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Preliminary observations on the reproductive strategies in five early-flowering species of *Gagea* Salisb. (*Liliaceae*)

Abstract

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The field and laboratory studies about *Gagea bohemica* (Zauschn.) Schult. & Schult. f., *G. chrysantha* Schult. & Schult. f. s. l., *G. granatellii* (Parl.) Parl., G. lutea (L.) Ker-Gawl., *G. fragifera* (Vill.) Ehr. Bayer & G. Lòpez are carried on community structure, phenology and reproductive biology. The studied species show differences in terms of community characteristics (species composition, structure), time and duration of flowering, and seed production. The species of rocky environment (*G. granatellii* and *G. bohemica*) show the highest number of vegetative individuals with respect to the flowering ones, the shortest duration of flowering and the lowest seed-set. It appears as a consequence of the low resource availability. The species of meadows (*G. chrysantha*, *G. fragifera* and *G. lutea*) appear instead to suffer overall the biological competition for resources and pollinators. Gross morphological analysis of the pollen grains revealed that deformed pollen is more abundant in triploid (*G. chrysantha, G. granatellii*) and heptaploid (*G. fragifera*) species, compared to the tetraploid (*G. bohemica*) and the hexaploid (*G. lutea*) ones. Seed production was very low. There is a positive correlation between amount of morphologically regular pollen and number of produced seeds. In most of the studied species we observed a trend to increase the seed production in individuals with many flowers; probably this is the result of a strategy aimed diminishing the effects of pollen- and pollinator-limitation.

Introduction

The species of the genus *Gagea* Salisb. appear typically insect-pollinated plants, which offer nectar (Orueta & Viejo 1996) and/or pollen (Petanidou & Vokou 1990) as reward. In terms of sexual reproduction, the performance of these plants is affected by their early flowering and probably by intrinsic factors (i.e. ploidy levels), which can reduce the sexual efficiency and favor the vegetative propagation. Early flowering plants have a short suitable period for their reproductive efforts. Indeed, in woodland areas, the extent of this period goes from the first prolonged phase of weather suitable for pollinators activity to the time of the canopy closure (Schemske & al. 1978). The rigidly preset time suitable for reproduction can produce in the flowering of these plants the strong seasonality observed in Mediterranean communities (Petanidou & al. 1995). Moreover, early flowering plants are often subject to unpredictable pollinator activity and to problems of pollen limitation. For these reasons, they adopted strategies for the reproduction enhancement, as for instance the increase of the number of flowers which sequentially occur for each inflorescence (Harder & Thomson 1989; Nishikawa & Kudo 1995; Nishikawa 1998). Another aspect influencing the reproduction success in early flowering plants is the resource availability, overall in order to promote or suppress flowering. Consequently, these plants balance the resource allocation among the different phases of their life-cycle (Dafni & al. 1981), eventually varying from year to year their flowering rates.

Considering the special scientific interest of early flowering plants, we studied the behavior of five *Gagea* species in Calabria (S Italy), which in that region typically occur in small and isolated populations (Peruzzi & Gargano 2005). We attempted to understand the possible link between ecological conditions and population dynamics. In this way, we used also a community level approach to estimate the effects of pollen and resource competition on the reproductive strategies of the studied species.

Here are presented the preliminary results on community structure, phenology and reproductive biology research of *G. bohemica*, *G. chrysantha*, *G. fragifera*, *G. granatellii* and *G. lutea*.

Material and Methods

This study was carried in four localities, whose data were recorded using a G.P.S. The population of *G. bohemica* occurs on calcareous rocky grounds at 1010 m asl (loc. Ospedaletto, Campotenese, province of Cosenza); *G. chrysantha* population is in a *Quercus pubescens* open maquis at 225 m asl (loc. Salerno, Montalto Uffugo, province of Cosenza); *G. granatellii* population occurs along the rocky slope of a river valley at 603 m asl (loc. Giancorella, Rose, province of Cosenza), and finally, at 1283 m asl, in meadows surrounded by beech woods, there are the populations of *G. fragifera* and *G. lutea* (loc. Piano di Mezzo, Masistri, province of Cosenza).

