# Does flowering synchrony contribute to the sustainment of dry grassland biodiversity?

Edy Fantinato [a,*], Silvia Del Vecchio [a], Antonio Slaviero [a], Luisa Conti [b], Alicia Teresa Rosario Acosta [b], Gabriella Buffa [a]

[a] Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy
[b] Department of Sciences, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy

A B S T R A C T

Phenological relationships among entomophilous species for pollination may play an important role in structuring natural plant communities.

The main aim of this work was to test whether in dry grassland communities there is a non-random flowering pattern and if the pattern influences the species richness, and the richness of subordinate and common species.

Field sampling was carried out in temperate dry grasslands in NE Italy. Species composition and the flowering phenology were monitored in 45 2 m × 2 m plots randomly placed over dry grasslands.

To quantify the degree to which insect-pollinated species overlap in their flowering time we developed a "co-flowering index" (CF-index). The significance of the observed flowering pattern was tested using a null model.

A positive correlation was found between the synchronic flowering and the number of subordinate species. Subordinate species showed shorter flowering length than the common species and a mostly specialized pollination system.

Our findings suggest that flowering synchrony might be a key characteristic which may contribute to shape dry grassland composition by favouring the long lasting maintenance of rare species populations within the community.

The comprehension of such functional relationships between species of different trophic levels is of great importance for the conservation of dry grasslands and the maintenance of the ecosystem services that pollination provides.

## 1. Introduction

Biotic interactions have long been investigated as a possible mechanism governing and maintaining species richness within a community (Brooker et al., 2008; Bonanomi et al., 2011; Hallett et al., 2014). Among the wide variety of biotic interactions, phenological relationships among entomophilous species for pollination have been hypothesized as playing an important role in structuring natural plant communities (Feldman et al., 2004; Moeller, 2004; Ghazoul, 2006; Callaway, 2007; Brooker et al., 2008). Pollinators are assumed to exert comparable selection pressures, thus generating correlations among floral traits (e.g., color, long and narrow corolla tubes, or particular nectar quantities and concentrations) (Armbruster et al., 1999, 2000; Fenster et al., 2004). Although generalist pollination systems are frequent on a global scale, also specialization is common and is assumed to have been integral to angiosperm diversification (Johnson and Steiner, 2000). Most angiosperms produce recognizable suites of convergent floral traits and reward sources that recur in flowers of different evolutionary origin but that share similar pollinators (Rodríguez-Gironés and Santamaría, 2004; Thomson and Wilson, 2008; Willmer, 2011; Sonkoly et al., 2016). In this way, plant-plant relationships may have been modified in order to ensure and/or enhance the visitation rate, eventually influencing community structure and dynamics. Especially, the sharing of the same temporal niche (synchronous flowering periods) by different plant species increases the abundance of floral resources. This in turn could enhance the possibility

