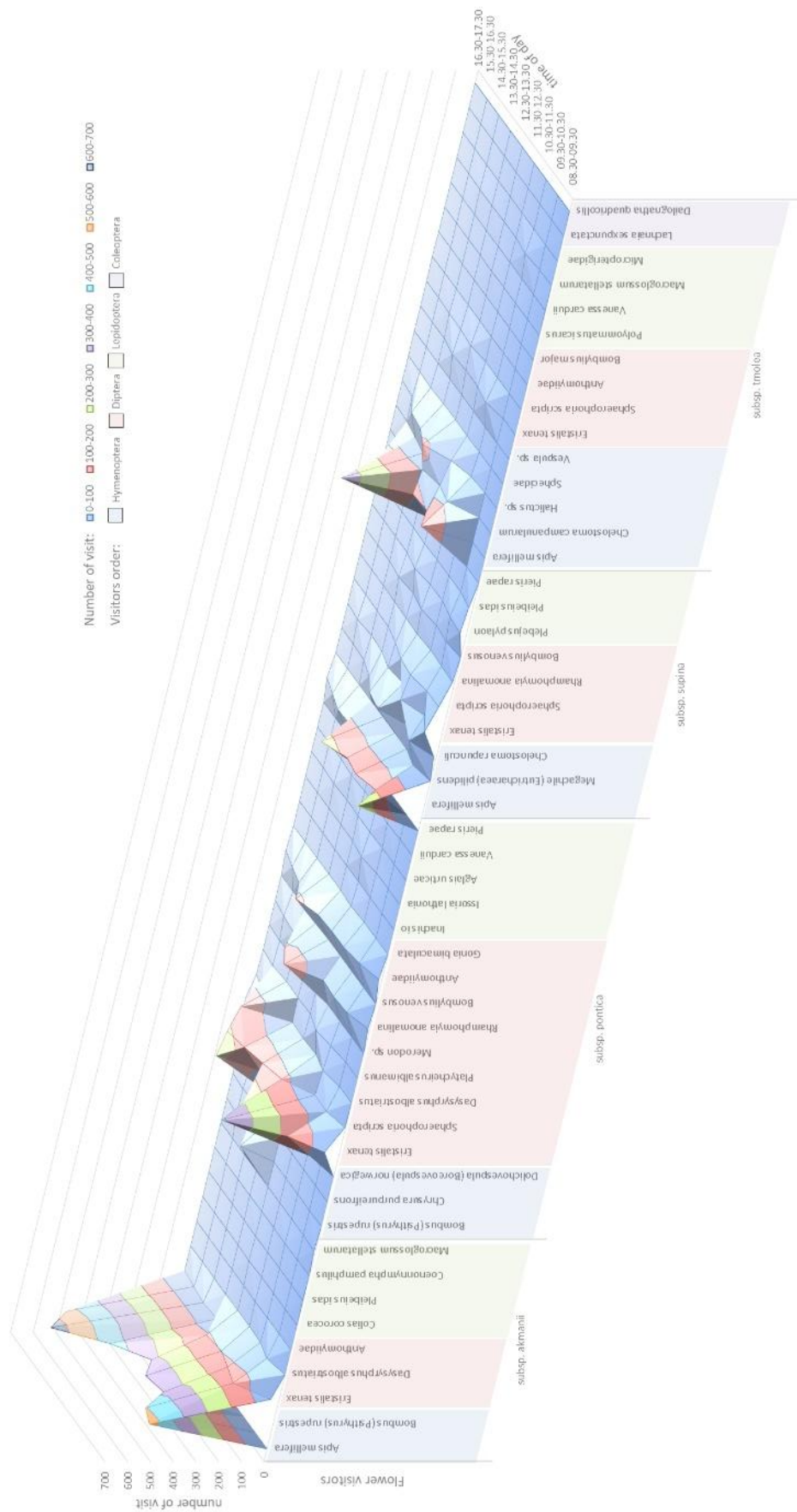


Figure 5. 3D surface graph showing hourly visits by insect taxa visiting subspecies *Jasione supina* over 9 h of observations.

