

Sexual phenotype of *Capparis herbacea* (Capparaceae)

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Abstract: Floral morphology coupled with the morphometry of androecium revealed that *Capparis herbacea* Willd. has an andromonoecious sexual system, producing both male and perfect flowers on the same plant. Functionally male flowers develop more stamens with larger anthers than bisexual ones (63.6 ± 0.6 and 4.1 ± 0.01 mm; 58.1 ± 0.7 and 3.6 ± 0.02 mm, respectively). The ratio of male to perfect flowers ranges from 0.5 to 2.6 during the flowering season. The development of dense trichomes on the adaxial surface of the cavity made by variegated connate petals is presumably adaptive and serves to protect nectary exudate from evaporation in a hot and dry environment. Air temperatures below 30 °C and successful cross pollination—rather than light and relative humidity—limit florescence time and duration. No preference for flower morphs was observed during pollinator visitations. These data confirm the pollen donation hypothesis regarding the role of male flowers in andromonoecious plants. It is a primary benefit of effective pollination in *Capparis herbacea*, a species with a short blooming period. Results also indicate that *Capparis herbacea* is the second member of the section *Capparis* L., after *Capparis spinosa* L., in which the coexistence of male and hermaphrodite flowers on the same plant has been reported.

Key words: Capparaceae, *Capparis herbacea*, andromonoecy

1. Introduction

Capparis herbacea Willd. (*C. spinosa* auct. non L.) is a perennial pendulous shrub distributed in the Irano-Turanian floristic region, extending into the Euro-Siberian region, the Middle East, Central Asia, and the Caucasus (Inocencio et al., 2006). Capers from the section *Capparis* L. (which also includes *C. herbacea*) usually inhabit desert and semidesert areas (Fakhireh et al., 2012). In Georgia this species grows in dry open woodlands, xerophytic scrublands, semideserts, and secondary steppes. As a native plant it is of considerable importance for erosion control and revegetation of degraded habitats in Georgia, due to its drought tolerance and ability to withstand desertification in the fragile semiarid ecosystem.

Capparis herbacea differs from the Mediterranean *C. spinosa* L. by straight stipules, prominent innervations of the abaxial leaf surface, strong zygomorphy of the flower, and star-like shape of the nectary. Like *C. spinosa*, *C. herbacea* is an economically important species; capers, pickled flower buds and young fruits, are often used as seasoning or garnish. The available data on the sexual system of the section *Capparis* indicate that at least one species from the section *C. spinosa* is an andromonoecious plant (Zhang & Tan, 2008).

Andromonoecy is a particular sexual system in which both perfect (hermaphrodite, bisexual) flowers are produced with male (staminate, female sterile) flowers on the same individual. This rare phenomenon is described only in 1.7% of Angiosperms (Yampolsky & Yampolsky, 1922; Diggle, 1991; Miller & Diggle, 2003). Our preliminary observations indicate that *C. herbacea* produces both hermaphrodite and staminate flowers on the same individual.

The aims of the present work were to determine morphological and morphometric differences between 2 flower morphs, study variations in the proportion of male and hermaphrodite flowers during the flowering season, and investigate whether andromonoecy occurs in *Capparis herbacea*. According to the classification of section *Capparis* L. revised by Inocencio et al. (2006), *C. herbacea* is regarded as *C. sicula* Veill. subsp. *herbacea* (Willd.) Inocencio, D. Rivera, Obón & Alcaraz. The number of stamens and the size and shape of the anther and nectary are regarded as important diagnostic characters. The species is known to be a nocturnal plant; however, factors affecting the time and duration of the blooming period have not yet been identified. Therefore, the second purpose of this study was to identify the characters mentioned in the studied

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plants and examine the influence of temperature, light, and pollination type on the floral longevity of *C. herbacea*.

2. Materials and methods

The study was conducted at the Tbilisi Botanical Garden and the adjacent Narikala Fortress, where a natural population of *Capparis herbacea* forms scattered shrubs on the dry hills and narrow clefts of stony walls. Most of the individuals grow on the steep vertical walls and are inaccessible for detailed observation. A total of 21 individuals, 702 flowers, 174 anthers, and gynophores were analysed in the study. Pollen grain diameter was measured for 200 grains per sexual type. Flowers were counted every 10 days during the flowering season from May to August 2012. Collected flowers were immediately measured under a stereomicroscope (Carl Zeiss DV4) equipped with a Canon PowerShot A580 digital photo camera. Pollen grains were transferred from the dehiscent anthers to glass slides with 2 drops of 1% eosin water solution. Temporary preparations were measured and microscopically examined with a light microscope (AxioVision, Zeiss, Germany). To determine the influence of temperature and light on the time and duration of blooming period, cut branches with both male and perfect flower buds were placed in containers of water under controlled air temperature and humidity. The effect of pollination type (self, geitonogamy, and xenogamy) on the occurrence of floral closure was also examined. Differences between the 2 flower morphs in gynophore length, anther

length, and number of stamens were statistically assessed using correlation analysis, independent samples t-test, and 1-way ANOVA using the SPSS 20 statistical package.