* Corresponding author.
E-mail addresses: edy.fantinato@unive.it (E. Fantinato), silvia.delvecchio@unive.it (S. Del Vecchio), antonio.slaviero@unive.it (A. Slaviero), luisa.conti@gmail.com (L. Conti), aliciateresarosario.acosta@uniroma3.it (A.T.R. Acosta), gabriella.buffa@unive.it (G. Buffa).
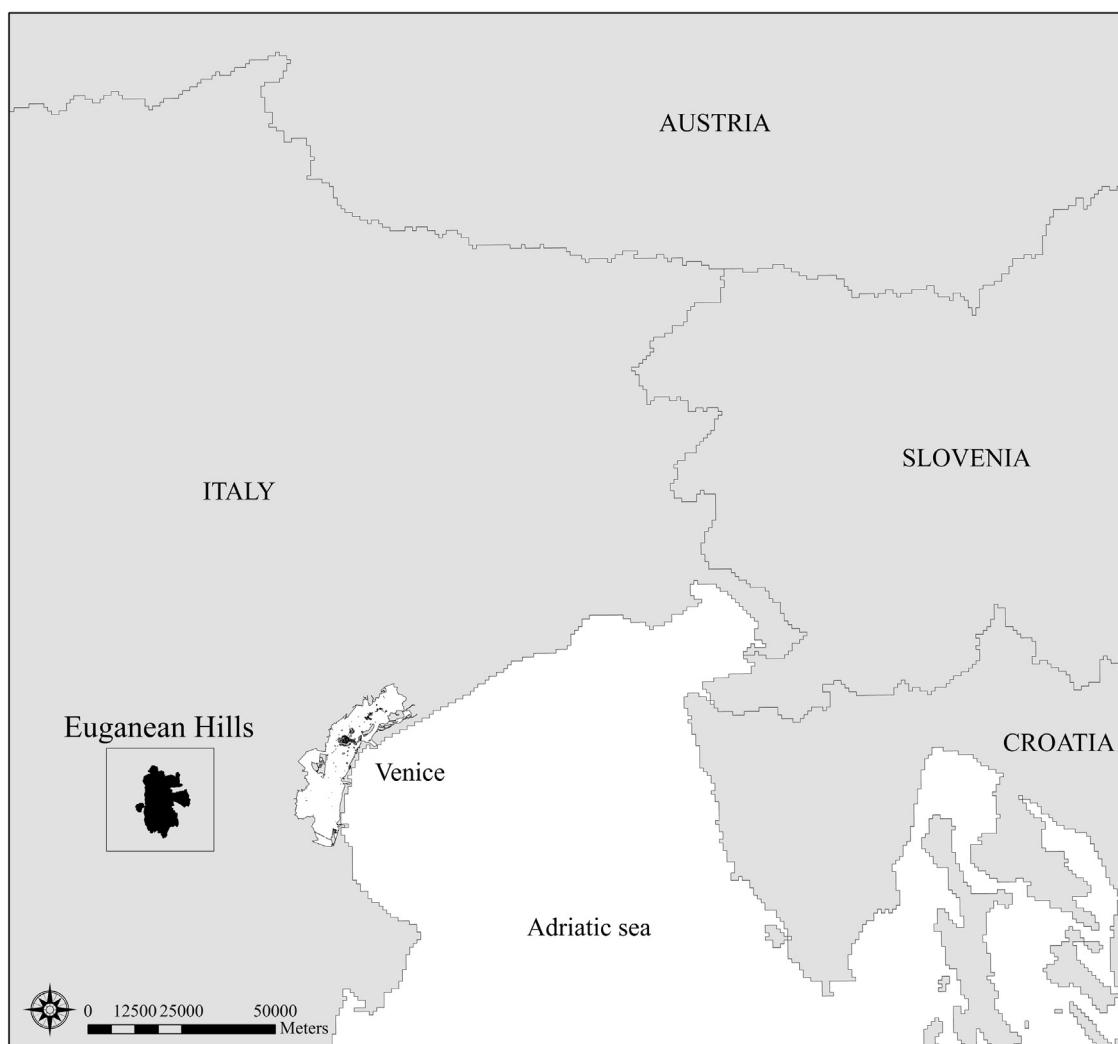
**Fig. 1.** Study area. Euganean Hills are enclosed within the black square.

of pollinator sharing and ensure the survival of rare and extremely selective entomophilous species (Laverty, 1992; Johnson et al., 2003; Ghazoul, 2006).

The phenological synchronization of flowering and fruiting is supposed to affect inter-specific patterns at several scales, from population to community and landscape (Frankie et al., 1974; Ollerton and Lack, 1992, 1998; Bronstein, 1995; Vilela et al., 2014; Borchert et al., 2005). For example, asynchronous ripening of fruits among certain species in a community was interpreted as a mechanism for avoiding competition for seed dispersers (Wheelwright, 1985). Conversely, flowering synchrony of individuals in a given population has been proved to influence both the quantity and genetic quality of their offspring, by affecting the number of potential mates and the foraging efficiency of pollinators (Schemske, 1977). At a different scale of observation, Dante et al. (2013) revealed how relationships among co-flowering entomophilous species drive species assemblage and distribution within an old-field plant community, highlighting the importance of pollination interactions in influencing ecosystem structure and functioning.