A total of 51 floral visitor taxa were observed across the four subspecies of *Jasione supina*, showing significant variation in composition and visitation frequency (see Figures 4 and 5 and Table 4). In subsp. *akmanii*, 4,462 floral visits involving nine visitor taxa were recorded. Of these, 86.03% were carried out by two Hymenoptera morphospecies, 13.43% by three Diptera morphospecies and 0.54% by four Lepidoptera morphospecies. The most frequent visitor was *Bombus rupestris*, accounting for 85.63% of all interactions. Five taxa visited flowers for both nectar and pollen, while four taxa collected only nectar. In subsp. *pontica*, 17 visitor taxa were recorded, with a total of 3,670 floral visits. Diptera constituted the majority at 95.48% (nine morphospecies), followed by Hymenoptera at 3.32% (three morphospecies) and Lepidoptera at 1.2% (five morphospecies). The dominant visitor was *Eristalis tenax*, responsible for 37.93% of visits. Eight taxa collected both pollen and nectar, while nine taxa visited flowers solely for nectar. In subsp. *supina*, ten visitor taxa made 2,278 visits, 71.28% of which were by three Hymenoptera morphospecies, 24.24% by four Diptera morphospecies and 4.48% by three Lepidoptera morphospecies. *Apis mellifera* was the main floral visitor, accounting for 55.27% of all interactions. Six taxa were pollen and nectar foragers, while four were exclusively nectar collectors. Subspecies *imolea* had the lowest visitation frequency, with 1,680 interactions involving 15 visitor taxa. Of these, 93.33% were carried out by five Hymenoptera morphospecies, 5.24% by four Diptera morphospecies, 1.31% by four Lepidoptera morphospecies and 0.12% by two Coleoptera morphospecies. The dominant pollinator was once again *Apis mellifera*, accounting for 54.76% of visits. Six taxa visited for both pollen and nectar, seven for nectar only and two were observed feeding directly on flower and bud tissues.

Table 4. Total number, percentage and collected resources (p=pollen, n=nectar, of taxa visiting flowers of *Jasione supina* subspecies. Flower visitors of *Jasione supina* total flower visit, percentage of the total number of visits and collected resources. (p = pollen, n = nectar, pre = predator).

