Integrated conservation of bee pollinators of a rare plant in a protected area near Bologna, Italy

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SUMMARY

An integrated approach was proposed for the conservation of the bee pollinators of the locally rare plant dittany *Dictamnus albus*. Based on previous studies that revealed the most efficient pollinators, we performed three related actions to improve their presence in the area: (i) we provided artificial nests for bumblebees and solitary bees; (ii) we added bee plants to support local populations of pollinators throughout their life cycle, and (iii) we reared and released bumblebee colonies from wild queens collected in the area. Artificial nests were occupied at high rates by cavity nesting species such as mason bees, leafcutter bees and carpenter bees, while we did not observe any ground nesting bees. Artificial nests for bumblebees did not attract any wild queens. The bee plants established at different rates: transplanted adult individuals survived better than seeds directly sown at the site. In three consecutive years we reared and released several colonies of buff-tailed bumblebees, which survived through the flowering season but only one developed new gynes.

BACKGROUND

In the last decades much attention has been given to honey bee colony collapse, but wild bees are also declining worldwide, both in abundance and species richness (Burkle *et al.* 2013, Ollerton *et al.* 2014). The main drivers of the decline are habitat fragmentation, land-use change, pollution and climatic changes, which may affect pollinators and reduce floral resources and nesting sites (Vanbergen *et al.* 2013, Goulson *et al.* 2015). Although the honey bee has been generally considered the most important insect pollinator, recent studies have demonstrated the importance of wild bees in the pollination of several crops (Garibaldi *et al.* 2013, Mallinger & Gratton 2014), wild plants (Ollerton *et al.* 2011) and floral resources in urban landscapes (Lowenstein *et al.* 2015). The provision of artificial nesting sites is among the most common methods used to support local populations of pollinators, and appropriate food resources are needed to ensure their sustenance throughout colony development.

From 2011 to 2015 a LIFE+ Biodiversity demonstration project (www.pp-icon.eu) has been carried out focusing on the conservation of an isolated population of dittany *Dictamnus albus* and its wild pollinators, within the Natural Park "Gessi Bolognesi e Calanchi dell'Abbadessa", located near Bologna, Emilia Romagna, Italy. While not threatened at global level, dittany is considered rare and it is locally protected in many European Countries (Italy, France, Germany, Spain, Czech Republic, Slovakia, Poland; Schnittler & Günther 1999). In Emilia Romagna, dittany is protected under Regional Law (L.R. 2/1977), and the studied area is included within a Natura 2000 site (SCI-SPA IT4050001) protected under the EU Habitats Directive.

The study site is mainly composed of abandoned coppice and abandoned pastures, interspersed with rural buildings and private land. The natural vegetation is dominated by downy oak *Quercus humilis* and manna ash *Fraxinus ornus*, and by mesophilous scrub of blackthorn *Prunus spinosa*, dog-rose *Rosa canina* and common dogwood *Cornus sanguinea*. Residual ungrazed grasslands are dominated by cock's-foot grass *Dactylis glomerata*, common meadow-grass *Poa pratensis* and couch grass *Agropyron repens*. The vertebrate fauna of the Natural Park is well known; by contrast, no inventory of arthropods of this area is currently available.

In recent studies Fisogni *et al*. (2011, 2016) reported that the great majority of insect visitors and pollinators of dittany in the area are bees (Hymenoptera, Apoidea), including both social (honey bees *Apis mellifera* and bumblebees *Bombus* spp.) and solitary bees (e.g. mason bees *Osmia* spp., carpenter bees *Xylocopa* spp., mining bees Andrenidae, digger bees Anthophorinae, sweat bees Halictidae).

One of the main risk factors for the target population of dittany is pollination limitation: seed production may suffer from a reduced pollen supply, indicating a deficit in the pollination service (Fisogni 2010, Fisogni *et al.* 2016).

To support the local bee fauna and consequently favour the pollination of dittany, we pursued three actions: enhancing the number of native bee plants, providing artificial nesting sites for solitary and social bees, and reinforcing the bumblebee population through the rearing and releasing of wild colonies.