During the last decade, there has been a consensus in ecological and environmental sciences about the interdependence between species richness and ecosystem functioning (Sachs et al., 2009; Hooper et al., 2005), although with contrasting views. Schwartz et al. (2000) proposed that this relationship generally follows an asymptotic trend, suggesting that only few species are needed to sustain most processes and functions. Accordingly, increased attention was paid to processes involving relatively dominant species (Schwartz et al., 2000; Lawler et al., 2001; Cardinale et al., 2006). Recently, however, several authors have emphasized the role of the whole diversity, comprising less common species, in assuring ecosystem stability and functioning across time (Allan et al., 2011; Mouillot et al., 2013).

According to Tilman (2004), the abundance of a species is proportional to the amount of the habitat that has the environmental conditions that match the species requirements. Thus, less common species are those better adapted to less common environmental conditions in a habitat and are likely to possess functional traits distinct from those of common species. Indeed, rare or subordinate species have been recognized as increasing both species richness (Wellstein et al., 2014) and the functional diversity of communities (Richardson et al., 2012); they are also expected to support ecosystem functioning under future environmental conditions (Mouillot et al., 2013). At the same time, in the majority of ecological communities, rare species are expected to be at higher risk of extinction than dominant ones (Purvis et al., 2000). Therefore, the identification of patterns related to the maintenance of species diversity and, in particular, of the rare or subordinate species in ecological communities should be mandatory when investigating ecosystem functioning (Allan et al., 2011; Mouillot et al., 2013).

European temperate calcareous dry grasslands provide a particularly suitable example to this specific issue. Their biological diversity is high and includes a variety of rare and endangered species from different taxonomic groups (Van Helsdingen et al., 1996; Ssymank et al., 1998). They are among the most diverse plant communities of the world at small spatial scales, mirroring intricate coexistence mechanisms (Wellstein et al., 2014; Dengler et al., 2014). A high species richness is also found for butterflies and other invertebrates (Bobbink and Willems, 1988; Van Swaay, 2002). In Europe, semi-natural dry grassland make up the majority of High Nature Value farmland and harbor the majority of EU farmland biodiversity (Collins, 2012; Habel et al., 2013). Given their importance they are listed in Annex I of the EU Habitat Directive (CE 43/92) as priority habitats, i.e. "natural habitat types in danger of disappearance".

In light of these considerations, this work's main aim was to analyze flowering synchrony among entomophilous species in temperate dry grassland communities and to elucidate the relationship between the flowering pattern and the structure of the community, in terms of rare and common species.

Specifically, we addressed the following questions (i) is there a non-random flowering pattern among entomophilous species in dry grassland communities? (ii) Does the flowering pattern influence the total species richness and the relative richness of subordinate and common species at community level? And (iii) do subordinate and common plant species show different patterns in the flowering time length and in the pollination system (i.e. generalized vs. specialized).

## 2. Material and methods

### 2.1. Study area and site selection

Field sampling was carried out in the Euganean Hills (NE Italy), a group of volcanic hills extending for 15096 ha (Fig. 1; pictures in Supplementary material). The area is a historically cultivated landscape with a mosaic of arable fields, semi-natural grasslands and deciduous forests. The site has been under protection since 1989 and was recently included in the Natura 2000 network as SCI/SPA. Bioclimatic classification, performed according to the Worldwide Bioclimatic Classification System (http://www.globalbioclimatics.org/), shows a Temperate-Oceanic type.