We counted the number of flowering and vegetative individuals of *Gagea* species in plots of 1 m^2, and also identified all the other species recorded within the sample areas and counted their individuals. These data were used to characterize communities and to evaluate their structure by means of Shannon's Index H, a widely used diversity index which takes into account both the number of species and their relative abundances (Shannon 1948; Pielou 1966, 1966a). We checked the flowering of *Gagea* species and other species weekly by counting the buds, flowers and fruits. The plants size and the diameter of open flowers were measured by digital caliper.

The evaluation of fitness in experimental individuals of *Gagea* (9 for each species) was maid by ovule/seed ratio. We related data on reproductive fitness to some morphological aspect (plant size, number and diameter of flowers), analyzed pollen grains and calculated a morphologically regular/deformed pollen ratio, and this parameter was also related to reproductive efficiency and plant morphology. The evaluation of relationships among the different parameters was maid by Pearson's correlation test.

We performed flower manipulations on some individuals for each species, aimed to test the effects of induced cross- and self- pollination. For individual isolation, in order to exclude pollinators and to verify spontaneous self-pollination, we used the cages with fine nylon mesh. Finally, we emasculated and isolated some individuals to test the occurrence of agamospermy.

Results and discussion

Community characterization

G. chrysantha community occurs in Mediterranean belt and it is dominated by early-flowering herbs, i.e. *Scleranthus annuus* L., *Cerastium glomeratum* Thuill., *Sherardia arvensis* L., *Trifolium pratense* L. Data regarding the three plots with *G. chrysantha* are reported in Table 1. There is a substantial variation in the ratio between reproductive and vegetative individuals of *Gagea* among the three plots. Also the *H* value is variable among the sample areas and this indicates differences in terms of number of species and contribution of each species to the total number of community individuals.

G. granatellii occurs also under Mediterranean condition, but on rocky and temporarily wet ground, with many bryophytes and few vascular plants, such as *Theligonum cynocrambe* L., *Sedum hispanicum* L. and *Euphorbia helioscopia* L. The structural characteristics of these communities are summarized in Table 1, which shows also the increasing young *Gagea* individuals percentage at the lowest values of *H*.

G. bohemica grows on rocky substrate. Its community is characterized by other geophytes and some species typical of rocky grasslands, i.e. *Muscari neglectum* Guss., *Allium* sp., *Orchis* sp., *Sesleria calabrica* (Deyl) Di Pietro. We recorded the highest rate of flowering *Gagea* individuals in the plot c, where the low *H* value results from the few species occurring and from the *Gagea* dominance, whose individuals represent more than 79% of the total (Tab. 1).

G. fragifera and *Gagea lutea* occur nearly together in mesophylous grasslands of mountain belt. Their communities are characterized by nitrophylous species, such as *Stellaria media* (L.) Vill., *Urtica urens* L., *Cruciata laevipes* Opiz, *Capsella rubella* Reuter and some grasses (*Poa annua* L., *Milium effusum* L.). Table 1 shows the data related to the plots placed within these communities. The low *H* values appear due to the grasses dominance and we recorded few *Gagea* individuals in the sample areas.

Table 1. Main characters recorded from the plots placed within the communities including *Gagea* populations.

Gagea	*bohemica*			*chrysantha*			*fragifera / lutea*			*granatelli*	
Plot	a	b	c	a	b	c	a	b	c	a	b
N° of species	10	28	6	12	21	32	12	12	15	17	13
Total of individuals	96	194	63	169	233	381	296	437	307	618	374
H	1.88	2.77	0.78	2.03	1.99	2.86	1.40	1.90	1.46	1.27	1.70
N° repr. *Gagea*	0	5	20	1	6	15	3	9	1	8	3
N° veg. *Gagea*	17	45	30	9	6	7	2	24	1	128	46

Table 2. The communities including *Gagea* populations: a) mean *H* value, b) number of co-flowering species.