ACTION

Providing nests for pollinators: We built artificial nests for bumblebees using upside-down terracotta flower pots, with a diameter of about 20 cm, filled with straw and bedding for caged hamsters (which is known to be attractive for bumblebee queens). Nest entrance was provided by a 25-30 cm long piece of garden hose (internal diameter 18 mm). The base of the pot and the hose were buried and covered with soil and leaves, leaving only the outer end of the tube free (Figure 1a). An accurate description of bumblebee nest construction can be found in Bortolotti *et al.* (2015). Nest materials cost €5 per

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ISSN 1758-2067

Figure 1. Artificial bumblebee nest: a) in the field, b) periodic upkeep.

nest. Ten bumblebee nests were placed in the area in spring 2011, preferably in sheltered places, such as tree bases. Nests were rearranged and supplied with new litter every year in early spring to increase their attractiveness (Figure 1b), and the occupancy was checked during the season by periodical observations of the nest entrance, and at the end of the season, by opening the nest to search for signs of bumblebee presence. Nests were then cleaned and left in the field until next year.

Artificial nests for solitary bees were modified throughout the project in response to project results (i.e. identification of the best pollinators of dittany), and to avoid the problem of ant colonisation experienced during the first year (see below). In 2011, we assembled 15 nests for cavity-nesting solitary bees. Each nest contained 28 holes of seven different sizes, ranging from 0.2 to 1.4 cm diameter. In March 2011 nests were placed on trees and fixed to branches and trunk with wire at a minimum height of 1.5 m to avoid mammal predation (Figure 2a). Since 10 out of 15 nests were colonised by acrobat ants *Crematogaster scutellaris*, which consumed or threw out the larvae and the pollen the nests contained, in April 2012 we put six different nests on a pole fixed in the ground and spread with ant glue (Glu arboricole Pelton 2) (Figure 2b). Each nest contained eight wooden cubes presenting cavities of different size, from 0.6 to 1.4 cm diameter. The smallest holes were no longer used because they hosted small bees that act as pollen and nectar robbers in dittany. Nest materials cost €80 per nest. Nest occupancy was assessed by visual inspection until October 2013.

Figure 2. Progression in solitary bee artificial nest site designs during project: a) 2011 nests, b) 2012-2013 nests, c) 2014-2015 bee hotels.

In spring 2014 we installed two “bee hotels” (40 × 70 × 150 cm) in the area: each one contained the above described wooden cubes and canes of various length and diameter. Canes of at least 60 cm length and 1.2 cm diameter were added for the mating and nesting of carpenter bees (Vicedomini 2009). In addition, to favour digger bees, we added perforated clay bricks filled with mud, and cleared the ground of the bee hotels (about 80 × 160 cm) from wild plants, turned it over, covered it with soil and sand and surrounded it with bricks (Figure 2c). Bee hotel legs were fixed in the ground and covered by ant glue. The material and manufacturing costs amounted to €600 for each bee hotel. Nest occupation was not recorded in 2014-2015, but nest holes were periodically inspected to check for damage by predators or the presence of nest intruders. Every year new canes and perforated wooden blocks were added to increase nesting sites or to replace the old and damaged ones. Fresh glue was spread on bee hotel legs several times from March to October to prevent predation by ants.

Planting of bee plants: In order to ensure and increase food resources to pollinators throughout their life cycle, we planted 17 native bee plants (i.e. plants that provide nectar and pollen for bees) with different flowering phenologies (Table 1). Selection of species was based on their attractiveness to bees, flowering period and environmental suitability (Mossetti 2015). We limited as much as possible the use of species that flower in May, in order to reduce the possibility of competition with dittany for pollination services. The propagation strategy comprised seed and/or adult plant collection (depending on species lifespan), seed germination in the greenhouse, seed propagation, and juvenile and/or adult transplantation to the abandoned pastures or to the wood fringes in the study site. Seeds and adult individuals were collected from local (regional) wild populations, except for deadnettle *Lamium* spp. and lungwort *Pulmonaria vallarsae* that were taken from the Bologna Botanic Garden, and were of wild regional provenance.