3. Results

Phenological observation. Vegetative growth started in mid April. Branchlet elongation continued throughout the summer season with a slower growing rhythm at the beginning of September when the last fruits were recorded. Flower buds developed from May to August, reaching the maximum amount before the onset of the warmest 10-day periods (Figure 1).

Flower morphology. Solitary flowers of 2 morphological types were observed in all studied plants. The first type is represented by perfect flowers with well developed stamens of different lengths, 5–7-cm long, green gynophore rising above the stamens. The second flower morph is represented by male flowers with long stamens and short, rudimentary yellowish gynophores (Figures 2a, b). The gynophore of the perfect flower is easily distinguishable at the bud stage (Figure 2c). We found that gynophore degeneration in male flowers begins at the latest stages of ontogenesis when floral organogenesis is almost completed. Thus, stem necrosis was observed in the distal part of the gynophore just under the ovary at the flower bud stage (Figure 2d). Both flower morphs were produced throughout the season and were intermixed on branches. Flower shape is typical for the section *Capparis*. In addition to the above-mentioned features, we found

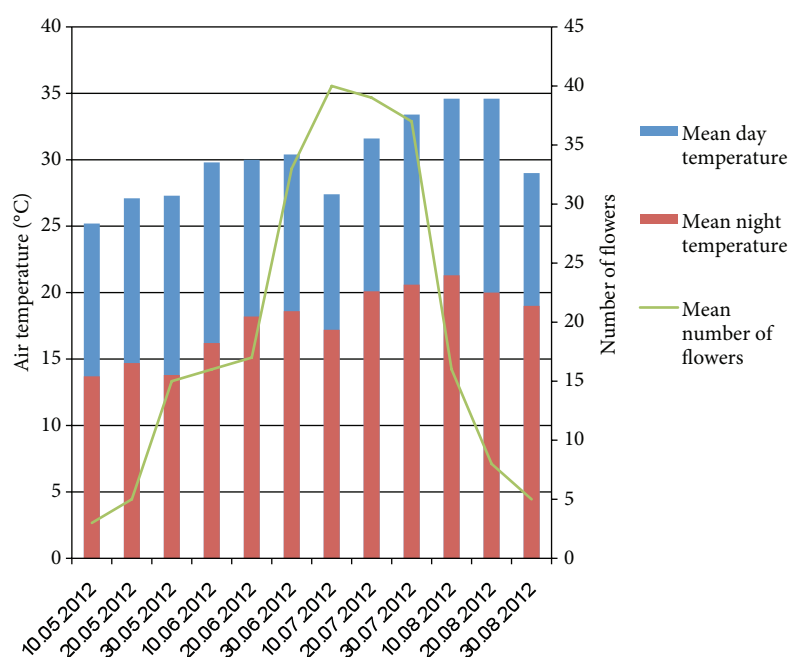


Figure 1. Seasonal variation in the number of simultaneously blooming flowers and air temperature (May–August).

that *C. herbacea* differs from *C. spinosa* by the occurrence of abundant trichomes on the inner surface of the small cavity formed by the connate petals surrounding the nectary (Figures 2e, f).

Morphological and morphometric analysis revealed that in all examined samples of *C. herbacea* growing at the Tbilisi Botanical Garden and Narikala Fortress, the number of stamens was <70, anther length was 3.5–4.2 mm, and the anther apex was rounded.