Community with	a	b
G. bohemica	1.81	3
G. chrysantha	2.29	9
G. fragifera/G. lutea	1.59	8
G. granatelli	1.49	6

Two species groups can be identified, according to their habitats: a first one which live in meadow communities (type A: *G. chrysantha*, *G. fragifera* and *G. lutea*) and a second one living in rocky environments (type B: *G. granatellii* and *G. bohemica*). In both types it is possible to trace a further distinction. Indeed, within type A, *G. chrysantha* occurs – as early element – in secondary open spaces of Mediterranean woodland, i.e. shrubbylands and garigues. On the contrary, both *G. fragifera* and *G. lutea* are included within mesophylous meadows which, according to Maiorca & Spampinato (1999), belong to the *Molinio – Arrhenatheretea*. The latter community probably originates from beech woods fragmentation. As far B type species are concerned, *G. granatellii* appears related to ruderal communities typical of shaded and seasonally wet soils, that Brullo & al. (2001) include in the *Stellarietea mediae*; while *G. bohemica* prefers sites strongly exposed like mountain ridges within *Sesleria calabrica* communities, referable to the *Festuco – Brometea* pastures (Biondi & al. 1988; Abbate & al. 1994; Maiorca & Spampinato 1999).

Comparative phenology

The large overlapping between the phenology of *G. chrysantha* and *G. granatellii* (species occurring in the same climatic belt, but in different habitats) could be seen on Figure 1. The flowering peaks are from the beginning to the half of March. *G. bohemica*, shows the maximum flowering at the beginning of April. Both *G. fragifera* and *G. lutea*, instead, show their flowering peaks at the end of April.

The flowering period is between twenty (*G. granatellii*) and thirthy-five (*G. chrysantha*) days. The large overlapping between flowering times of the species of Mediterranean belt – *G. chrysantha* and *G. granatellii* – can be explained by the necessity to bloom as soon as suitable temperatures permit and before canopy closure (Schemske & al. 1978).

The results of comparison of the flowering phenologies in five species of *Gagea* respect the other flowering species recorded in their communities is shown on Figure 2.

G. chrysantha shows an intermediate flowering pattern comparing with those of other species in its own community. In fact, its blooming is placed between the earliest species (*Romulea bulbocodium* Sebast. & Mauri, *Senecio vulgaris* L., *Cardamine hirsuta* L., *Erophila verna* (L.) Chevall.) and others (*Erica arborea* L., *Cerastium glomeratum* Thuill., *Sherardia arvensis* L., *Geranium molle* L.). As a consequence when *G. chrysantha* is at the blooming

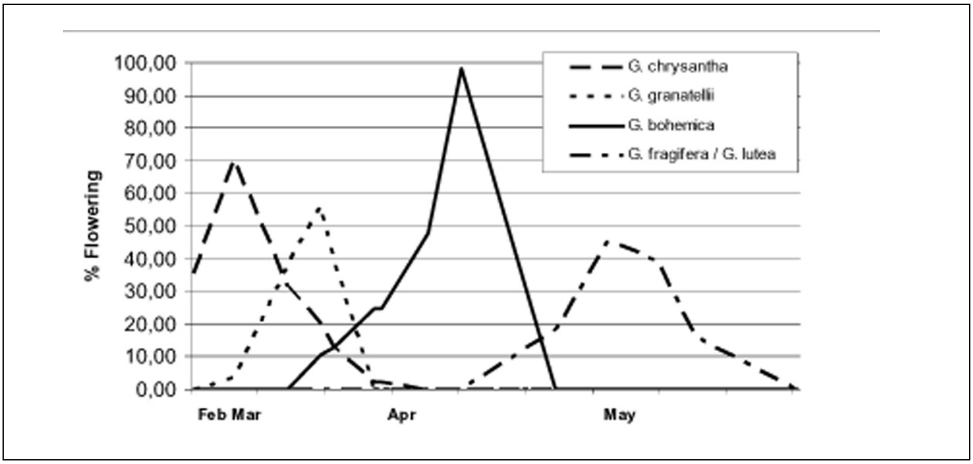

Fig. 1. Comparative flowering phenology of the studied *Gagea* species.

peak its flowers make 31.5% of total flowring, while of *Senecio vulgaris* represent 45%.

The flowering of *G. granatellii* follows *Romulea bulbocodium* and *Cardamine hirsuta*; at maximum flowering, *Gagea* represents 48.4% of the total amount of open flowers. Co-flowering species are *Euphorbia helioscopia* (29%), *Veronica cymbalaria* Bodard (12.9%) and *Vicia sativa* L. var. *angustifolia* (L.) H. T. Ho (6.5%).

G. bohemica is the earliest flowering species within its own community; indeed, other plants (*Senecio vulgaris*, *Saxifraga tridactylites* L., *Muscari neglectum*) start to flower only after the peak of *G. bohemica*.