In November 2011 we directly dispersed in the target area some diaspores collected during summer and we planted adult individuals of long-lived perennials; in early spring 2012 we transplanted adults and plantlets germinated at the Botanic Garden (Table 1). During late spring and summer 2012, we collected mature seeds of the selected plants following “ENSCONET Seed Collecting Manual for Wild Species” recommendations (http://www.bgci.org/resources/news/0632). A technical data form was compiled for each source population. From July 2012, the collected seeds were both potted at the Botanic Garden and directly dispersed in the target area, in order to increase the possibilities of propagation. To reinforce populations introduced the previous years, during autumn 2012 we repeated transplantations of green hellebore *Helleborus viridis*, deadnettle and lungwort, and in April 2013 we also transplanted a few individuals from newly germinated juveniles at the Botanic Garden.

Rearing and releasing of bumblebee species: Queens of the most common bumblebee species were collected every year in the surroundings of the target area and reared in controlled conditions; the resulting colonies were released in the area before the beginning of the flowering season. The protocol applied for bumblebee rearing and releasing is described in Bogo & Bortolotti (2015). In September 2011, 15 queens of the buff-tailed bumblebee *Bombus terrestris* and eight queens of the common carder bee *B. pascuorum* were collected and

ISSN 1758-2067

Table 1. Details of the bee plants transplanted and established to benefit bee populations. Year = date of sowing or transplantation of individuals; flowering period refers to the study area.

Species	Flowering period	Sampling[1]	Provenance[2]	Year	Total number of seeds and planted individuals	Number of established plants
Helleborus viridis	Feb, Mar	Adult	Wild	2011-2012	30 plants	30
Pulmonaria vallarsae	Mar, Apr	Adult	BG, Wild	2012	>25 plants	8
Lamium purpureum	Mar, Apr	Adult	BG	2012	>25 plants	> 20
Lamium maculatum	Mar, Apr	Adult	BG	2012	>25 plants	> 20
Vicia sativa	May-Jul	Seeds	Wild	2012	≈ 70 seeds, 5 plants	5
Lathyrus latifolius	May-Aug	Seeds	Wild	2012-2013	≈ 60 seeds, 5 plants	5
Securigera varia	May-Aug	Seeds, adult	Wild	2012-2013	> 300 seeds	> 10
Hedysarum coronarium	Jun, Jul	Seeds, adult	Wild	2011	15 plants	2
Trifolium pratense	Jun, Jul	Adult	BG, Wild	2012	≈250 seeds, >25 plants	> 25
Scorpiurus muricatus	Jun, Jul	Seeds	Wild	2012-2013	> 60 seeds, 5 plants	5
Trifolium repens	Jun-Aug	Seeds	Wild	2012-2013	> 300 seeds	> 25
Prunella laciniata	Jun-Aug	Seeds	Wild	2012	> 60 seeds, 5 plants	> 25
Melilotus officinalis	Jun-Sep	Adult	Wild	2012-2013	> 200 seeds, 5 plants	5
Veronica spicata subsp. barrelieri	Jun-Sept	Seeds, adult	Wild	2011	15 plants	15
Vicia cracca	Jun-Sept	Seeds, adult	Wild	2013	> 60 seeds, 10 plants	> 10
Cephalaria transsylvanica	Jul-Sept	Seeds	Wild	2012-2013	10 plants, ≈ 30 seeds	> 10
Clinopodium nepeta	Jul-Sept	Adult	Wild	2012	5 plants	> 5