The study was conducted on semi-natural, oligo-to mesotrophic, *Bromus erectus*-dominated dry grasslands. They establish on poorly developed, shallow and skeletal, calcareous soils characterized by low water retention capacity and high leaching rates (Bini, 2001). The community structure is mainly determined by hemicryptophytes and, subordinately, chamaephytes. The floristic composition of the Euganean dry grasslands reflects their particular geographic position, in a transitional connection area among the Mediterranean, the Alpine and the South-Eastern European phytogeographical regions (Villani et al., 2016). The vegetation is dominated by few, highly covering, anemophilous species (e.g. *B. erectus*, *Artemisia alba*, *Catapodium rigidum*, *Koeleria pyramidata*, *Carex hallerana*, *Bothriochloa ischaemum*) and numerous, scarcely covering, entomophilous species, the most common of which include *Bupleurum gussonei*, *Scabiosa triandra*, *Fumana procumbens*, *Helianthemum nummularium* ssp. *obscurum*, *Convolvulus cantabrica* and *Globularia bisnagarica*. Based on recent syntaxonomic revision (Terzi, 2015), Euganean dry grasslands can be included in the *Festuco-Brometea* Br.-Bl. & Tx. ex Klika & Hadač 1944 class and the SE-European-Illyrian order *Scorzoneretalia villosae* Kovačević 1959 (=*Scorzonero-Chrysopogonetalia*), represented by several diagnostic species, with high frequency values (*Chrysopogon gryllus*,

*Dorycnium pentaphyllum* ssp. *herbaceum*, *Eryngium amethystinum*, *Salvia pratensis* ssp. *bertolonii*, *Sanguisorba minor*, *S. triandra*, etc.). Given the high percentage of Mediterranean and Illyrian taxa and the phytogeographical and ecological context, the community can be referred to the alliance *Saturejion subspicatae* Tomić-Stanković 1970.

Originally, sampled grasslands had been managed by low-intensive mowing practices. Since the foundation of the Park and the SPA/SCI management plan, they have been subject to yearly mowing and grazed every three years.

### 2.2. Data collection

We selected 15 dry grassland areas extending from 0.27 ha to 6.95 ha (ranging from 68 m a.s.l. to 284 m a.s.l.). Altogether 45 plots of $2\,m \times 2\,m$ ($4\,m^2$) were selected using a stratified random sampling design. For each plot, species composition was recorded and flowering phenology of all entomophilous species was monitored every ten days (1st April–26th September in 2015) for a total of eighteen monitoring intervals. Flowering was considered started when the first flower was observed to be open (Pleasants, 1980) and ended when individual plants no longer possessed any flower with anthers (Dante et al., 2013). All species surveyed in the plots flowered within the monitoring period and no differences in flowering time were observed among individuals of the same species occurring in different plots. Species nomenclature was standardized following Conti et al. (2005).

Plant traits have been widely used to detect general ecological patterns irrespective of species identity (Lavorel and Garnier, 2002; Díaz et al., 2004). When applied to floral characters (e.g. position of the nectar in the flower, flower shape, floral symmetry, flower openness and accessibility of floral rewards), floral traits combinations allow the identification of functional groups of flowers and corresponding functional groups of flower visitors. This, in turns, allows to define the type of pollination system and determine plant species degree of specialization. Following Ellis and Ellis-Adam's classification (in Corbet, 2006), all the entomophilous species recorded were grouped on the basis of the accessibility of nectar. Three categories of entomophilous plant species were recognized: (a) allophilous species, which have fully exposed nectar; (b) hemiphilous species, with partly concealed nectar, and (c) euphilous species, that have deeply concealed nectar. Allophilous and hemiphilous species can be visited by a high number of small, short tongued insect species, and some of medium size and tongued length (Corbet, 2006; Willmer, 2011); thus, their pollinator spectrum is wide and the pollination system can be regarded as generalized. Euphilous species, on the contrary are mainly visited by large, long tongued insect. Given the restricted spectrum of effective pollinators, their pollination system can be considered as specialized (Corbet, 2006; Willmer, 2011). On this basis, entomophilous species were grouped according to the pollination system, as "generalists" (allophilous and hemiphilous) and "specialists" (euphilous).