Finally, *G. fragifera* and *G. lutea* flower together with other species such as *Ranunculus ficaria* L. and *Scilla bifolia* L.; when *G. fragifera* and *G. lutea* reach the blooming peak, their flowers are 81.3% of the total. The decrease of *Gagea* blooming is instead accompanied by the beginning of flowering for *Capsella rubella*, *Cruciata laevipes*, *Stellaria media* and *Taraxacum officinale* Weber.

Two different patterns resulted from the phenology of the studied species. The first one includes *G. chrysantha* and *G. fragifera* / *G. lutea*; it is characterized by a flowering time of thirty-four/thirty-five days and by similar times of blooming increase and decrease. These species occur in sites with good moisture availability but possibly high competition for resources and pollinators, as shown by *H* values and by the high number of co-flowering species (Tab. 2). This is true even if *G. fragifera* and *G. lutea* communities have lower *H* values than those with *G. chrysantha*, because this is a consequence of grasses dominance which reduces the diversity but not the resource competition. Two species with a very similar floral model (both with yellow, stellate flowers/inflorescences) are blooming together with *G. fragifera* and *G. lutea*: *Ranunculus ficaria* and *Taraxacum officinale* group (sect. *Ruderalia*)

The second one includes *G. granatellii* and *G. bohemica*; it shows a flowering time of twenty/twenty-four days and a faster blooming decrease phase. The habitat of these species has low and quickly decreasing resources (because of the drying), so producing a shorter

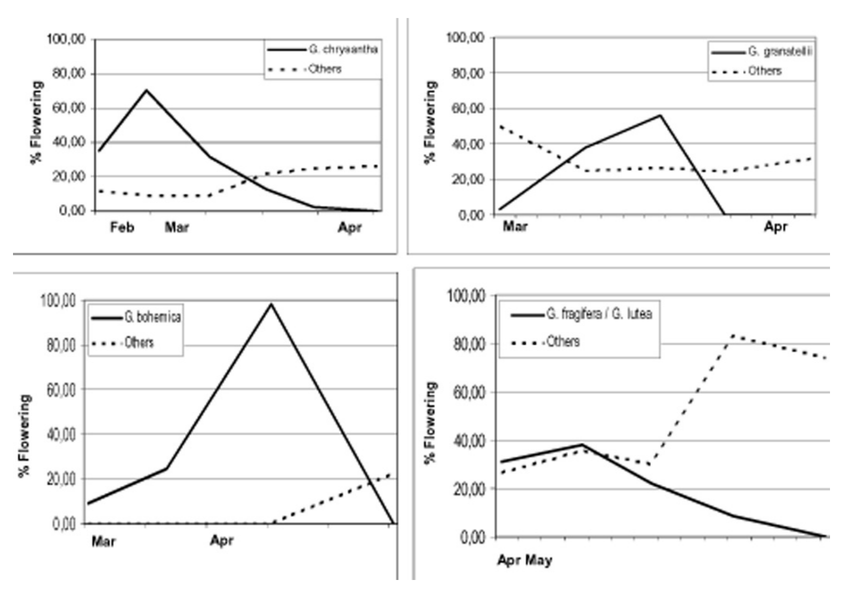

Fig. 2. Comparative flowering phenology among the species of *Gagea* and the other flowering species occurring in their communities.

suitable time for flowering, low *H* values and low number of co-flowering species (Tab. 2). Moreover, in this case, all the co-flowering species show different floral models respect to *Gagea*, i.e. different flower color, shape and size.

We believe that the two patterns result from ecological characteristics of the biotopes and communities in which the species occur. Indeed, it is proved as moisture availability (Dafni & al. 1981; Bell & Stephens 1984; Zimmerman & al 1989; Friedel & al. 1993) and interspecific competition for resources and pollinators (Ratchke 1983; Bawa 1983; Waser 1983) can affect the blooming patterns.

Reproductive performance

Considering morphological aspects, Table 3 shows the results of correlation tests among size of plants, number of flowers and diameter of flowers. All species show a number of flowers increasing with plant size (in *G. lutea* and *G. bohemica* this trend is less evident than in the others). The flower size is negatively related to plant size in *G. lutea* and *G. chrysantha*, while this relation is positive in *G. fragifera*, *G. bohemica* and, less evidently, also in *G. granatellii*. As far the correlation between flower size and number is concerned, Table 3 shows a clear positive relation in *G. lutea* and *G. granatellii*, in *G. fragifera* this relation is not evident, while the trend becomes negative in *G. bohemica* and *G. chrysantha*.