[1]Adult = vegetative adult plants; [2]Wild = local populations; BG = Botanic Garden

hibernated individually inside plastic Petri dishes (diameter 6 cm) placed in a fridge at 5°C, but they failed to survive, probably because of the low humidity. In the following years the hibernation protocol was gradually improved, using queens from commercial colonies, by putting them in groups inside plastic boxes filled with untreated topsoil, and placing them first at 15°C for a week (as transition period) and then 3 months at 5°C. Consequently, the percentage of diapause survival increased to 85% (Bogo *et al*. submitted). However, for the whole project duration we decided to collect in the field only post-diapausing bumblebee queens, to avoid depletion of wild populations. Nine *B. terrestris* queens were collected at the end of March 2012, but the colonies obtained did not develop adequately, probably due to an early spring and consequently a delay in our collecting campaign. As a consequence, in 2012 we purchased three commercial colonies of buff-tailed bumblebees from a local supplier (Bioplanet Soc. Coop. A.R.L., Cesena, Italy), to implement the pollination service on dittany. Each commercial colony cost €50. Colonies were removed before the emergence of males and queens, to avoid genetic contamination of the native populations. Conversely, in 2013 spring was late and rainy, so we could collect only eight post-diapause buff-tailed bumblebee queens and two post-diapause common carder bee queens at the end of March (Figure 3). Queens of the common carder bee did not develop a colony due to breeding difficulties, since this is a "pocket-maker" bumblebee species and does not feed larvae individually from pollen lumps (Ptáček *et al*. 2015). Therefore we proceeded in the following years only with buff-tailed bumblebees, which are easier to rear artificially. Colonies were periodically inspected both outside, to verify the presence of flying bumblebees, and inside, to check for survival and the presence of nuisances (Figure 4).

Figure 3. Management of bumblebee queens: a) collection in the field; b), buff-tailed bumblebee *B. terrestris* in the laboratory; c) common carder bee *B. pascuorum* in the laboratory.

CONSEQUENCES

Providing nests for pollinators: Bumblebee artificial nests were not occupied by queens for any of the five years of the project duration. Occupancy of solitary bee nests increased through years, particularly in medium- and large-sized cavities (Table 2). Canes with 0.6 cm diameter were the most frequently occupied in both 2011 and 2012, while there was a slight reduction in the proportion that were occupied in 2013. By contrast, there was a substantial increase in the occupation rate of larger canes across years, especially in the last year of monitoring.

The placement of bee hotels considerably increased the number and shape of cavities compared with previous availability of different types of nest. In 2014 for the first time we observed occupation of larger canes by carpenter bees, both during the early mating season and at later stages (Figure 5). During spring 2014, a random sample of individuals nesting in

Figure 4. An artificially-reared bumblebee colony a) bees in the colony; b) bees being released; c) periodical checking for colony development and presence of pests.

ISSN 1758-2067

Table 2. Proportion of artificial nests for solitary bees occupied during the first three years of the project.

Cavity diameter (cm)	Occupied cavities 2011		Occupied cavities 2012		Occupied cavities 2013	
	No./total	%	No./total	%	No./total	%
0.2	31/60	52	---	---	---	---
0.4	25/60	42	---	---	---	---
0.6	13/60	21.7	117/294	39.8	83/294	28.2
0.8	3/60	5	7/216	3.2	60/216	27.8
1	1/60	1.7	10/150	6.7	31/150	20.7
1.2	0/60	0	3/48	6.2	16/48	33.3
1.4	0/60	0	1/48	2.1	2/48	4.2
ALL	73/420	17.4 (5.7)*	145/756	19.2	192/756	25.4

* percentage calculated excluding the smallest (0.2 and 0.4 cm) cavities.

canes of different size were collected soon after emergence for identification. We found mason bees, leafcutter bees *Megachile* spp. and wool carder bees *Anthidium manicatum*. We did not observe any ground nesting bees at the base of the bee hotels nor digger bees in the mud-filled bricks. Solitary bee nests were partly colonised by nest competitors, parasites and predators in varying amounts (Figure 6). In the first group, we found grass-carrying wasps *Isodontia mexicana*, which feed their larvae on paralysed crickets stored in the nest cavities; nests of different spider catching wasps (Sphecidae, Eumenidae and Pompilidae) were also found inside holes. Among parasites, we mainly observed the bee-fly *Anthrax anthrax*, which lays eggs in the open cells of solitary bees. During the first year of the study 67% of nests of solitary bees were predated by acrobat ants.

