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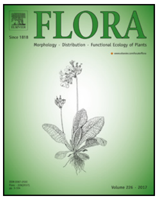

Ten years of progress in the study of *Hadena*-Caryophyllaceae nursery pollination. A review in light of new Mediterranean data☆

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ABSTRACT

Nursery pollination interactions are widespread between Caryophyllaceae species and the pollinating-seed predator *Hadena* moths (Noctuidae). A previous revision of this system was based mainly on widely distributed species in the north and center of Europe. However, there was no information from the Mediterranean region, one of the global diversification centers of both taxa. The aim of this work is to review the progress on the knowledge of this nursery pollination system since the first revision, providing unpublished data of Caryophyllaceae-*Hadena* associations from Spain. Furthermore, we conduct a preliminary network analysis to illustrate the advantages of this approach to explore nursery pollination systems.

In the last 10 years, most of studies have focused on selective forces exerted by *Hadena* on the plant reproductive traits through pollination and predation. *Hadena* moths are selectively attracted by flower scents, flower sizes and number of flowers per plant are also crucial for attraction of the moths. Caryophyllaceae species may have developed some phenological, chemical, morphological and physiological adaptations to avoid overexploitation by larvae. The evolution of sexual dimorphism in Caryophyllaceae may be a consequence of mutualistic and antagonistic interactions. Other pollinators as well as an anther smut fungus and larval parasitoids are important selective agents that can shift this interaction between mutualism and parasitism. Whereas most studies highlighted the parasitic nature of the *Hadena*-Caryophyllaceae interaction, we need further analyses on the pollinator effectiveness of *Hadena* and on the spatio-temporal variation of the interaction outcome. Based on our field surveys and bibliographic records we found evidence of nursery pollination between 22 noctuid species (mostly *Hadena*) and 70 Caryophyllaceae species from 11 genera (mostly *Silene* and *Dianthus*). From these interactions, 26 were new for the Iberian Peninsula and 18 were not described before. Results of our preliminary network analysis suggest that these interactions are constrained by phylogenetic, geographical and ecological filters.

1. Introduction

Nursery pollinators, i.e., pollinating seed predators, are insects that use the reproductive structures of the plant they pollinate to feed their offspring (Dufaÿ and Ansett, 2003). These interactions can be very specialized, reciprocally obligate, and mutualistic, including classical examples like yuccas and yucca moths in North America (Pellmyr, 2003), *Ficus* and Agaonidae waps in tropical environments (Herre et al., 2008; Wiebes, 1979), and Phyllanthaceae trees and *Epicephala* moths in Asia (Kato et al., 2003; Kawakita and Kato, 2004). However, in some nursery pollination systems other pollen vectors contribute to the pollination of the host plant, as in *Lithophragma* and *Greya* moths (Thompson and Pellmyr, 1992), and *Trollius* globeflowers and *Chiastocheta* flies (Suchan et al., 2015). In these facultative systems, the interaction between the nursery pollinator and its host plant may shift between being mutualistic and parasitic, depending on the pollination-predation net outcome and on the importance of co-pollinators (Dufaÿ and Anstett, 2003). These non-obligate systems are very interesting for the study of the origin, evolution and maintenance of mutualisms (Thompson et al., 2013), and are widespread between *Hadena* moths (Noctuidae) and Caryophyllaceae species, especially *Silene* (Brantjes, 1976a,b,c; Giménez-Benavides et al., 2007; Kephart et al., 2006; Pettersson, 1991; Prieto-Benítez et al., 2016a; Reynolds et al., 2012).

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Both male and female *Hadena* moths visit and pollinate the flowers of Caryophyllaceae. Female additionally lays the eggs within or on the surface of the calyx of some flowers, and the larvae subsequently feed on the developing fruits (Bopp, 2003; Brantjes, 1976a). The review of this system by Kephart et al. (2006) found 58 interactions between 14 *Hadena* and 26 Caryophyllaceae species (14 *Silene* species). This work, though it is based on a small sample of documented cases, showed that the system has low specificity, with plant and moth species interacting with more than one partner suggesting a diffuse coevolutionary scenario. A special effort was made by these researchers to identify the selective forces that potentially operate on the plant and moth traits, independently or simultaneously.

The aim of this work is to review the current state of knowledge of the nursery pollination system between caryophyllaceous plants and *Hadena* moths ten years after the first review (Kephart et al., 2006). This is an excellent opportunity to assess the progress since then, identify major gaps, and discuss further directions for research. This model system has been a growing field of research over the last decade (Fig. 1). We conducted a search in the Web of Knowledge in 11 October 2016 with keywords "Hadena" and ("Silene" or "Dianthus" or "Caryoph*") that returned 54 matches. We also included several related publications by cross-reference, for a total of 59 works. Publications on this topic are only scattered from 1976 (Brantjes, 1976a,b,c) until the beginning of 2000s; and then got more frequent. However; most of the studies were conducted in Central Europe and North America (77%); and many of them exclusively centered on *Silene vulgaris*; *S. latifolia* and *H. bicruris* (83%); despite both plant and moth genera are highly diversified and widely distributed throughout the globe. To partially fill the gap we present new field data of Caryophyllaceae-*Hadena* associations from Spain; and conduct a preliminary analysis of complex networks to illustrate how this tool is a relevant framework to explore the structure and the degree of specialization of the nursery pollination systems.

2. Literature survey

2.1. *New evidence on the mutualism–parasitism dilemma and the evolutionary implications of plant-moth interactions*

Most *Hadena* moths are major or common pollinators of its Caryophyllaceae hosts (Kephart et al., 2006; Kula et al., 2014). Both male and female moths visit flowers and provide pollination services, and the plant obtains only positive outcome from male moths, but positive and negative (due to larval predation) outcomes from female moths (Kephart et al., 2006; Labouche and Bernasconi, 2010). Nevertheless, the net effect of pollination vs. predation have been quantified in few cases because it depends on the pollination service provided by other pollinators, and may change with time and among populations (Thompson, 2005; Kula et al., 2014). Nevertheless, in the scarce studies it was found that *Hadena*-Caryophyllaceae interactions are mostly parasitic, with predatory activity exceeding pollination services (Pettersson, 1991; Giménez-Benavides et al., 2007; Reynolds et al., 2012; Kula et al., 2014; Prieto-Benítez et al., 2016a).