### 2.3. Data analysis

#### 2.3.1. Flowering synchrony

Several quantitative methods have been proposed to measure flowering synchrony (hereafter FS) (Sargent and Ackerly, 2008). Most of them have been developed to quantify overlap in flowering time at the level of population or individual plants within a population (e.g., Refs. Primack, 1980; Augspurger, 1983; Marquis, 1988; Mahoro, 2002; Freitas and Bolmgren, 2008). On the contrary, the measurement of FS at the community level has received less attention. Dante et al. (2013) suggested the use of a niche overlap index based on the number of flowering plants per species (Schoener,
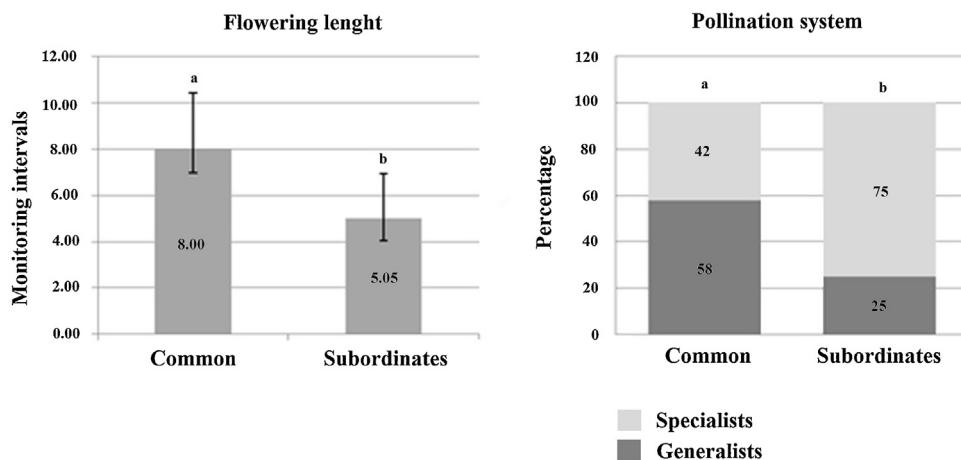
**Fig. 2.** Flowering length and pollination system of common and subordinate species. Values of the flowering length written on bars are the means of the monitoring intervals ± standard deviation. Values of the pollination system are the ratio "generalists: specialists" within common and subordinate species. Different letters indicate significant differences according to post-hoc Tukey HSD test.

1970; Pleasants, 1980). As an alternative, we propose to quantify interspecific FS by using a co-occurrence index. Co-occurrence indexes, such as V-score, are commonly used to evaluate the degree to which species co-occur spatially (Lepš and Šmilauer, 2003). We transposed this concept to flowering phenology to assess the temporal co-occurrence of flowering in the studied species. We called this co-occurrence flowering index "CF-index".

Similarly to V-score, to calculate CF-index we created a presence-absence matrix (flowering matrix), where rows were species (101 entomophilous species) and columns were the monitoring intervals (18 monitoring intervals). Entries represented the presence (1) or the absence (0) of the flowering event. We then calculated the CF-index by correlating the strings of each sampled species to each other through Pearson's correlation (Lepš and Šmilauer, 2003). As a result, species that tend to flower in the same time interval (i.e. in a completely synchronic pattern) have a positive CF-index (CF ~ 1) while species that tend to flower in a diachronic pattern have a negative CF-index (CF ~ −1). Finally, no correlation in the flowering span (CF ~ 0) arises between those species which show an incomplete FS or no synchrony at all. We then calculate the mean of the CF-indexes for all the species pairs in the matrix in order to assess the mean flowering pattern in our data.