Subspecies	Order	Visitors	% visitation	Total visits	Resource collected	Subspecies	Order	Visitors	% visitation	Total visits	Resource collected
subsp. <i>akmanii</i>	Hymenoptera	<i>Apis mellifera</i>	0,4	18	p/n	subsp. <i>supina</i>	Hymenoptera	<i>Apis mellifera</i>	55,27	1259	p/n
	Hymenoptera	<i>Bombus rupestris</i>	85,63	3821	p/n		Hymenoptera	<i>Megachile pilidens</i>	1,05	24	p/n
	Diptera	<i>Eristalis tenax</i>	12,62	563	p/n		Hymenoptera	<i>Chelostoma rapunculi</i>	14,97	341	p/n
	Diptera	<i>Dasysyrphus albobriatus</i>	0,76	34	p/n		Diptera	<i>Eristalis tenax</i>	10,76	245	p/n
	Diptera	Anthomyiidae	0,04	2	p/n		Diptera	<i>Sphaerophoria scripta</i>	10,27	234	p/n
	Lepidoptera	<i>Colias coroceae</i>	0,09	4	n		Diptera	<i>Rhamphomyia anomalina</i>	1,32	30	p/n
	Lepidoptera	<i>Pleibeus idas</i>	0,09	4	n		Diptera	<i>Bombylius venosus</i>	1,89	43	n
	Lepidoptera	<i>Coenonympha pamphilus</i>	0,22	10	n		Lepidoptera	<i>Plebejus pylaon</i>	3,47	79	n
	Lepidoptera	<i>Macroglossum stellatarum</i>	0,13	6	n		Lepidoptera	<i>Pleibeus idas</i>	0,97	22	n
subsp. <i>pontica</i>	Hymenoptera	<i>Bombus (Psithyrus) rupestris</i>	3,02	111	p/n	subsp. <i>imolea</i>	Lepidoptera	<i>Pieris rapae</i>	0,04	1	n
	Hymenoptera	<i>Chrysura purpureifrons</i>	0,22	8	n		Hymenoptera	<i>Apis mellifera</i>	54,76	920	p/n
	Hymenoptera	<i>Dolichovespula norvegica</i>	0,08	3	n		Hymenoptera	<i>Chelostoma campanularum</i>	18,69	314	p/n
	Diptera	<i>Eristalis tenax</i>	37,93	1392	p/n		Hymenoptera	<i>Halictus</i> sp.	19,17	322	p/n
	Diptera	<i>Sphaerophoria scripta</i>	23,3	855	p/n		Hymenoptera	Sphecidae	0,65	11	n
	Diptera	<i>Dasysyrphus albobriatus</i>	6,29	231	p/n		Hymenoptera	<i>Vespula</i> sp.	0,06	1	n
	Diptera	<i>Platycheirus albimanus</i>	1,55	57	p/n		Diptera	<i>Eristalis tenax</i>	3,51	59	p/n
	Diptera	<i>Merodon</i> sp.	0,16	6	p/n		Diptera	<i>Sphaerophoria scripta</i>	1,43	24	p/n
	Diptera	<i>Rhamphomyia anomalina</i>	13,11	481	n		Diptera	Anthomyiidae	0,24	4	p/n
	Diptera	<i>Bombylius venosus</i>	0,14	5	n		Diptera	<i>Bombylius major</i>	0,06	1	n
	Diptera	Anthomyiidae	12,83	471	p/n		Lepidoptera	<i>Polyommatus icarus</i>	0,18	3	n
	Diptera	<i>Gonia bimaculata</i>	0,16	6	p/n		Lepidoptera	<i>Vanessa cardui</i>	0,06	1	n
	Lepidoptera	<i>Inachis io</i>	0,03	1	n		Lepidoptera	<i>Macroglossum stellatarum</i>	1,01	17	n
	Lepidoptera	<i>Issoria lathonia</i>	0,41	15	n		Lepidoptera	Micropterigidae	0,06	1	n
	Lepidoptera	<i>Aglais urticae</i>	0,25	9	n		Coleoptera	<i>Lachnaia sexpunctata</i>	0,06	1	pre
	Lepidoptera	<i>Vanessa cardui</i>	0,35	13	n		Coleoptera	<i>Dailognatha quadricollis</i>	0,06	1	pre
	Lepidoptera	<i>Pieris rapae</i>	0,16	6	n						

4 DISCUSSION

This study comparatively evaluated the morphological, physiological and reproductive biology traits of four subspecies of *Jasione supina*. Significant differences were revealed among the subspecies in key reproductive parameters such as flowering structure, pollen viability, ovule number and fertilisation success. These differences provide valuable insights into the ecological adaptations and potential evolutionary divergence of the subspecies.

The highest reproductive success was observed in subsp. *supina*. This subspecies had a clear advantage in terms of the number of corymbose inflorescences and flowers per individual, as well as the number of ovules and fertilised ovules per ovary, compared to the others. Additionally, its high pollen viability at the pre-anthesis stage may have contributed directly to enhanced fertilisation success. Its most pronounced style and stamen elongation, traits associated with secondary pollen presentation, suggest efficient interaction with pollinators and effective pollen transfer may further support its reproductive performance. In contrast, subsp. *tomlea*, despite having lower numbers of inflorescences and flowers, stood out due to its high post-anthesis pollen viability and relatively high fertilisation rates. Although the shorter style and stamen lengths, as well as the reduced pollen-collecting hair structures, may limit secondary pollen presentation, the high physiological quality of the pollen appears to partially compensate for these limitations. This finding highlights the compensatory role of producing fewer, higher-quality gametes, and emphasises how distinct reproductive strategies can evolve at the subspecies level. *J. supina* subsp. *pontica* and subsp. *akmanii*, on the other hand, demonstrated comparatively weaker reproductive performance, with lower fertilisation rates and relatively shorter floral structures. Interestingly, despite being the highest pollen producer, subsp. *akmanii* exhibited the lowest pre-anthesis pollen viability, likely reducing the functional impact of its high pollen output. This observation highlights that pollen quantity alone is not a sufficient determinant of reproductive success, and that viability and timing also play critical roles.