Whether mutualistic or parasitic, a recurrent topic on this system was to assess which functional traits of plants and moths are susceptible to coevolutionary adaptation. Kephart et al. (2006) suggested that some traits related to attraction of pollinators and efficient pollen transfer may have evolved in response to the moths. This is partially motivated by the traditional classification of *Silene* and sister taxa in two contrasting pollination syndromes, diurnal and nocturnal (Lindman, 1897; Greuter, 1995). Nocturnal species typically have white or pale petals and the beginning of flower anthesis and scent emission are synchronic in the evening or night (Faegri and van der Pijl, 1979; Prieto-Benítez et al., 2016a). Diurnal species have pink or red corollas, the flowers remain open during day and night, and scent changes are imperceptible by the human nose (Greuter, 1995; Jürgens et al., 2002; Jürgens, 2006). Kephart et al. (2006) detected a significant association between these flower traits and nocturnal pollination, and we also know that female *Hadena* moths select by flower color, shape and scent when two host plants coexist (Castillo et al., 2014; Page et al., 2014). Kephart et al. (2006) showed a clear segregation of a few diurnal and nocturnal species by their scent composition. However, an inherent bias exists as most of the published scents of the European species reflected data captured only during day (species classified a-priori as diurnal) or night (species classified as nocturnal). Recent progress on the diel variation of flower scent in a larger sample of caryophyllaceous species have shown that most species emit volatile compounds with attractive potential to pollinators during both day and night (Castillo et al., 2014; Dötterl et al., 2012; Giménez-Benavides et al., 2007; Martinell et al., 2010; Waelti et al., 2008; Prieto-Benítez et al., 2015) with diurnal and nocturnal species having similar scent compositions at the respective times of the day (Prieto-Benítez et al., 2015). Interestingly, Prieto-Benítez et al. (2016b) found that flower scent in the tribe Sileneae is phylogenetically constrained to some extent, and the strength of phylogenetic signal is stronger in night than in day emissions, suggesting that the selective forces exerted by diurnal and nocturnal flower visitors on floral scents differ. Many species substantially change the amount and composition of scent from day to night, but sometimes in the opposite way as expected based on other flower traits, especially flower color (Prieto-Benítez et al., 2015, 2016a). These results help to explain why most caryophyllaceous species show a mixed suite of day and night flower visitors, including Hymenoptera, Diptera and Lepidoptera, and suggest the prevalence of a mixed pollination strategy (Dötterl et al., 2012; Prieto-Benítez et al., 2015, 2016a). For instance, in hybrids from *S. latifolia* (white petals) and *S. diclinis* (pink petals), the diurnal and nocturnal pollinators visited more frequently larger flowers, but they did not show clear preferences for flower color (Brother and Atwell, 2014). In any case, this is not in direct conflict with the pollination syndrome concept because floral specialization may reflect adaptations to the most effective pollinators, and pollination effectiveness may shift in space and time (Fenster et al., 2004; Ollerton et al., 2007). To understand the role of *Hadena* and any other pollinator as selective agents of caryophyllaceous floral traits, Kephart et al. (2006) encouraged the research community to gather detailed data on the pollination effectiveness of each functional group of flower visitors, in combination with phenotypic selection studies. Unfortunately, after ten years, the number of studies on this topic can be counted on one hand and results are not conclusive. *Silene sennenii* and *S. stellata* are white campions that produce higher rates of scent at night and have moth species as most effective pollinators (Martinell et al., 2010; Reynolds et al., 2009). *Silene ciliata* also has white flowers with crepuscular opening and dominance of night scent, but diurnal pollination provided greater female fitness in a pollination exclusion experiment, although pollination effectiveness was not really measured (Giménez-Benavides et al., 2007). In hybrids between *S. latifolia* (white petals) and *S. diclinis* (pink petals) nocturnal pollinators provided higher seed set than diurnal pollinators (Brother and Atwell, 2014). To our knowledge, there is only one published study quantifying the strength and direction of pollinator-mediated selection in *Silene*, but it was focused in *S. virginica*, a hummingbird-pollinated species (Reynolds et al., 2010).

Regarding the question whether or not *Hadena* fruit predation also shapes flower traits, some evidence supports moths as potential selective agents for traits minimizing the frequency and intensity of attacks. Some contributions in the last decade suggest

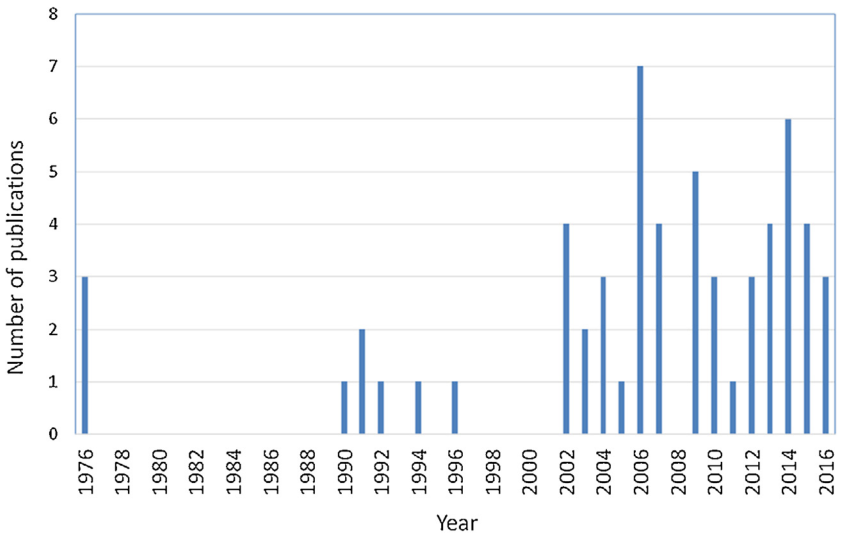

Fig. 1. Evolution of the number of publications devoted to the *Hadena*-Caryophyllaceae nursery pollination system extracted from a search in the Web of Knowledge (11 October 2016).