Planting of bee plants: Of the long-lived adult individuals directly transplanted in the site, green hellebore and spiked speedwell *Veronica spicata* subsp. *barrelieri* were most successful, with 30 and 15 established individuals respectively (100% success rate for both species); the planting of French honeysuckle *Hedysarum coronarium* resulted in the establishment of two out of five individuals (Table 1). In 2012, the majority of individuals belonging to deadnettle and lungwort, despite flowering during spring, did not survive the summer due to the combination of an extremely dry season and presumed eradication by boars. In 2013 there was a recovery of deadnettle plants after spontaneous germination by seed, and by the end of the project several individuals were well established in the area. By contrast, only three plants of lungwort were observed in 2014 and 2015, likely due to disturbance by boars.

Several individuals planted in 2012 from seeds germinated at the Botanic Garden were observed in the following seasons (2013-2015). All the species transplanted in April 2013 survived to some extent (Table 1), and during surveys performed in 2014 and 2015 several individuals were in bloom and actively visited by bees (e.g. mason bees, carpenter bees and bumblebees).

Figure 5. Solitary bee species nesting in artificial nests: a) mason bees *Osmia cornuta* b) *Osmia* sp. using artificial nests, c) carpenter bee *Xylocopa violacea*.

Bumblebee rearing: The rearing process and release of bumblebee colonies in the project area improved from 2013 to 2015 (Table 3). In 2013, three out of 10 buff-tailed bumblebee queens produced a medium-size colony (10-20 workers and large brood) that were released at the beginning of May. Two other queens with a reduced brood (only few larvae and no workers) were placed in two of the artificial bumblebee nests that were not previously occupied, but they did not produce a colony. The first three colonies survived during the flowering of dittany, and one of them developed until gynes production. At the beginning of May 2014 we released nine colonies. After one month, six of them were still in good condition, but during summer they were severely attacked by parasites and predators, and failed to produce new gynes. In 2015, all seven colonies released survived throughout the season, but again they suffered attack by parasites and predators and did not survive until gyne production.

Among parasites we observed larvae and puparia of the fly *Brachycoma devia* and the lesser house fly *Fannia canicularis*, and larvae of the bee moth *Aphomia sociella*. Among predators we mainly observed the hornet *Vespa crabro*.

DISCUSSION

The introduction of artificial nests and bee plants to enhance and support wild pollinators is quite a widespread practice in conservation programs, as well as in bee-friendly gardening. In this project we proposed an integrated approach that combines habitat management, such as the plantation of bee plants, and conservation actions towards pollinators (Bogo *et al.* 2015).

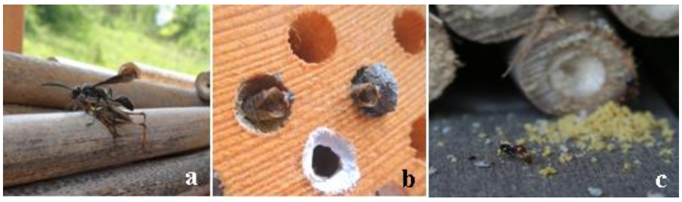

Figure 6. Nest intruders, parasites and predators of solitary bee nests: a) adult grass-carrying wasp *Isodontia mexicana* nesting in bee hotel, b) bee-fly *Anthrax anthrax* exuviae emerging from nest cavities, c) acrobat ant *Crematogaster scutellaris* feeding on the stored pollen.

ISSN 1758-2067

Table 3. Number of wild queens collected in the field, and resultant colonies released and surviving in the field until the end of May in each year of the project.