The reproductive performance of the *Jasione supina* subspecies revealed substantial differences between subspecies in terms of floral traits, gamete viability and seed set success. These findings are partially consistent with those observed in other *Jasione* taxa. Compared to *J. montana*, which produces an average of five corymbs per plant and approximately 40 flowers per corymb (Parnell 1985), *J. supina* subsp. *supina* displayed markedly higher inflorescence density and comparable flower numbers, suggesting enhanced reproductive potential. However, ovule counts in *J. supina* were generally lower than in *J. montana* (37 ovules per capsule; Andreychuk and Odintsova 2021) and *J. maritima* (33 ovules; Mendess et al. 2022). Nevertheless, *J. supina* subsp. *supina* achieved the highest ovule and fertilised ovule numbers among all the subspecies analysed in this study. This implies that, while ovule numbers per flower may be lower in certain taxa, reproductive efficiency through fertilisation success can compensate for this discrepancy under favourable pollination conditions. In terms of pollen viability, our findings support the variability observed across the genus. While *J. bulgarica* demonstrated exceptionally high pre-anthesis pollen viability (93.3%; Yankova-Tsvetkova and Vladimirov 2024), the *J. supina* subspecies exhibited a broader range. Again, subsp. *supina* showed the highest pre-anthesis pollen viability (86.5%), while subsp. *akmanii* showed the lowest (56%). These differences may reflect either intrinsic genetic divergence or local adaptation to distinct environmental conditions. The consistent absence of fertilisation following self-pollination in all *J. supina* subspecies is consistent with the strong self-incompatibility previously described in *J. maritima* (Siopa et al. 2020) and supports the hypothesis that obligate outcrossing is a conserved trait within the genus. The high success rate of cross-pollination in subsp. *supina* further emphasises its superior reproductive efficacy, which is likely facilitated by a combination of high floral output, pollen quality, and a compatible reproductive strategy.

This study observed that stimulating the pollen-collecting hairs on the style of *Jasione supina* subspecies flowers significantly accelerated their transition from the male to the female phase. In individuals where the stylar hairs were touched after anthesis, the female phase began within 6 to 6 hours and 45 minutes; in the absence of such contact, however, this transition was delayed by up to 22 to 26 hours. These findings suggest that the stylar hairs' function is not limited to presenting pollen, but that they also trigger the onset of the female reproductive phase. A similar observation was reported in *Campanula trachelium* by Vranken et al. (2014): individuals subjected to manual stimulation of the stylar hairs transitioned to the female phase in an average of 1.9 ± 0.1 days, whereas unstimulated flowers required up to 2.7 ± 0.2 days. In both studies, stylar hairs appear to initiate a physiological response through pollinator contact or mechanical disturbance, thereby regulating the timing of the onset of the female phase. This similarity suggests that, in species within the Campanulaceae family with secondary pollen presentation, stylar hairs are not merely passive structures, but rather sensory components that direct a critical phase of floral phenology. Although this transition occurs on an hourly scale in *J. supina* and on a

daily scale in *C. trachelium*, the underlying mechanism and functional role are largely analogous between species. Floral phenology observations corroborate earlier reports on capsule development and seed dispersal timing in *Jasione* species (Parnell 1985), with seed release occurring approximately two to three weeks after fertilisation. However, the prolonged phenological phases observed in subsp. *supina* suggest a temporal reproductive extension that may confer ecological or evolutionary advantages in specific habitats.