that Caryophyllaceae species have evolved phenological, chemical, morphogical or physiological adaptations to avoid overexploitation by *Hadena* larvae. Diurnal *Silene* species experience significantly less predation than nocturnal species. Thus, floral phenotypes enhancing diurnal attractiveness could represent a strategy of predation avoidance (Kephart et al., 2006; Prieto-Benítez et al., 2016a). Early flowering plants of *Dianthus sylvestris* had higher fruit set, the fruits had more and heavier seeds and they suffer less predation by *H. compta* than later flowering plants (Collin et al., 2002 Collin and Shykoff, 2010), showing that changes in phenology are also a mechanism by which plants can influence predation rates. In *S. latifolia*, pollination and moth oviposition rapidly decrease the amount of scent and change its composition in the affected flowers and neighboring plants, reducing the attractiveness of flower clumps to *Hadena* moths (Dötterl et al., 2005; Muhlemann et al., 2006; Piesik et al., 2014). Interestingly, the higher emission of (*Z*)-3-hexenyl acetate and β-ocimene after herbivore attack increased nocturnal pollinator attraction (Cozzolino et al., 2015). Several works showed that bigger *S. latifolia* plants with large flowers had higher pollinator visitation rates but were also more prone to predation by *H. bicruris* (Biere and Honders, 2006; Burkhardt et al., 2012; Brothers and Atwell, 2014). However, Labouche and Bernasconi (2013) found that flowers with longer corolla tubes constrain egg laying on the safest parts of the flowers, so it takes place more frequently on the petals, raising larval mortality. Those flowers that receive *Hadena* eggs have more probabilities of abortion and/or abscission, decreasing the survival probability of larvae and avoiding secondary attacks (Jolivet and Bernasconi, 2006; Burkhardt et al., 2009). Fruit abscission was proposed as a strategy to avoid overexploitation by fruit predators in some obligate nursery pollination systems (Holland and DeAngelis, 2006). Also, when *S. latifolia* flowers were experimentally exposed to fruit attack, larval growth was dependent on the identity of maternal plant's family, suggesting the existence of hereditary chemical defense mechanisms (Jolivet and Bernasconi, 2006). An alternative mechanism of defense was proposed when observing that the walls of fruit capsules are thicker in the native range of *S. latifolia* than in the invasive range, where *H. biscruris* is absent (Blair and Wolfe, 2004; Wolfe et al., 2004). However, Jolivet and Bernasconi (2006) demonstrated that wall thickness did not represent an adaptive advantage because thinner fruits were not significantly more prone to attack. In summary, Burkhardt et al. (2012) suggested that the selective forces exerted by *Hadena* in *S. latifolia* are higher by their predation role than their pollination role. Up to now most of these defense mechanisms have been shown only in *S. latifolia,* and highlight the degree of specialization of the *Silene-Hadena* interaction system (Jolivet and Bernasconi, 2006; Bernasconi et al., 2009).

Another promising avenue of research with this model system is the origin and evolution of plant sexual systems and the evolution of sexual dimorphism (Bernasconi et al., 2009). The genus *Silene* has a rich diversity of sexual systems, i.e., hermaphroditism, dioecy, gynodioecy and gynodioecy–gynomonoecy (reviewed by Casimiro-Soriguer et al., 2015), and a remarkable sexual dimorphism in some species (Geber et al., 1999). The probable evolutionary pathway to dioecy from hermaphroditism, with gynodioecy or monoecy as intermediate steps (Charlesworth, 1999), have been addressed with *Silene* due to its high frequency of gynodioecious species (13.3%, by only 7% of total angiosperms are gynodioecious, Casimiro-Soriguer et al., 2015; Renner, 2014). These research lines, including the evolution of sexual chromosomes in *Silene*, were reviewed recently by Bernasconi et al. (2009), thus we here focus only on the studies exploring the pollinating seed predators as selective agents. Collin and Shykoff (2010) explored whether females of *Dianthus sylvestris* have a selective advantage if they are less prone to *Hadena compta* herbivory than hermaphrodites. Generally, in reproductive systems where female and hermaphroditic individuals coexist, females must have some fitness advantage to persist, in terms of quantity (higher fecundity) or quality (absence of inbreeding depression) of the offspring (Shykoff et al., 2003; Collin and Shykoff, 2003). Nevertheless, other factors providing female advantage can involve mutualistic and antagonistic interactions, but they have been less explored (Ashman, 2002). Collin and Shykoff (2010) found that *Hadena compta* ovoposits preferentially in hermaphrodite flowers, but fruit predation did not differ between sexes because the larvae, after consuming the primary fruit, move to eat additional fruits independent of sex. Another hypothesis of the sexual selection theory state that, in dioecious plant species, males are selected to invest more than females in flower attractive traits because males compete among them to fertilize the ovules (Willson, 1979). Waelti et al. (2009) tested this prediction in *Silene latifolia* and found that male individuals had more but smaller flowers, but the scent emission was higher than that of female individuals, especially for the volatile compounds involved in moth-attraction. They also found that male naïve moths preferred male over female flowers, suggesting that flower dimorphism may be mediated by sexual selection and moth preferences