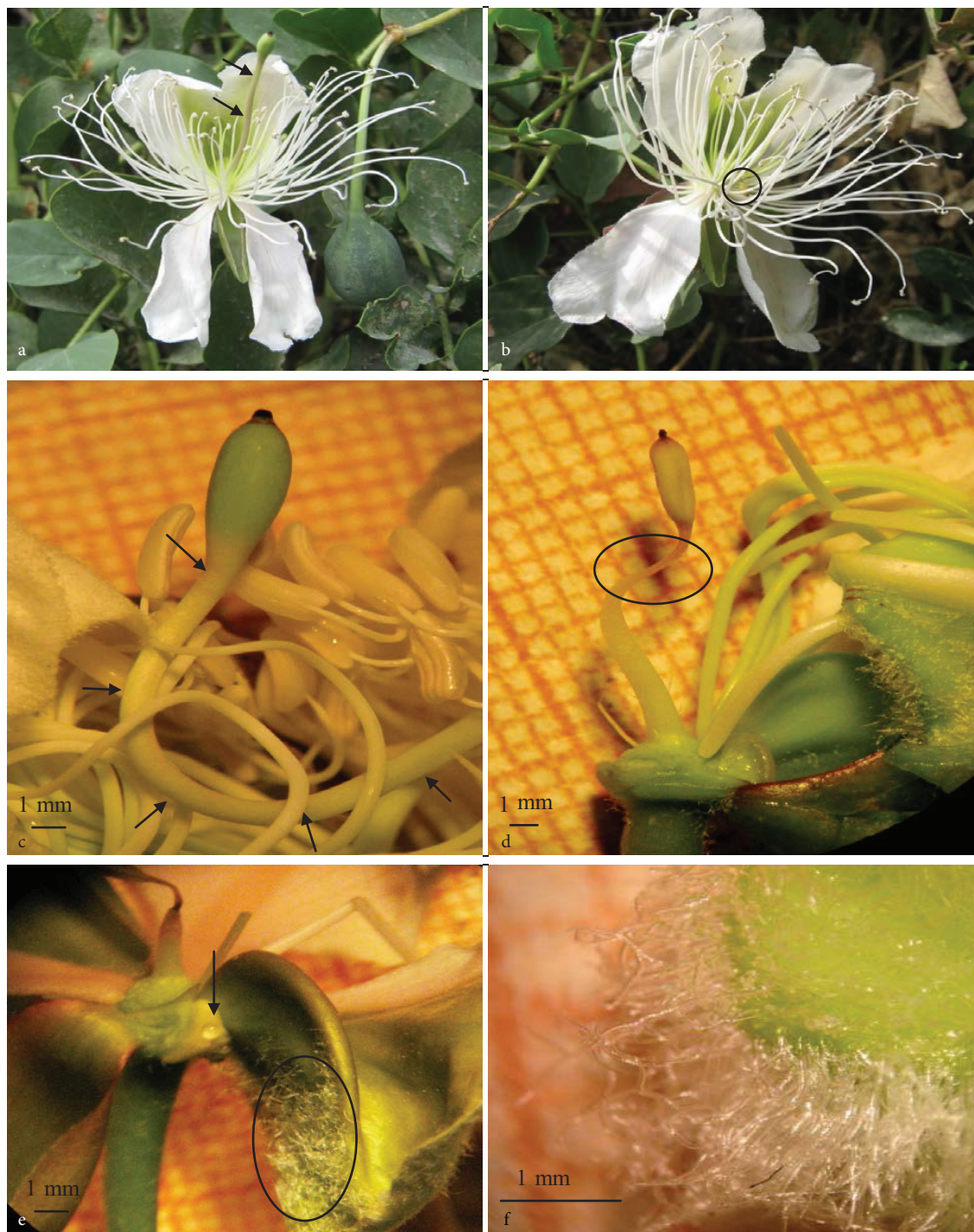


Figure 2. Floral dimorphism in *Capparis herbacea*. a - perfect flower with long gynophore (arrows); b - functionally male flower with underdeveloped yellowish gynophore (oval); c - green, long gynophore at the bud stage; d - underdeveloped gynophore of the male flower with degenerated distal part of the gynophore stem (oval); e - nectary with exudate drop (arrow) and abundant trichomes (oval) on the inner surface of connate petals; f - trichomes on high magnification.

The corolla consists of 2 white and 2 variegated petals. The green parts of the variegated petals are overlapped and fused with green, helmet-shaped sepals at the base of the floral disk. Stamens range in length from 1 to 6 cm. Each flower develops stamens with white and green filaments. As a rule, green ones are shorter, and they develop in the centripetal direction around the gynophore. The anthers are white before dehiscence. The timing of pollen release relates directly to the change of anther colour from white to light pink. Bithecal anther dehiscence is through longitudinal fissures (Figure 3a). Spherical pollen grains are about 30 to 40 μm in equatorial diameter in both flower morphs (Figure 3b).