Table 4 shows the results from pollen observation and seed-set. The ratio between morphologically regular and deformed pollen is the lowest in *G. granatellii*, while it reaches

Table 3. Values of Pearson's coefficient related to the following correlation tests: a) plant size - number of flowers, b) plant size - flowers diameter, c) flowers diameter - number of flowers.

Species	a	b	c
G. bohemica	0.16	0.58	-0.09
G. chrysantha	0.43	-0.21	-0.10
G. fragifera	0.67	0.24	0.18
G. granatelli	0.51	0.12	0.49
G. lutea	0.16	-0.30	0.73

Table 4. Pollen features and seed production: a) morphologically regular/deformed pollen ratio, b) seed/ovule ratio.

Species	a	b
G. bohemica	3.98	0.003
G. chrysantha	2.46	0.019
G. fragifera	1.51	0.009
G. granatelli	0.21	0.000
G. lutea	8.38	0.028

the maximum value in *G. lutea*. The amount of deformed pollen seems to be related to the ploidy level: 3*x* (*G. chrysantha*, *G. granatellii*) and 7*x* (*G. fragifera*) species show pollen more deformed than 4*x* (*G. bohemica*) and 6*x* (*G. lutea*) ones (for information on the ploidy levels of the studied populations cfr. Peruzzi, 2003; Peruzzi & Aquaro, 2005). Seed set is very low in all the species and totally absent in *G. granatellii* (Tab. 4). Although the low seed set, there is a clear positive correlation (P = 0.76) between the amount of morphologically regular pollen and the number of seeds produced. Table 5 summarizes the results originated from correlations among seed production and morphological parameters in each species; for *G. chrysantha* seed-set is negatively related to all the considered morphological traits. In other species, the amount of produced seeds is positively linked to the number of flowers and (weakly) to the size of flowers.

The flower manipulations, aimed to test effects of spontaneous or provocated self- and cross-pollination and occurrence of agamospermy, did not produce seed-set. This occur-

Table 5. Percentage of plants producing seeds - a, Pearson's coefficients for correlations between seed production and: b) plant size, c) number of flowers, d) diameter of flowers.

Species	a	b	c	d
G. chrysantha	55.56	-0.60	-0.17	-0.22
G. granatelli	0.00	//	//	//
G. bohemica	25.00	-0.03	0.85	0.16
G. lutea	37.50	0.19	0.75	0.40
G. fragifera	35.71	0.45	0.44	0.24

rence may be due to the low sexual fitness of the studied populations, at least at the time of our study. Indeed, it is well known as sexual efficiency may vary among different *Gagea* species and/or populations either for weather conditions at flowering time (Orueta & Viejo 1996; Kudo & al. 2004) or the ploidy level (Guerlesquin 1965; Heyn & Dafni 1971; Zarrei & Zarre 2005).

The seed production in the studied populations seems to be affected by a strong pollen limitation which can be related to the severe condition which limit pollinators activity in the early flowering plants (Schemske & al. 1978). Considering the absence of seed production also in the hand-pollinated plants, we think that the pollen limitation was also accompanied by a strong resource limitation. This was more evident in the two species of rocky environment, in which the lowest seed-production can be referred to the more unstable habitat (Dafni & al. 1981). Regarding to the increase of seed production observed in the plants with many flowers, overall in the species of meadows (but for *G. chrysantha*), this occurrence agrees with Harder & Thomson (1989), which consider the extension of anthesis by sequential flowering as a strategy for promote pollination success under pollinator-limited condition, although in the later flowers the seed production can decrease with respect to the early ones of the same plant (Nishikawa 1998).

Conclusions

A single year of observation is not sufficient to generalize the discussed results, because climatic variation from year to year can produce differences in flowering times (Schemske & al. 1978) and these variations in the springtime can also strongly affect the efficiency of sexual reproduction (Kudo & al. 2004). However, our preliminary data suggest the effects of extrinsic ecological factors (i.e. climatic condition, stand characteristics and community features) and intrinsic ones (morphology, ploidy level) as affecting the results of the reproductive efforts in *Gagea* species.

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