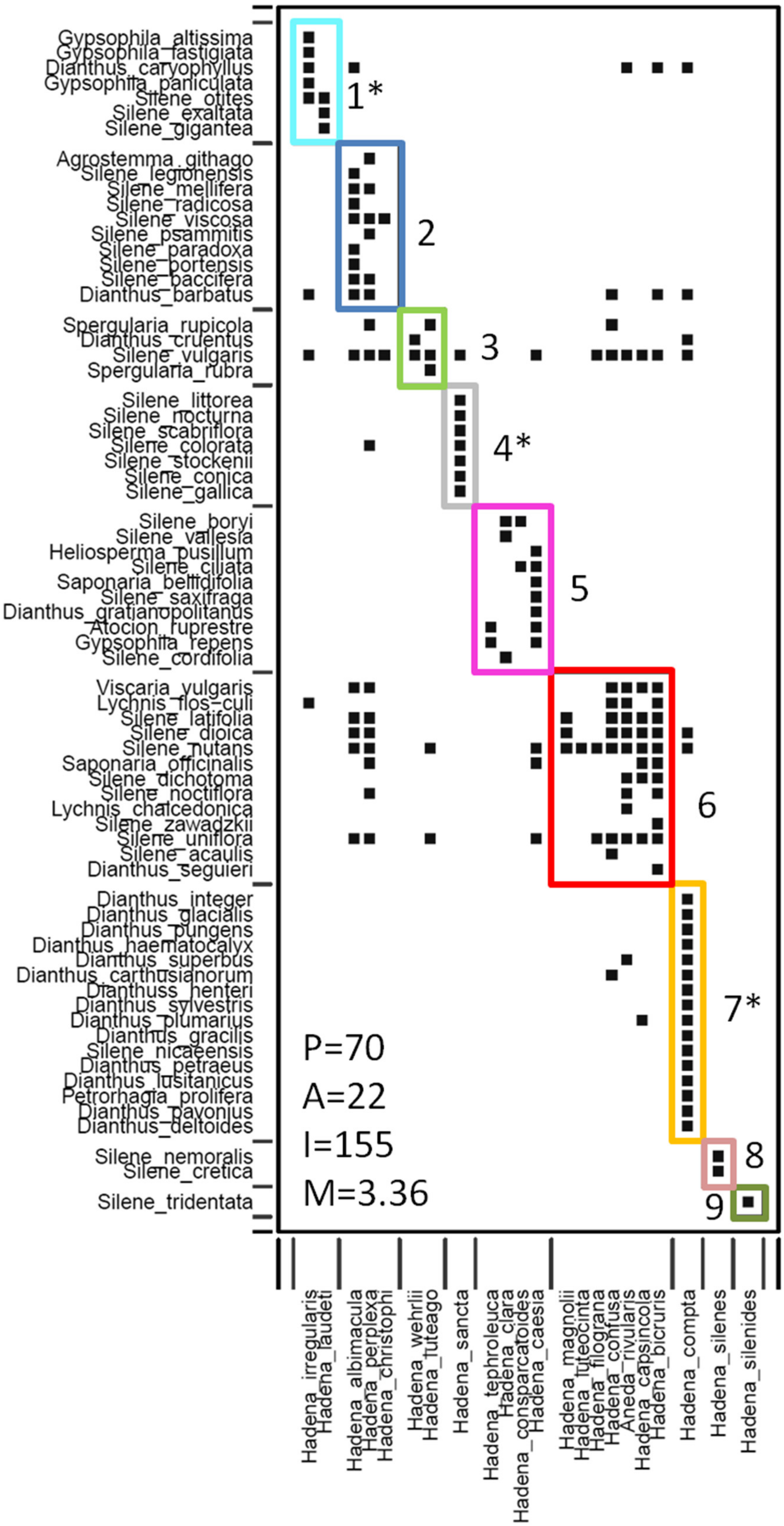

Fig. 2. Result of the modularity analysis of the nursery pollination network. Plant species are in rows and moth species in columns. Black cells denote a nursery pollination association found in the bibliographic review or in our field work. Species are sorted according to their modular affinity. Black cells outside modules are interactions linking the nine modules together into a coherent network, and black cells inside are linking the species within modules. (*) denote modules with phylogenetic signal.

Table 1

Summary of the nursery pollination interactions found in Spain during field surveys in 2007–2012 between species of *Silene, Dianthus* and *Hadena*. (1) denotes species interactions and (*) denote interactions which have not been described before.

	H. albimacula	*H. bicruris*	*H. clara*	*H. compta*	*H. confusa*	*H. consparcatoides*	*H. perplexa*	*H. sancta*	*H. wehrlii*
D. lusitanus	–	–	–	1*	–	–	–	–	–
S. boryi	–	–	1*	–	–	1*	–	–	–
S. ciliata	–	–	–	–	–	1	–	–	–
S. colorata	–	–	–	–	–	–	1*	1*	–
S. conica	–	–	–	–	–	–	–	1*	–
S. gallica	–	–	–	–	–	–	–	1*	–
S. latifolia	–	1	–	–	–	–	–	–	–
S. legionensis	1*	–	–	–	–	–	–	–	–
S. littorea	–	–	–	–	–	–	–	1*	–
S. mellifera	1*	–	–	–	–	–	1*	–	–
S. nicaeensis	–	–	–	1*	–	–	–	–	–
S. nocturna	–	–	–	–	–	–	–	1*	–
S. nutans	–	1	–	–	–	–	–	–	–
S. portensis	1*	–	–	–	–	–	–	–	–
S. psammitis	–	–	–	–	–	–	1*	–	–
S. scabriflora	–	–	–	–	–	–	–	1*	–
S. stockenii	–	–	–	–	–	–	–	1	–
S. vulgaris	–	1	–	1	1	–	1	1*	1*

As we have seen, several studies in the last decade have addressed the floral evolution of Caryophyllaceae hosts in response to *Hadena* species as pollinators and predators. However, the opposite way, the evolution of morphological and behavioral traits of moths in response to the plant features is almost unexplored (Kephart et al., 2006). Castillo et al. (2013) and Kula et al. (2013) proved that female *H. ectypa* have developed an optimal oviposition and pollination strategy because they lay eggs in young and virgin flowers of *S. stellata*, and these flowers have a high chance of set fruit. Since the larvae prefer to feed on immature fruits, female moths maximize the fitness of their progeny with this behavior. In summary, we have provided many cues highlighting that both plants and moths can potentially evolve in response to their partners. However, up to now we do not have any direct confirmation that *Hadena* and caryophyllaeous species have been under reciprocal selection. The case study that gets closest to show evidence was performed by Magalhaes and Bernasconi (2014). They found that flower tube length and moth proboscis length correlate in *S. latifolia*-*H. bicruris* coexisting populations. Nevertheless, many authors have stated that correlation between two traits does not necessarily imply the existence of reciprocal selection and a coevolutionary process (Nuismer et al., 2010; Thompson, 1994).