The star-like nectary develops in the floral disk. It appears in the form of a 3-armed star; the upper arm narrower and more long than broad, and rounded basal arms (Figure 3c). The initially swollen nectary shrinks when nectar secretion stops (Figure 3d). As a rule, the nectary is odourless during the daytime even if the flower is open; however, it becomes functional at dusk, filling the air with a strong odour. It must be emphasised that the micropilar papilla of the gynophore forms the hole after successful pollen transfer (Figures 3e, f). We analysed the decadal ratio of male to perfect flowers during the flowering season. It ranges from 0.5 to 2.6, with the lowest values occurring at the beginning or end of flowering season and the highest values coinciding with the peak of simultaneous blooming in early July (Figure 4).

Usually, both male and hermaphrodite flowers open in the evening after sunset. Blooming is nocturnal and lasts 16–20 h, after which flowers start to wilt. However, night flowering is somehow conditional. Thus, in cloudy weather we observed flowers open during the daytime as well. We tested the relationships between these parameters. Under experimental conditions (i.e. controlled temperature and humidity with air temperature below 30 °C) flowers were open during the daytime. When the air temperature exceeded 30 °C all flowers were closed, imitating wilting. After lower temperature conditions were reintroduced, flowers reopened. In the case of artificial cross pollination (both xenogamy and geitonogamy), flowers wilted within the first 24 h. Self-pollinated hermaphrodite flowers stayed open for 2–3 days, after which they wilted, although the temperature was below 30 °C (Figure 5). In the male flowers, wilting started after anther dehiscence.

Flowers of *C. herbacea* are visited by various insects. The most frequent visitors are bees, ants, wasps, and moths. It seems likely that, for some butterflies, *C. herbacea* is a host species throughout the larvae stage. Our observations revealed that ants did not come into contact with the gynophore effectively. They only settled inside the cavity, tightly closing access to the nectaries for larger insects. Presumably, most of the insects, with the exception of the ants, are the potential pollinators. Usually pollinators land

on the platform formed by connate variegated petals just in front of the nectary, touching the filaments of the stamens. They provide pollen transfer from one flower to another by visiting a large number of flowers, without displaying any visual preference between the 2 different floral morphs. Our observations also show that *C. herbacea* is the host for some floral herbivore insects that eat the ovaries and young fruits. Thus, we observed butterfly larvae inside the maturing fruits and *Mantis religiosa* laying eggs on a receptacle. Preliminary results indicate that the number of damaged seeds is high.

Androecium morphometry. We measured the number of stamens and anther and gynophore length in male and perfect flowers (Table). ANOVA results detected significant ($P < 0.001$) differences in number of stamens ($F = 30.9$), anther length ($F = 246.4$), and gynophore length ($F = 2152.9$) between the male and perfect flowers groups. Gynophore length was negatively correlated with stamen number ($r = -0.414$) and anther length ($r = -0.785$) ($P < 0.01$).

4. Discussion

According to our results the sexual system of *Capparis herbacea* is andromonoecious. Measurements indicate that tested floral traits differ significantly in male and perfect flowers. The male flowers develop significantly more stamens and bigger anthers with rounded tips (63.6 ± 0.6 and 4.1 ± 0.01 mm, respectively) compared to bisexual ones (58.1 ± 0.7 and 3.6 ± 0.02 mm, respectively). To compare, in *C. sicula* subsp. *herbacea* the number of stamens varies from 100 to 150, mean anther size does not exceed 2.3–3 mm, and the anther apex is acutely sharp. The rounded anther apex is characteristic of *C. orientalis* Veill. and *C. aegyptia* Lam. These species are also included in the section *Capparis* and are distributed in Mediterranean Europe and North Africa (Inocencio et al., 2002; El-Ghani, 2013). However, other diagnostic characters (number of stamens, anther length, and shape of nectary) differ from those of *Capparis herbacea*. From our qualitative observations no pollinator preferences were noted between the 2 flower morphs. Between male and perfect flowers there was no difference in the shape and size of mature pollen grains from anthers with white and green filaments. The relationship between anther size and male sexual phenotype was also found in another species of section *Capparis*, the Mediterranean *C. spinosa*, in which male flowers have larger anthers with larger pollen grains (Zhang & Tan, 2009). According to these authors, the male flowers of *C. spinosa* function as pollen donors, promoting cross pollination. Similar observations have been made in other andromonoecious species (Cuevas & Polito, 2004). However, another study indicates that neither the resource savings hypothesis nor the increased pollen donation hypothesis explain the evolution and maintenance of