To test for the significance of the observed mean CF-index, we performed a null model test. We built a null model which, in each randomization of the matrix, held constant (constrained) the sum of entries across each rows ("Frequency" null model, Gotelli and Graves,1996; "picante" R package). In this way, by constraining the sum of entries, the flowering span of each species stayed constant in each randomization. Furthermore, to distinguish early flowering from late flowering species, permutations between columns were allowed only within groups belonging to similar flowering periods. Therefore we constrained the randomization only inside two sub-matrices (from April to July and from July to September). The randomization of each sub-matrix was replicated 999 times, after each time the CF-index and its mean were calculated. We then compared the observed mean CF-index to the distribution of randomized mean CF-indexes in order to assess the statistical significance (p-value) of the mean co-flowering pattern observed.

To reveal fine scale variations in the flowering pattern, we also generated a flowering matrix for each plot. Since no differences in flowering time were observed among individuals of the same species occurring in different plots, these matrices can be considered as sub-matrices of the general flowering matrix. For each plot flowering matrix, as for the total matrix, the CF-index was calculated between each species pair and averaged over the plot. In this way, we obtained 45 mean CF-indexes, one for each plot, which reflect the degree of FS among species co-occurring in the same plot.

CF-index calculation and null model tests were performed on R statistical environment (R Development Core Team, 2008).

### 2.3.2. Plot level relationships between FS and total species richness and subordinate and common insect-pollinated species richness

Although extensively used in ecology and conservation biology, a sound definition of the rarity concept is still lacking (Landi and Chiarucci, 2014), since it involves both issues related to threatened or biogeographically rare species and simple abundance/frequency measures. We proceeded dividing species into common and subordinate according to their occurrence in the plots. The threshold level was set at 50% and corresponded to a natural break in species frequencies, in fact few species (12 species) were widespread and present in more than 23 plots (51% of the plots), while most species (89 species) occurred in less than 16 plots (36% of the plots). Accordingly, species with a frequency equal to or below the threshold level (but present in more than one plot) were classified as subordinate, while those which frequency was above the threshold were classified as common.

Spearman's Rank correlation was used to analyze the influence of the mean CF-index on the total insect-pollinated species richness, and the richness of subordinate and common species.

### 2.3.3. Flowering pattern and pollination system in subordinate and common species

To test for significant differences in the flowering time length and in the pollination system (generalized vs. specialized) between subordinate and common species, we performed a PERMANOVA test with 9999 randomization (Anderson and Ter Braak, 2003). We used species frequencies (subordinate vs. common) as a grouping variable and the length of flowering span (number of monitoring intervals) and the pollination system as dependent variables. Post hoc Tukey test was performed by STATISTICA software (version 8, 2008) to identify significant differences.

**Table 1**
Plot level (n = 45) relationships between the CF-index and the total species richness, and the subordinate and common insect-pollinated species richness (Spearman's rank correlation coefficients (Rs) and P-values). Significant results are in bold.

|  | $R_s$ | P |
|---|---|---|
| Total species richness | 0.215 | >0.05 |
| Subordinate species richness | **0.314** | **<0.05** |
| Common species richness | 0.036 | >0.05 |

**Table 2**
Differences in the flowering length and in the pollinators range, between subordinate and common species, evaluated by PERMANOVA. In bold the significance.

| PERMANOVA |  |
|---|---|
| Permutation N: | 9999 |
| Total sum of squares: | 428.3 |
| Within-group sum of squares: | 337.7 |
| F: | 30.69 |
| P (same): | **<0.001** |

## 3. Results

### 3.1. Flowering synchrony

When all entomophilous species were considered, their mean overlap in flowering span (CF-index) was significantly higher than expected by the null model (P-value$_{CFobs > CFexp}$ < 0.001), showing that entomophilous species tend to synchronize their flowering.

Many species pairs showed almost a complete FS (470 species pairs; CF > 0.75), many others shared at least part of their flowering time (1201 species pairs; CF > 0.50) whereas, only 10 species pairs showed a complete separate flowering period (CF < −0.75). Most common combinations of co-flowering species (r > 0.75) were between *Lamiaceae* and *Fabaceae*, *Asteraceae* and *Lamiaceae*, *Geraniaceae* and either *Lamiaceae* or *Fabaceae*, *Orchidaceae* and either *Lamiaceae* or *Fabaceae*.