2.2. *Third parties, enemies and allies*

To understand the context in which nursery pollination systems develop, it is also critical to assess the relationships with other organisms influencing both plant and moth partners. Caryophyllaceous species have to face another threat derived from *Hadena* and co-pollinators' visits, the transmission of the fungus *Microbotryum violaceum*. This fungus causes anther-smut disease that produces sterile flowers, and it is present in 80% of perennial *Silene* and related species (Hood et al., 2010). Infected flowers emit less scent and are less attractive than healthy flowers for *Hadena* moths (Dötterl et al., 2009). Although to a minor extent, moths visit the infected flowers and transmit the spores (Antonovics, 2005). *Hadena* moths lay eggs preferably in healthy flowers, producing more harm through fruit predation in uncontaminated plants (Biere and Honders, 2006; Dötterl et al., 2009). Therefore, *M. violaceum* is a third element to be considered in the net balance of positive and negative outcomes between Caryophyllaceae and *Hadena*. Moreover, recent molecular analysis showed that *M. violaceum* is a complex of cryptic species specialized on groups of closely related hosts (Le Gac et al., 2007). They have experienced frequent host shifts, and thus, strict cospeciation was ruled out (Refrégier et al., 2008). The possible specialization of *Hadena* species onto different plant hosts, and the segregation of pollination niches in sympatric populations of Caryophyllaceae species, thus may affect evolution of *Microbotryum* by reducing interspecific spore transfer and promoting host race differentiation (van Putten et al., 2007; Bernasconi et al., 2009).

Besides the antipredatory strategies mentioned before, Caryophyllaceae species also have insect allies with potential to regulate the *Hadena* populations. Hymenopteran parasitoids from Braconidae and Ichneumonidae parasitize larvae of *Hadena*, reducing fruit predation (Elzinga et al., 2003; Elzinga et al., 2007). The frequency of parasitoids is greater in large plant populations, regardless of larval density (Elzinga et al., 2007). These parasites attack caterpillars when the latter are too large to being protected by the fruit capsules (Elzinga et al., 2005). It can be expected that plant species, populations and individuals with smaller fruits raise the chances of parasitoid attack, being the fruit size another potential trait to be indirectly selected by parasitoids to minimize fruit predation by *Hadena*.

In summary, caryophyllaceous species face a complex suite of mutualistic and antagonistic organisms, from *Hadena* nursery pollinators and genuine pollinators to fungal pathogens, parasitoids, and even nectar robbers and herbivores. The evolution and maintenance of nursery pollination, the selective forces operating on both plants and moths, and the ecological context shifting the interaction from mutualism to parasitism cannot be assessed if these partners and the complex trade-offs they generate are excluded.

2.3. *Diversity and specificity of Caryophyllaceae-moth interactions*

In contrast to obligate nursery pollination systems, most pollinating-seed predation systems like *Caryophyllaceae-Hadena* are facultative and not very specific (Dufaÿ and Anstett, 2003; Kephart et al., 2006; Pellmyr, 2003; Thompson and Pellmyr, 1992). For example, female moths of *H. bicruris* oviposit on both *Silene latifolia* and *S. dioica*, though they prefer *Silene latifolia* over *S. dioica* when coexist in mixed stands (Bopp and Gottsberger, 2004). A rearing assay showed that there are differences in the profitability of these fruits because pupae weight change depending on the *Silene* species used as food. However, larvae do not select between them (Boop and Gottstberger, 2004). The previous review on this system revealed that one-to one associations of plant and moth species are not frequent (Kephart et al., 2006). However, it was difficult to find a general pattern because most case studies included plant and moth species with a wide geographic distribution throughout North and Central Europe (e.g., *Silene latifolia*, *S. dioica*, *S. vulgaris*, *H. bicruris, H. compta* and *H. perplexa*), and it is rather expected that widely distributed species will have a suite of alternative partners through their ranges. On the contrary, little to no published information exists on European *Silene-Hadena* associations involving narrowly distributed species, or from the diversification centers of both taxonomic groups, such as the Mediterranean region. Therefore, there is a high number of potential interactions and a subjacent coevolutionary scenario that remain unexplored.

3. A mediterranean survey

*3.1. Sampling of Caryophyllaceae-*Hadena *interactions in Spain and range size estimates*

To start filling the gap, we conducted extensive field surveys throughout Spain in the period 2007–2015. The low visitation rates and the lack of naked-eye visibility at night make direct observations of *Hadena* on Caryophyllaceae unproductive for an extensive study. For this reason we used fruit predation as evidence of the nursery pollination interaction. Unripe fruits of 19 species of *Silene* and one *Dianthus* were collected from natural populations. As the identification of *Hadena* from larval stage is difficult, larvae were fed *ad libitum* with frozen fruits (from the host species) until they pupated. At the beginning of the next spring the emerged moths were used to identify the species. Larva growing conditions are in the Supplementary Material 1.

To obtain the distribution and range size of plant and moth species included in the network, we use different sources of data (GBIF–Global Biodiversity Information Facility, Flora Europaea, Noctuidae Europaeae, The Lepidoptera of Europe and a *Hadena* review) (Hacker, 1992; Hacker et al., 2002; Nowacki and Fibiger, 1996; Tutin et al., 1993). Since georeferenced data are very scarce, especially for some *Hadena* species, we use distribution data at country level. To standardize and compare species we use the geographical scheme proposed by Brummitt et al. (2001), which identifies biogeographical units at four levels. Political countries are considered the basic recording units, and are hierarchically arranged in biogeographical countries, regions and continental levels. We classified the species distribution in 3 categories: small (species with a small distribution within one or two regions), medium (species spread in several countries of two or three regions), and widespread (species present in four, five or six regions) (Supplementary material 2). Owe to the nature of the data used, most of the references were based mainly on widespread species and this may produce a bias in our network analysis. We explored if the distribution of the species were biased by the "study identity" (the reference where each interaction is cited) or by the "country of origin" (the country where the interaction was found). We made glms with Poisson family for the Caryophyllaceae and *Hadena* species included in the matrix (published data and records of present study; Supplementary material 2). We also made Log-linear analysis of frecuency tables to figure out if the modules (see below network analysis) are composed by species from the same provenance (countries).