Figure 3. Functional activity of floral organs at pollination. a - anther dehiscence; b - mature pollen grains stained in eosin solution; c - swollen nectary; d - exudate (arrow) beneath the shrunken nectary; e - gynophore tip before pollination; f - open stigma at the moment of pollination.

andromonoecy in the case of *Solanum carolinense* L. (Vallejo-Marín & Rausher, 2007). Given that pollinators visit both flower morphs, and the morphology of pollen

grains is similar in male and perfect flowers, we assume that these data support the pollen donor hypothesis of male flowers in the case of *C. herbacea*. However, a special

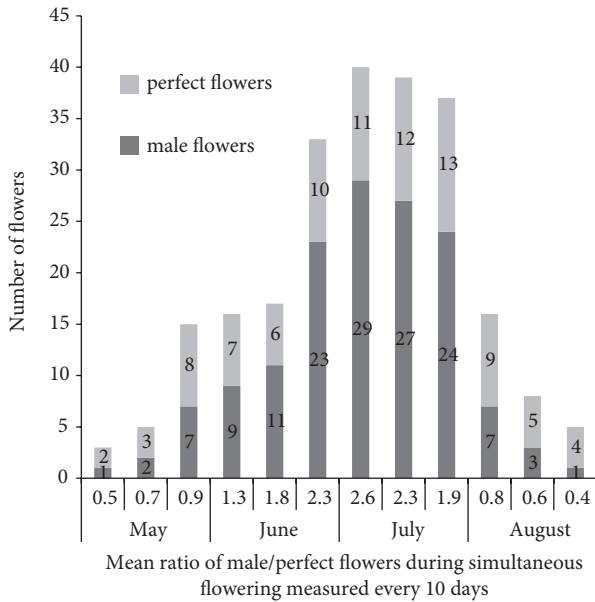


Figure 4. Sex expression dynamics of *C. herbacea* during the flowering season.

investigation of pollen viability in both flower morphs is necessary to prove our assumption.

Floral longevity is a substantial moment in plant reproduction (Bingham & Orthner, 1998; Ishii & Sakai, 2000). Floral behaviour of *Capparis herbacea* revealed different patterns of flower opening time and duration. Namely, the nocturnal blooming does not end with dawn; it can be prolonged up to 3 days under experimental conditions if temperatures are below 30 °C and/or cross pollination is not successful. Our observations reveal that morphology of the nectary changes from swollen to shrunken as the odorous secretion accumulates under the nectary at night. We suggest that these data reflect changes

in the functional activity of the nectary. The development of dense trichomes on the adaxial surface of the cavity made by the variegated connate petals just over the nectary is presumably adaptive and serves to protect nectary exudate from evaporation in a hot and dry environment, especially during the daytime. It seems that pollination time could be limited by several ecological factors, but not necessarily by the small group of nocturnal pollinators. Flowers wilted within 24 h after geitonogamous and xenogamous pollination. These observations suggest that there is no preference in cross pollination type. The development of gynophores longer than stamens in perfect flowers and observed protandry support our assumption regarding self-incompatibility. In the case of self-pollination, floral closure occurred much later (52–74 h after pollination). The maximal floral lifespan in *C. herbacea* does not exceed 3 days. However, if successful cross-pollination occurs the flower starts to wilt, thus minimising the costs of flower maintenance. The temporal closure of unpollinated flowers caused by rising air temperatures also reflects the plasticity of the floral lifespan. These facts support the assumption presented here, that the time and duration of the optional nocturnal flowering of *Capparis herbacea* reflect complicated interactions between flower internal rhythm (night odorous nectary) and exogenous ecological factors (temperature and effective pollination).

The fact that sexual dimorphism is phenotypically well-expressed in open flowers but quite inconspicuous at the early bud stage was an unexpected result. However, this can be explained as follows: genetically determined hermaphroditism of *Capparis herbacea* causes the initial development of bisexual floral primordia. Establishing functionally male or perfect sexual phenotypes may depend on environmental conditions or available resources to achieve reproductive success. There are various kinds of resource-dependent sex allocation, including age- or size-