The majority of floral visits observed in the *Jasione supina* subspecies were carried out by Hymenoptera, accounting for 63.33% of all visits. This was followed by Diptera (34.6%), Lepidoptera (1.88%) and Coleoptera (0.03%). As with *J. supina*, it has been reported that *J. montana* flowers were visited by species belonging to these four orders, with the majority of visits being made by Hymenoptera (36%) and Diptera (25%) (Parnell 1985). Likewise, in *J. maritima* var. *sabularia*, an Iberian Peninsula endemic, it has been reported that species belonging to these four orders visited the flowers, with the majority of visits being carried out by Diptera (39.8%) and Hymenoptera (38.9%) (Mendess et al. 2022). Among the subspecies studied, it was only in *J. supina* subsp. *pontica* that visits by Diptera exceeded those by Hymenoptera. Similarly, while Diptera were the primary pollinators in *J. maritima* var. *sabularia*, Hymenoptera were observed to be the primary pollinators in *J. montana*. Similar pollinator orders observed across different *Jasione* taxa suggest that these plants have evolved a 'generalist pollination strategy' towards these groups. However, variation in the relative contributions of these orders indicates that factors such as microhabitat differences, environmental conditions, phenological timing and floral traits influence these interactions. This suggests that pollinator community structure is highly dynamic, with significant variation existing even among subtaxa of a single plant species. Similarly, differences in pollinator visitation rates, nectar properties, and floral morphology were found between two populations of *Oenothera epilobiifolia* located at 2000 m and 4450 m altitude, respectively (Rodríguez-Sánchez et al. 2024). These differences demonstrate that environmental heterogeneity substantially affects pollinator interactions and plant reproductive strategies. It has been reported that 96% of visits by the rare butterfly species *Parnassius apollo* were to four plant species (*Jurinea humilis*, *Thymus bracteata*, *Jasione montana* and *Armeria arenaria*). During the pollination period, the butterfly initially used *Jasione montana* as its main resource, but shifted to *Jurinea humilis* towards the end of the flowering period (Baz 2002). This indicates that pollinators can change their preferences depending on the flowering of other plant species present in the same area. Based on our results, we hypothesise that the difference in main pollinator groups between *J. supina* subsp. *akmanii* and subsp. *pontica*, despite their highly similar secondary pollen presentation mechanisms and morphological characteristics, may be due to environmental heterogeneity and different floristic compositions in their respective habitats, causing pollinators to prefer different plant species.

Overall, the reproductive divergence observed among the subspecies of *Jasione supina* extends beyond morphological and phenological traits to encompass functional and ecological dimensions. This study's findings demonstrate substantial variation in reproductive structure development, pollinator interactions, and pollen presentation strategies across subspecies. This suggests that these differences are closely shaped by the specific environmental contexts in which each taxon occurs. These results highlight the importance of evaluating intraspecific taxa in terms of their reproductive ecology as well as from a systematic perspective.

This study clearly demonstrates that there are significant reproductive differences among the four subspecies classified under *Jasione supina*. Despite their morphological similarities, these taxa have evolved distinct reproductive strategies with respect to floral phenology, pollen viability, style and stamen development, ovule fertilisation success and pollinator assemblages. The variation observed in secondary pollen presentation mechanisms at the subspecies level highlights the evolutionary plasticity of such traits and their potential to evolve in response to microevolutionary pressures. Furthermore, environmental heterogeneity and local floristic composition appear to directly influence pollinator behaviour and consequently reproductive outcomes.

Beyond elucidating the reproductive ecology of the *Jasione supina* complex, this study emphasises the importance of analyses at the subspecies level in understanding plant evolution, adaptation and conservation. The results emphasise the need for integrative approaches that consider local adaptation and ecological specificity when developing strategies for the sustainable management of endemic and range-restricted plant taxa.

ACKNOWLEDGEMENTS

This study, which we carried out within the scope of our PHD thesis titled "Pollination biology of *Jasione supina* subspecies" at the Ege University Institute of Science, was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK Project No: 112T851). We would like to extend our sincere thanks to Dr

Bekir Keskin of the Biology and Zoology Department at the Faculty of Science, Ege University, for his valuable contributions to the identification of pollinators.

AUTHOR CONTRIBUTIONS

Volkan Eroğlu completed the main experiments, designed the study, and wrote the main manuscript text; Serdar Gökhan Şenol revised the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the study are available from the authors on reasonable request.

DECLARATIONS

Conflict of interest All authors of this manuscript hereby declare that they have no conflicts of interest.