*3.2. Specificity of Caryophyllaceae-*Hadena *nursery pollination*

With our field data and the bibliographic survey, we have largely improved the knowledge of the Caryophyllaceae-*Hadena* associations with respect to the previous review ten years ago (Kephart et al., 2006). The number of *Hadena* and Caryophyllaceae species have increased in 8 (57%) and 44 (169%) species, respectively, with an increase of 279% of the total number of associations. We found interactions between 22 species of Hadenini (21 *Hadena* and one *Aneda*, formerly considered as *Hadena*) and 70 species of Caryophyllaceae from 11 genera (*Agrostema, Atocion, Dianthus, Gypsophila, Heliosperma, Lychnis, Petrorhagia, Saponaria, Silene, Spergularia, Viscaria*) (Supplementary material 2). *Silene* and *Dianthus* were the dominant genera (36 and 19 species, respectively). Only in our field survey in Spain, we found 26 interactions between 18 Caryophyllaceae (17 *Silene* and 1 *Dianthus*) and 9 *Hadena* species, which is 16.4% of known associations. It is important to note that 18 (11.3%) of these associations had not been described before (Table 1). This points to the relevance of the Mediterranean basin as one of the diversification centers of this interaction system.

Of 22 Hadenini species using Caryophyllaceous hosts, only two occur on a single host plant, whereas 43 of 70 plants species are known to have only one moth species associated. Eleven Caryophyllaceae species found in Spain have only one *Hadena* species associated, but all *Hadena* species have more than one host. As expected, the single 1: 1 association corresponded to species with narrow distributions (i.e. *H. silenides-S. tridentata*), whereas most widespread species are more generalist and interacted with (many) alternative partners (e.g. *S. vulgaris*, *S. nutans, S. uniflora*; *H. compta*, *H. perplexa* and *H. albimacula*). A special case is *H. compta*, the species with the highest number of interactions but almost exclusively with *Dianthus* species. *Hadena compta* is widely distributed in the Palearctic region, such as other generalist species (i.e. *H. bicruris* and *H. confusa*) that are nursery pollinators of generalist and widespread *Silene* (i.e. *S. dioica* and *S. nutans*). The remarkable host switch of *H. compta* to *Dianthus* has allowed the moth to nurse its offspring without the potential competition of other *Hadena* species. Moreover, if selection of *Dianthus* as host has a fitness advantage for *H. compta*, the nursery pollinator might show specific adaptations to exploit this genus, such as behavioral and morphological traits for effective recognition, egg-laying and pollination (Kephart et al., 2006; Pellmyr, 2003), but this needs to be assessed in future studies.

4. A preliminary assessment of Hadenini-Caryophyllaceae interactions with network analysis

In the last decades, species interactions have been assembled and analyzed as complex networks, in which species are nodes connected to each other by links when they interact (Bascompte and Jordano, 2007). The properties of ecological networks have been studied in plant-animal mutualistic systems (i.e., pollinators and seed dispersers), and in antagonist networks (i.e., food webs and host-parasite relationships) (Bascompte et al., 2003; Dunne et al., 2013; Godfrey, 2013; Gómez et al., 2013; Jordano et al., 2003; Olesen et al., 2007; Vázquez et al., 2005). Therefore, the network analysis is an excellent tool to study the architecture of the nursery pollination systems that shifts between parasitism and mutualism. Despite differences between mutualist and antagonist network architectures, there are consistent patterns between them, such as the frequent occurrence of modularity (Olesen et al., 2007; Thébault and Fontaine, 2010). In a modular or compartmentalized network, some subsets of species (called modules) are more linked among them than to species in other modules (Fortuna et al., 2010; Olesen et al., 2007). Recent studies of plant-animal and host-parasite networks have shown that modules are frequently composed of closely related species (Dupont and Olesen, 2009; Krasnov et al., 2012; Mello et al., 2011), that is, network modularity has a significant phylogenetic signal. The importance of phylogenetic resemblance to explain module composition will depend on the specificity of interactions, with host-parasite networks being the best examples (Krasnov et al., 2012). A strong phylogenetic signal in module composition would indicate potential coevolutionary vortices (sensu Thompson, 2005). In contrast, module networks without phylogenetic signal will be assembled randomly, or maybe determined by convergent adaptations, opportunistic interactions, or dispersal limitations (Krasnov et al., 2012).

We built a presence/absence bipartite network with the associations found in bibliographic records and our field data, linking each plant species with their nursery pollinators (Bascompte et al., 2003; Gómez et al., 2013; Nielsen and Bascompte, 2007). In the literature search we considered only the records that 1) contained information about the pollination and predation processes, or 2) contained information of the fruit predation by *Hadena* larvae, assuming that *Hadena* moths pollinated previously. References used are in the Supplementary Material 2. *Aneda rivularis* (Noctuidae) was described previously as *Hadena rivularis* and has been considered as pollinating-seed predator of 10 Caryophyllaceae (Kephart et al., 2006). For this reason it was included in this revision. The information of American species is scarce and based on few species, some of them introduced as *S. latifolia*, so we built the network only with Palearctic species, and more specifically with European species. We analyzed several properties of the network: number of plant species (*P*), number of moth species (*A*), total number of interactions (*I*), and modularity (M) (Fig. 2). To calculate the modularity in the presence/absence bipartite web (Barber, 2007), we used the QuanBiMo algorithm (Dormann and Strauss, 2014). We also explored the phylogenetic signal of each module with a Caryophyllaceae tree assembled for this purpose, using the D statistic index for presence/absence data (Fritz and Purvis, 2010) with the function *phylo.d* in package *caper* (Orme et al., 2013). We gathered information about the distribution and range size of all plant and moth species of the network from different sources (see Supplementary material 2). To explore whether references used to build the network were biased to plant and moth species with wide, medium or small distribution, we tested the effect of reference identity and reference country on the speciesí range size. We did not find influence of the study identity ($X^2_{35,213} = 21.26$, $P = 0.97$ and $X^2_{35,213} = 5.12$, $P = 1$ for Caryophyllaceae and *Hadenini,* respectively) or the country of origin in the species range distribution ($X^2_{13,235} = 27.69$, $P = 0.35$ and $X^2_{13,235} = 7.99$, $P = 0.99$ for Caryophyllaceae and *Hadenini*, respectively). These results suggest that the references used were not biased to species with wide distribution, and within the studied countries the sampling was not biased by the rage size of species. All analyses were implemented in R (R Core Team, 2014). For further details see Supplementary material 1.