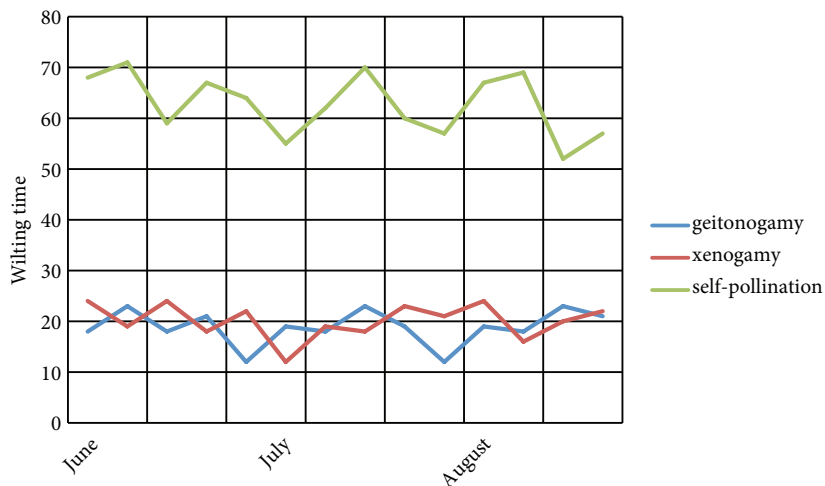


Figure 5. Relationship between pollination type and time of floral closure.

Table. Group statistics for floral traits in male and perfect flowers.

Floral traits	Sex	N	Mean	Std. error
Number of stamens	Male	87	63.60	0.641
	Perfect	88	58.18	0.731
Anther length	Male	87	4.092	0.0130
	Perfect	88	3.645	0.0252
Gynophore length	Male	87	1.4529	0.05663
	Perfect	88	5.9318	0.07798

dependent allocation (Cao & Kudo, 2008; Peruzzi et al., 2012). In the case of andromonoecious *Capparis herbacea*, male and hermaphrodite flowers develop simultaneously on the same branchlets of the same individual. The genetic mechanism underlying selective expression of male genes at the late stages of bud development in *Capparis herbacea* is still unknown. However, the study of sex-specific gene expression in andromonoecious poplar (Song et al., 2012) has shown that flower development is epigenetically regulated. We suggest that the development of a male or perfect flower from a bud with completed organogenesis (both stamens and gynophores, even ovules well developed at the bud stage) can be the result of the late epigenetic regulation of male/hermaphrodite gene expression. As a result, we cannot confidently exclude the effects of any factors affecting sex allocation.

The ratio of male to hermaphrodite flowers in *C. herbacea* is labile and reaches its highest levels in times of maximal simultaneous blooming, before the arrival of the hottest season in August. The tissue under attack by herbivores at this time plays a key role in the understanding of general plant responses in sex allocation patterns. Presumably, the response of *C. herbacea* to floral herbivory can be explained by the basic principles of allocating resources to the most profitable sex (Charnov, 1982). However, testing this hypothesis requires the study of individual sexual shifts under a controlled herbivory context.

Male flowers are favoured because pistils and their resulting fruit are costly to produce (Emms, 1993). However, andromonoecious species can optimise reproductive fitness by developing male and perfect flowers at the beginning of the reproductive stage when the supply rate of resources to maturing flower buds is high. For example, unpollinated treatments of andromonoecious plants lead

to an increase in the proportion of perfect flowers (May & Spears, 1988). The effect of flower occupancy on the foraging of flower visiting insects is expressed by damage to the perfect flowers and a shift in the sex ratio towards increasing the share of perfect flowers as fruits mature (Solomon, 1985).

Other studies showed that plants exposed to herbivory generally demonstrate a shift in sex expression (Hendrix & Trapp, 1981; Krupnick & Weis, 1999). Our preliminary observations indicate that a shift in the male biased sex ratio toward bisexual flowers (first 10 days of August) coincides with fruit development and visible damage caused by florivory and leaf herbivory (Figure 12). Based on the few cases analysed, we assume that the intensity of herbivory may influence the level of sex expression in *C. herbacea*. However, a special study must be conducted to prove our speculations.

In conclusion, the present study is a first attempt to describe the existence of an andromonoecious sexual system in *Capparis herbacea*. According to the data obtained, this species exhibits a labile floral sex ratio. Plant response to external stress (high temperatures and fruit damage caused by herbivory) is expressed in accordance with the principles of allocating resources to the most profitable sex in order to achieve reproductive success. In our opinion, the male-biased sex ratio at the beginning of flowering season may be due to the need for pollen overproduction to ensure successful pollination during the short period of individual flowering, which usually finishes within 24 h. With each successfully fertilised perfect flower resources are diverted toward seed maturation. The plant will continue to produce bisexual flowers until fruit set reaches a level high enough to inhibit flower development (July–August). Pollen limitation to the plant or damage to reproductive structures may lead to this altered sex ratio.

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