REFERENCES

- Andreychuk R, Odintsova A (2021) Morpho-anatomy of the gynoecium and fruit in three ornamental members of Campanuloideae (Campanulaceae). *Acta Agrobotanica* 74(1):7415. <https://doi.org/10.5586/aa.7415>.
- Armbruster WS, Pélabon C, Hansen TF, Bolstad GH (2009) Macroevolutionary patterns of pollination accuracy: a comparison of three genera. *New Phytol* 183(3):600-617. <https://doi.org/10.1111/j.1469-8137.2009.02930.x>.
- Arnold SJ, Pfrender ME, Jones AG. (2001) The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112:9–32. <https://doi.org/10.1023/A:1013373907708>.
- Barrett S (2002) The evolution of plant sexual diversity. *Nat Rev Genet* 3:274–284. <https://doi.org/10.1038/nrg776>.
- Barrett SC, Harder LD (2017) The ecology of mating and its evolutionary consequences in seed plants. *Annu Rev Ecol Evol Syst* 48(1):135-157. <https://doi.org/10.1146/annurev-ecolsys-110316-023021>
- Baz A. (2002) Nectar plant sources for the threatened Apollo butterfly (*Parnassius apollo* L. 1758) in populations of central Spain. *Biol Conserv* 103(3):277-282. [https://doi.org/10.1016/S0006-3207\(01\)00138-0](https://doi.org/10.1016/S0006-3207(01)00138-0).
- Borchardt KE, Morales CL, Aizen MA, Toth AL (2021) Plant–pollinator conservation from the perspective of systems-ecology. *Curr Opin Insect Sci* 47:154-161. <https://doi.org/10.1016/j.cois.2021.07.003>.
- Carolin RC (1960) The structures involved in the presentation of pollen to visiting insects in the order Campanales. *Proc Linn Soc, New South Wales*, 85, pp 197–207.
- Dafni A (1992) Pollination ecology: a practical approach. Oxford University Press, New York
- Damboldt J (1978) *Campanula* L. In: Davis PH (ed) *Flora of Turkey and the East Aegean Islands*, Vol. 6. Edinburgh University Press, Edinburgh, pp. 86–89.
- Erbar C, Leins P (1989) On the early floral development and the mechanisms of secondary pollen presentation in *Campanula*, *Jasione* and *Lobelia*. *Bot Jahrb Syst* 111:29–55.
- Erbar C, Leins P (2006) Floral ontogeny and systematic position of the Didiereaceae. *Plant Syst Evol* 261:165-185. <https://doi.org/10.1007/s00606-006-0432-y>.
- Faegri K, Van Der Pijl L (1979) Principles of pollination ecology, 3rd edn. Pergamon, Oxford
- Hamston TJ, Wilson RJ, de Vere N, Rich TCG, Stevens JR, Cresswell JE (2017) Breeding System and Spatial Isolation from Congeners Strongly Constrain Seed Set in an Insect-Pollinated Apomictic Tree: *Sorbus subcuneata* (Rosaceae). *Sci Rep* 7:45122. <https://doi.org/10.1038/srep45122>.
- Harder LD, Barrett SC (Eds.). (2006) Ecology and evolution of flowers. Oxford University Press, Oxford. <https://doi.org/10.1093/oso/9780198570851.001.0001>.