The resulting network was modular. The empirical modularity (mean (SE) *z*-score = 3.36 (0.036), N = 100 iterations, all *P* values <0.005) was higher than random values in 100 replicates with a mean modularity (SE) of 0.48 (0.002), N = 100 iterations. We detected 9 modules (Figs. 2 and 3) in 57% of the iterations. In module 7, *H. compta* was linked mainly with *Dianthus* species, whereas in the other modules there were associations between several genera of Caryophyllaceae and *Hadena.* Eighty five point seven percent of the network species were present in the Caryophyllaceae tree. Phylogenetic signal was detected (Table 2) only for modules 1, 4 and 7 (Table 2, Figs. 2 and 3). A strong phylogenetic signal in module composition and in the connectivity between them might indicate potential coevolutionary units in the network (Olesen et al., 2007). The modularity may be caused by a combination of phylogenetic and ecological processes, such as convergent adaptations, opportunistic interactions, habitat preferences or biogeographic limitations (Bellay et al., 2015; Brito et al., 2014; González and Oliva, 2009; Krasnov et al., 2012; Rezende et al., 2009). In this case, the phylogenetic signal was owed to the close relationship of Caryophyllaceae species within modules (*Dianthus* spp., section Silene *sensu* Oxelman et al., 2013 and *Gypsophila* spp.). These groups of closely related species interact mainly with one *Hadena* species in each module (*H. compta*, *H. sancta* or *H. irregularis*). Within modules 1 and 4, the significant phylogenetic signal arose from the close relationships of some of the species. These two modules contain some species that are not closely related with the rest, but all of them share habitat preferences (sandy or rocky soils) or a Mediterranean distribution, respectively (Hacker, 1992, 1996, 1999; Laínz and Garmendia, 1990; Lu et al., 2004; Wagner, 2015; Walters, 1996). The rest of the modules without phylogenetic signal can be partially explained by geographic distribution or habitat preferences. In fact, there were differences between modules in the countries where interactions come from ($X^2_{84} = 117.54$, $P = 0.009$; $X^2_{84} = 115.36$, $P = 0.013$ for Caryophyllaceae and *Hadenini*, respectively). The module 6 contains plant and moth species of wide distribution (i.e. *H. confusa, H. bicruris, H. magnolii* and *S. latifolia, S. nutans, Saponaria officinalis*). All these species show a high number of associations and constitute the central core of the most generalist species in the network (Fig. 3). Some of these plants are cosmopolitan because they are associated with roadsides, crop edges and disturbed zones (Laínz and Garmendia, 1990; Tutin et al., 1993). Module 3 also contained Caryophyllaceae species of wide distribution, but associated with *Hadena* species with more limited range size that overlaps partially among them (Walters, 1996; José Luis Yela, unpublished data). Module 8 and 9 had species with small range size in Central and South Europe, Asia and North Africa (Laínz and Garmendia, 1990; José Luis Yela, unpublished data). Module 2 has plant species with affinity for open zones, as grasslands and rocky grounds, whereas module 5 is a cluster of mountainous species (Laínz and Garmendia, 1990; Wagner, 2015). In summary, these results suggest that Caryophyllaceae-*Hadena* associations are constrained by phylogenetic, geographical and ecological filters, as described for some host-parasite networks mentioned above. This preliminary assessment is very far from being definitive, since it spans only over a small fraction of the diversity of *Silene* and *Hadena* species, but is useful to illustrate the potential of this approach to explore the structure of pollinating-seed predator systems.

Table 2
Analysis of phylogenetic signal for the plant species composition of modules, measured by the D statistic. (*) denote significant values ($p < 0.05$).

	D statistic
Module 1	0.41 *
Module 2	1.15
Module 3	1.08
Module 4	−0.11 *
Module 5	0.64
Module 6	0.63
Module 7	−0.35 *
Module 8	–
Module 9	–

Module 8 and 9 could not be analyzed because it had 1 and 0 species in the phylogenetic tree (Fig. S1), respectively. Values of D statistic ≈ 0 indicates a Brownian motion mode of evolution, D ≈ 1 suggests a random mode of evolution (Fritz and Purvis, 2010).