Bumblebee rearing progress	2012	2013	2014	2015
Collected queens	9	10	32	26
Egg laying queens	5	7	18	15
Colonies released in the target area	0	5	9	7
Colonies survived in the field	0	1	6	7

The pollinating species targeted by our actions are among the most common and efficient pollinators in the study area, and of dittany in particular. Among solitary bees, we observed good artificial-nest occupation by mason bees and leafcutter bees, and a sporadic but promising presence of carpenter bees at our artificial shelters. On the contrary, we did not observe the presence of digger bees in the artificial clay bricks or ground nesting bees in the turned soil below the bee hotel; other surveys also indicate a low effectiveness of artificial nests for these bees (Gibbs 2004).

The most common cavity nesting species are usually easily attracted by bee hotels and artificial nests, and several studies report encouraging results (reviewed in Dicks *et al.* 2010). Nevertheless it is complicated to calculate the benefits of these measures on local bee populations, and additional investigation to understand the pitfalls and benefits of bee hotels on bee biodiversity and pollination is needed (MacIvor & Packer 2015).

The high occupation rate of our artificial nests by medium-sized mason bees and leafcutter bees and by large carpenter bees suggests an increased availability of efficient pollinators for dittany, and consequently for the other flowering plants in the site. Accordingly, the survey on dittany pollinators during the four years of the project (Fisogni *et al.* 2016) showed a significant increase of mason and leafcutter bees in 2014 with respect to the previous years. By contrast, no increase in bumblebee visits to dittany were recorded, despite the strong efforts in colony release. This could be because the number of released colonies was not adequate, or bumblebees were attracted by other co-flowering plants. Other variations in dittany pollinators were probably independent of our actions: for example, the two abundance peaks observed in 2013 and 2014 for the digger bee *Habropoda tarsata*, a species which was not attracted by our artificial nests, and the high inter-annual fluctuation of honey bees.

The presence of parasites and predators in artificial nests apparently did not appear to severely affect the nest occupancy by solitary bees, which increased through years. The most aggressive predators in solitary bee nests were acrobat ants, which destroyed two-thirds of the nests in 2011. In the following years ant predation was easily prevented by placing nests on a pedestal covered by ant glue. Other methods of protection from ants are described in the literature (Zammit *et al*. 2008).

The artificial nests and bee hotels were left in the area to increase nesting places for solitary bees after the end of the project. Nests will be periodically cleaned and renovated for at least the next five years (2016-2020) to guarantee their continued functionality, as foreseen in the after-LIFE communication plan (http://www.pp-icon.eu/site/wp-content/uploads/Annex-7.3.2-After-Life-Plan.pdf).

Wild bumblebees did not use the artificial nests provided and these results support other works that highlight the low occupation success of artificial shelters by wild social species (reviewed in Dicks *et al.* 2010). However, a significant number of colonies of the buff-tailed bumblebee were released after rearing wild queens in controlled conditions. Artificial rearing is more expensive and time consuming than providing artificial nests outdoors, especially due to the maintenance of a climate room and the continued husbandry of the colonies, although despite costs it can be regarded as a good conservation measure for bumblebees (Goulson *et al.* 2002). Nevertheless, our released colonies struggled to survive through the season, likely due to the presence of parasites and predators that caused developmental arrest before the emergence of queens and males. Therefore, although we enhanced the bumblebee population during the flowering of dittany, we did not observe an increase in dittany flower visits by bumblebees (Fisogni *et al.* 2016) and we did not succeed in establishing a new bumblebee generation for the forthcoming years.

All the bee plants transplanted as plantlets or adults established some individuals in the area, while directly sown seeds showed a lower success. The plants that flowered attracted bees of several species throughout the year. In particular, early flowering species such as the green hellebore could represent an important food resource for bumblebee queens and early pollinators like mason bees, carpenter bees and other solitary bees. Considering the results obtained after three years from these actions, the introduced plants are expected to maintain themselves without further management, and eventually increase in abundance.

ACKNOWLEDGEMENTS

We thank the Regional Park Gessi Bolognesi e Calanchi dell'Abbadessa for permitting fieldwork. This work was performed within the Life+ Project PP-ICON (Plant–Pollinator CONservation approach: a demonstrative proposal – LIFE09/NAT/IT000212) funded by the European Union.

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ISSN 1758-2067