- Howell GJ, Slater AT, Knox RB (1993) Secondary pollen presentation in angiosperms and its biological significance. *Aust J Bot* 41:417–438. <https://doi.org/10.1071/BT9930417>
- Jost L (1918) Die Griffelhaare der Campanulablüte. *Flora* 111:478-489. [https://doi.org/10.1016/S0367-1615\(17\)32858-6](https://doi.org/10.1016/S0367-1615(17)32858-6).
- Lammers TG (2007) World checklist and bibliography of Campanulaceae. The Board of Trustees of the Royal Botanic Gardens, Kew.
- Leins P, Erbar C (1990) On the mechanisms of secondary pollen presentation in the Campanulales-Asterales complex. *Botanica Acta* 103:87–92. <https://doi.org/10.1111/j.1438-8677.1990.tb00131.x>.
- Ludwig S, Robertson A, Rich T, Đorđević M, Cerović R, Houston L, Harris S, Hiscock S (2013) Breeding Systems, Hybridization and Continuing Evolution in Avon Gorge *Sorbus*. *Ann Bot* 111:563–575. <https://doi.org/10.1093/aob/mct013>.
- Mendes S B, Timóteo S, Loureiro J, Castro S (2022) The impact of habitat loss on pollination services for a threatened dune endemic plant. *Oecologia* 198(1):279-293. <https://doi.org/10.1007/s00442-021-05070-y>.
- Morgan MT, Wilson WG (2005) Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* 59(5):1143-1148. <https://doi.org/10.1111/j.0014-3820.2005.tb01050.x>.
- Moyroud E, Glover BJ (2017) The physics of pollinator attraction. *New Phytol* 216(2):350-354. <https://doi.org/10.1111/nph.14312>.
- Murren CJ (2002) Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *J Ecol* 90(1):100-107. <https://doi.org/10.1046/j.0022-0477.2001.00638.x>.
- Nyman Y (1993) The pollen-collecting hairs of *Campanula* (Campanulaceae). II. Function and adaptive significance in relation to pollination. *Am J Bot* 80:1437-1443. <https://doi.org/10.1002/j.1537-2197.1993.tb15389.x>.
- Parnell JAN (1985) *Jasione montana* L. *J Ecol* 73(1):341–358. <https://doi.org/10.2307/2259787>.
- Paus A, Brooks SJ, Hafliðason H, Halvorsen LS (2023) From tundra to tree-birch; late-glacial and early Holocene environment and vegetation oscillations at the ecotonal positioned Bjerkreim, Dalane, SW Norway. *Quat Sci Rev* 320:108347. <https://doi.org/10.1016/j.quascirev.2023.108347>.
- POWO (2025) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. <https://powo.science.kew.org/>. Accessed 10 June 2025.
- Rocha J, Almeida P, Ramirez-Rodriguez R, Sales F, Amich F, Crespi A (2022) Phenotypic strategies for *Jasione* L. or the challenge that taxonomy face to recent floristic responses along western Eurasia. *Turk J Bot* 46(1):62-79. <https://doi.org/10.3906/bot-2104-15>.
- Rodriguez-Riano T, Dafni A (2000) A new procedure to asses pollen viability. *Sex Plant Reprod* 12: 241–244. <https://doi.org/10.1007/s004970050008>.
- Rodríguez-Sánchez GT, Pelayo RC, Soriano PJ, Knight TM (2024) Intraspecific variation in pollination ecology due to altitudinal environmental heterogeneity. *Ecology and Evolution* 14(6): e11553. <https://doi.org/10.1002/ece3.11553>.
- Serrano M, Dimitrova L, Ortiz S (2023) Nomenclature and Typification of the *Jasione* L.(Campanulaceae) Groups from the Eastern Mediterranean Basin. *Plants* 13(1):50. <https://doi.org/10.3390/plants13010050>.
- Sezer O, Ardic M (2022) Taxonomic assessment and rearrangement of endemic *Jasione supina*-complex from Turkey. *Pak. J. Bot* 54(2):439-451. [http://dx.doi.org/10.30848/PJB2022-2\(1\)](http://dx.doi.org/10.30848/PJB2022-2(1)).
- Shetler SG (1979) Pollen-collecting hairs of *Campanula* (Campanulaceae), I. Historical review. *Taxon* 28:205–215. <https://doi.org/10.2307/1219578>.

Siopa C, Dias MC, Castro M, Loureiro J, Castro S. (2020) Is selfing a reproductive assurance promoting polyploid establishment? Reduced fitness, leaky self-incompatibility and lower inbreeding depression in neotetraploids. *Am J Bot* 107(3):526-538. <https://doi.org/10.1002/ajb2.1441>.

Strauss SY, Irwin RE. (2004) Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annu. Rev. Ecol. Evol. Syst* 35:435–466. <https://doi.org/10.1146/annurev.ecolsys.35.112202.130215>

Vamosi JC, Goring SJ, Kennedy BF, Mayberry RJ, Moray CM, Neame LA, Tunbridge ND, Elle E (2007) Pollination, floral display, and the ecological correlates of polyploidy. *Functional Ecosystems and Communities* 1(1):1-9.

Vranken S, Brys R, Hoffmann M, Jacquemyn H (2014) Secondary pollen presentation and the temporal dynamics of stylar hair retraction and style elongation in *Campanula trachelium* (Campanulaceae). *Plant Biol* 16(3):669-676. <https://doi.org/10.1111/plb.12097>.

Yankova-Tsvetkova E, Vladimirov V (2024) Reproductive Traits in Two Alpine Endemic Species of Campanulaceae. *C R Acad Bulg Sci* 77(5):676-686. <https://doi.org/10.7546/CRABS.2024.05.05>.

Yeo PF (2012) Secondary pollen presentation: Form, function and evolution. Springer Science & Business Media, New York

Zapata TR, Arroyo MTK (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10(3):221–230. <https://doi.org/10.2307/2387907>.