5. Conclusions and future directions

The *Hadenini*-Caryophyllaceae nursery pollination systems seem to be parasitic, based on the few studies that have measured the net outcome. However, a possible drawback is that net outcome has been studied only for the female flower function (seed produced vs. seed predated). In the nursery pollination system between *Trollius europaeus* and Chiastocheta, a positive interaction has been postulated only for the male flower function because these flies are the best pollen dispersers, highlighting the different effects of the nursery pollinators over flower sexual functions (Suchan et al., 2015). Therefore, the pollinator importance of *Hadena* and co-polinators needs to be assessed in search for possible conflicting

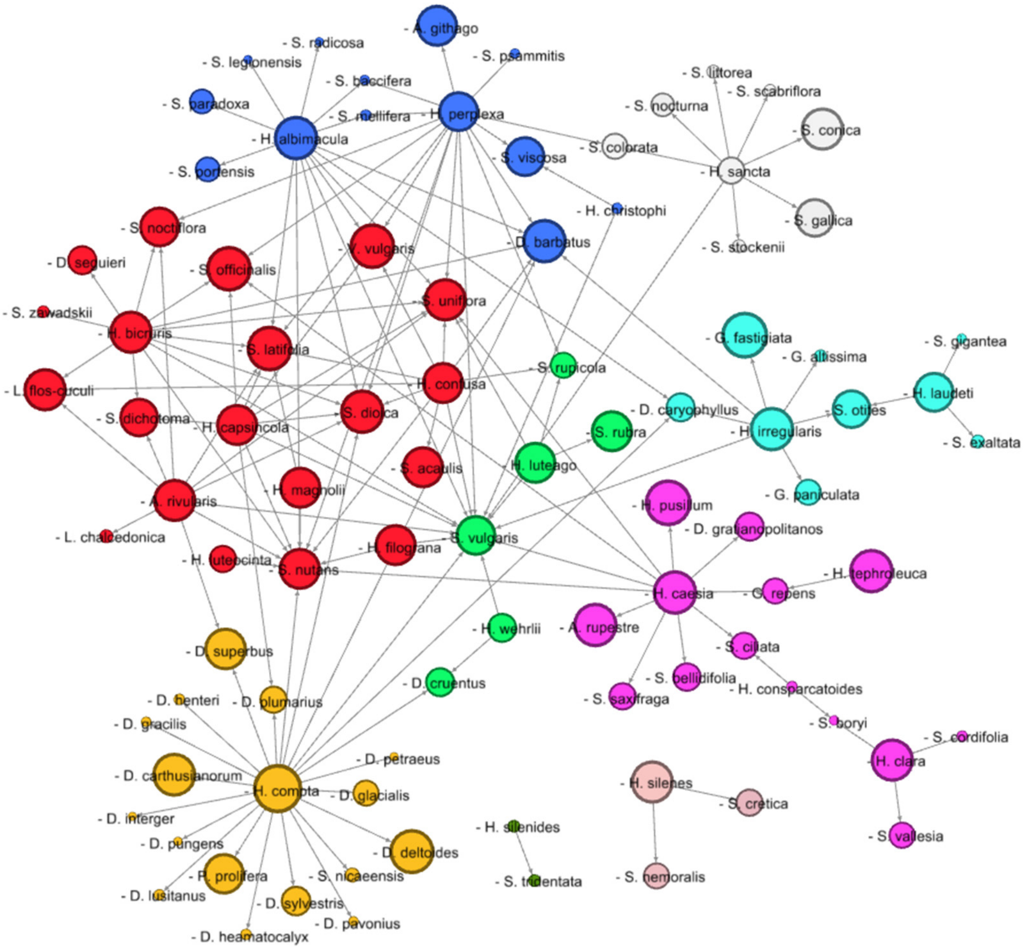

Fig. 3. Graphical representation of the *Caryophyllaceae-Hadena* nursery pollination network, with colors indicating the modules detected by the QuanBiMo algorithm (Dormann and Strauss, 2014). Size of nodes is proportional to geographic range size. A: *Aneda*, Ag: *Agrostemma*, At: *Atocion*, D: *Dianthus*, G: *Gypsophila*, H: *Hadena*, He: *Heliosperma*, L: *Lychnis*, P: *Petrorhagia*, S: *Silene*, V: *viscaria.* Graph created with Gephi v0.9.1 (Bastian et al., 2009).

selection between male and female flower functions. This may help to explain why *Hadena* nursery pollinators have not been excluded in presence of co-pollinators.

Despite there are few available studies to generalize that pollination syndromes correlate with the most effective pollinators in Caryophyllaceae, it seems reasonable to assume that some floral traits may have evolved in response to *Hadena* and other moths as selective agents. However, the special role of plant and moth as source of phenotypic selection on their partners' traits, whether mutualistic or antagonistic, has not been studied in detail and no direct evidence of reciprocal selection has been provided up to now. The unspecificity and apparent lability of the species' associations makes it difficult to study the selective forces acting jointly on plant and moth traits. Perhaps the study of the Caryophyllaceae-*Hadenini* systems that are phylogenetically more constrained (i.e associations between *H. compta* and *Dianthus*) may offer more robust conclusions about the coevolutionary history, avoiding the noise of the present time and local variation of selective forces. Since flower scent is a key trait for the maintenance of Caryophyllaceae-*Hadena* systems (Kephart et al., 2006) and scent composition is phylogenetically constrained (Prieto-Benítez et al., 2016b), we especially encourage the study of coevolutionary forces driving the composition of flower scent and the ability of its detection by *Hadena*. Finally, to gain an integrative view of this model system, we also need to adopt the framework of the geographic mosaic theory of coevolution, to explore the spatio-temporal variation of abiotic and biotic selective forces among nursery pollinators, legitimate pollinators, fungal pathogens, larval parasitoids and the rest of organisms interacting with Caryophyllaceae species.

With our preliminary analysis, we have shown that the network approach is a powerful tool to describe the structure of pollinating seed predation systems. Although the network is based in a limited number of associations, we found some interesting patterns to explore in the future. Three modules were constrained by the phylogenetic resemblance of host plant species, one of them likely evolved by a host shift from *Silene* to *Dianthus*. Other modules can be partially explained by the geographic distribution and habitat preferences of partners. In spite of our modest contribution to explore this system in the Iberian Peninsula, other diversification centers of both groups remain unexplored. For example, Turkey has a high richness and endemicity of *Hadena* (60 species, 12 endemic), as well as Caryophyllaceae (181 species, 31 endemic) (Greuter et al., 2015; José Luis Yela, unpublished data), which suggest promising avenues of future research. Future surveys of interacting species in unexplored regions, combined with complete co-phylogenetic analyses and information of the geographic distribution of species are necessary to go further in the understanding of the origin and evolution of this system.

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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.2017.02.004.

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