### 3.2. Plot level relationships between FS and total species richness and subordinate and common insect-pollinated species richness

No significant associations were found between CF-index and the total number of insect-pollinated species per plot (Table 1), demonstrating that richness in entomophilous species and the degree of flowering synchrony are totally independent.

A positive correlation was found between the number of subordinate species and the mean CF-index per plot (Table 1), while no significant correlation was observed for common species (Table 1).

### 3.3. Flowering pattern and pollination system in subordinate and common species

Significant differences in the flowering length and in the pollination system (generalized vs. specialized species) between subordinate and common species were revealed by PERMANOVA (Table 2). Post hoc Tukey test indicated that subordinate species are characterized by shorter flowering length than the common ones (Fig. 2). Moreover the pollination system was not equally distributed between subordinate and common species. In particular, most subordinate species showed a specialized pollination system (Fig. 2). Conversely common species did not evidence a comparable clear dominance.

## 4. Discussion

Dry grassland entomophilous species proved to overlap significantly in flowering time, revealing a non-random pattern of flowering, i.e. the degree of convergence in flowering time among species within the community differed from what would be expected if flowering spans were randomly attributed from a uniform distribution.

The observed synchrony may result from a combination of factors. On a broad scale, seasonal timing of flowering has been supposed to possess a phylogenetic component, with repeated patterns in the phenological events across plant lineages (Davies et al., 2013), i.e. the tendency for closely related species to be similar to each other more closely in their flowering phenology than expected by chance. Davies et al. (2013) also described similar phylogenetic patterns in long-term phenological response traits across geographically separated communities. Although phylogenetic studies have shown that especially flowering time is a particularly conserved trait within temperate phylads (Levin, 2006), we may assume that our results are not affected by the phylogenetic effect since the most common combinations of co-flowering species involved different plant families, well spread over published phylogenetic trees of angiosperm (Angiosperm Phylogeny Group, 2009).

A precise phenological timing is certainly linked to climate. Temperate habitats normally show flowering patterns with a strong seasonal bias, which may result from local resource availability peaking within a relatively narrow timeframe (Appanah, 1993; Dominguez and Dirzo, 1995). In particular, most temperate species flower in response to temperature, that defines the start and end of the growing season (Larcher, 2003; Schwartz, 2003; Inouye, 2008). Indeed, flowering pattern at community level broadly resembled the hump-shaped seasonal trend of temperature. Thus, local adaptation to environmental signals might have greater influence on species flowering phenology than taxonomic membership. Furthermore, temperate dry grasslands are mostly semi-natural communities maintained by mowing and grazing which have been proved to regulate species composition and richness (Valkó et al., 2011, 2012). With reference to the flowering time, it could be assumed that the timing of management practices, e.g. the time of hay-making and its regularity over years, could contribute to select species based on their reproductive cycle. However, species categorized upon their frequency evidenced significant differences in the flowering length which was also coupled with a different pollination system, providing further evidence supporting a non-random flowering pattern at community level.

A growing number of studies provided evidence that the flowering phenology is a crucial element of the ecology of plants and an important component of community assembly as it influences not only the relative abundance of species in a given ecosystem, but also their presence or absence (Sargent and Ackerly, 2008; Crimmins et al., 2011). The way in which flowering phenology affects the composition of plant communities is through its effect on species interactions, rising the potential for strong competition, but also facilitation, for pollination resources (Rathcke and Lacey, 1985; Callaway, 1995).

Indeed, several studies have investigated FS among plant species, although findings vary from study to study. Some research described reduced FS in response to competition for pollinators (e.g. Refs. Mosquin, 1971; Pleasants, 1980), to avoid heterospecific pollen transfer (Waser and Fugate, 1986; McLernon et al., 1996; Brown and Mitchell, 2001; Morales and Traveset, 2008) or the spread of pollinator transmitted diseases (Elzinga et al., 2006). Other studies have evidenced convergence in flowering time to ensure pollinator visits (Moeller, 2004; Ghazoul, 2006; Dante et al., 2013). The sharing of the same temporal niche (synchronous

flowering periods) by different plant species may increase the abundance of floral resources. This in turn could enhance the possibility of pollinator sharing to occur by increasing the visitation rate, leading to an amelioration of plant species reproductive success and, eventually, to the establishment of co-flowering species in the community (Waser and Real, 1979; Thompson, 1988; Moeller, 2004). Mutualistic interactions, such as pollination and seed dispersal, are good examples of the complex web of interactions that play a pivotal role in population establishment, reproduction and migration, and community development (Forup and Memmott, 2005). Flowering phenology can thus become a key characteristic in plant reproductive biology by favoring mutualistic interactions, thereby increasing the success of plant reproduction and their long lasting permanence in a site.

We found that the FS does not influence the total species richness, but when considering the richness of species with different frequency, FS turned out to positively influence the richness of subordinate species. Subordinate species are usually under stronger selective pressure to secure pollination than common plants (Gumbert et al., 1999; Dante et al., 2013). Population density is one of the most important spatial components influencing pollen flow and seed set (Morris, 1993; Richards et al., 1999; Brys and Jacquemyn, 2010). Plant density has been proved to influence the visitation frequency of pollinators per plant and the seed production, both of which tend to be lower in lower-density populations (Silander, 1978; Allison, 1990; Ohashi and Yahara, 1998). Moreover, our results reveled that subordinate species are mainly characterized by a specialized pollination system, and that they flower for particularly short time spans. On the other hand, common species showed both generalized and specialized pollination system, and flower for a longer periods than subordinate species. Thus, subordinate species may take advantage of flowering in the same periods of common species, which being visited by a consistent number of functional groups of flower visitors, could increase the attractiveness and the probability of pollination events to occur (McEwen and Vamosi, 2010). Thus, plant-plant relationships for pollinators may contribute to shape dry grassland communities composition, by maintaining subordinate insect-pollinated species which contribute for the most part to the overall diversity in species-rich grassland communities (Wellstein et al., 2014).

## 5. Conclusions and further perspectives

Species rich ecosystems have long fascinated ecologists. Our findings suggest to regard FS as an important process in influencing the species composition and richness of the community in temperate dry grasslands. Arguably, as plant-pollinator interactions play a central role in the reproduction of the plants and the life histories of the insects they likely have a marked influence in ecological community dynamics and diversity. Thus, the comprehension of the functional relationships between species of different trophic levels, which reciprocally influence each other, is certainly of great importance for the conservation of their populations and the maintenance of the ecosystem services that pollination provides.

Plant-pollinators relationships have been traditionally studied focusing on highly specific interactions between one or few plant species and constrained groups of pollinators. Little information is available at the community level. However, a community wide approach, i.e. in which patterns of interaction among all the plants and pollinators in a given area are studied simultaneously, is essential to account for community level processes (e.g. potential competitive or facilitative effects among species that belong to the plant-pollinator system, or patterns of communities assembly), which are often linked to species diversity. For example, our study suggests that the possibility for a subordinate specialist species to persist within a diversified community might depend on the sharing of pollination services from generalists.

Moreover, since pollinators establish populations only once their habitat requirements (i.e. food resources, nesting sites and nesting materials) have been met, a community-wide approach can represent a useful tool to define the "conservation status" of a given habitat, a concept that is central to the EC Habitats Directive (EC 92/43). The same approach might also be used for a better understanding of the actual functioning of restored communities and how these compare to a "reference state". Thus, the inclusion of biotic pollination-related interactions as an element in management or restoration plans could promote the conservation of the whole community, and guide monitoring programs.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.flora.2016.04.003.

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