PP-ICON - Plant-Pollinator Integrated CONservation approach:
a demonstrative proposal

Technical manual

DICTAMNUS

CONSERVIAMO, IMPOLLINIAMO, RACCONTIAMO

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Technical handbook

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INTRODUCTION

Biodiversity cannot be safeguarded without an ecosystemic vision

Giovanni Cristofolini

Biodiversity, or biological diversity, is generally considered and quantified as the set of various species populating an environment or region of the Earth: the more varied the species, the higher the level of biodiversity. This is true to a certain extent, yet it is also a limited vision.

It is well known that no species – plant or animal – could live in a fully desert environment, meaning without any other living species. Interactions among species are far more numerous and complex than they might appear at first glance. Limiting the discussion to plant species (and, in this context, not taking into account the "adverse" actions of consumers and parasites), we can find interactions and symbioses on all levels: through the secretion of specific metabolites, roots interact with other plant species, bacteria and fungi; leaves and sprouts interact with bacteria and arthropods, mainly (but not only) through glands, extrafloral nectaries and other structures that produce a wide range of secondary metabolites; flowers interact mainly (but not only) with arthropods, as is discussed extensively in other sections of this manual; lastly, fruit and seeds owe their dispersion to vertebrates and invertebrates.

Awareness of the complexity and importance of these interspecific relationships led to the concept of "ecosystem". First proposed by the British ecologist Arthur Tansley in 1935, for decades it was the main approach to interpreting the environment and the biological diversity it hosts.

In an ecosystemic vision, biodiversity is not reduced to the mere number of different species present in an environment, but is better represented by the number and complexity of their interactions, because interactions are the condition for the life of the species and the very stability of the system.

Based on the above, it follows that the practice of conservation of the species must be considered from this standpoint. While conservationism in the first half of the twentieth century focused above all on the species per se, as if it were conceivable to conserve a species without considering the conservation of the species with which it interacts, in the second half of the century there was growing awareness that no species can survive if its environment is not preserved or – in short – if its ecosystem is not maintained. For any species to survive, it is not enough to maintain its physical environment: the species with which it interacts, and thus the network that mutually unites them, must also be preserved.

The pollination process, which is the core of this programme, is a clear example of the interdependence of different species, in this case of plant species and the animals that pollinate them.

PREFACE

Why plant-pollinator interactions are important

Marta Galloni

The stability of ecosystems is closely tied to the action of pollinators: they influence the floristic diversity of habitats, the genetic variability of plant communities and, in general, ecological relationships as a whole, determining evolutionary phenomena of adaptation, selection and speciation.

What are pollinators?

The pollination process, meaning the transfer of pollen from the anthers (male part) to the stigma (receptive female part) of flowers, is fundamental for the sexual reproduction of flowering plants (angiosperms): once the pollen reaches the stigma, it can germinate, launching the subsequent process of fertilization, which ends with the development of seeds and fructification.

In some plants pollination within the same flower or between flowers of the same plant (self-pollination) can lead to the production of seeds through self-fertilization. Nevertheless, in many plant species this is greatly limited or prevented entirely by intrinsic mechanisms such as morphological traits, the staggered maturation of male and female reproductive structures, and physiological systems of self-incompatibility, which encourage the cross-breeding of different individuals. Allogamous plants, which cannot self-pollinate or self-fertilize, necessarily require a pollination "service", meaning a vector that will transfer pollen from one flower to another among different individuals that are genetically distinct.

In some cases, pollen transport occurs thanks to wind (anemophily) and more rarely water (hydrophily), while for most plants (around 90% of known species), the vectors are animals (zoophily).

The pollination of flowers through animals effectively constitutes dependency between two partners – plants and pollinators – that, by mutually exerting pressure, determine the parallel evolution of both. This explains why the rapid "evolutionary radiation" that, starting with their appearance on Earth around 150 million years ago, led angiosperms to their great current diversity (approximately 300,000 estimated species) largely depended on this co-evolution with pollinators.

Insects currently represent the most important group of pollinators (entomophily). Among them, from standpoint of plants, bees (in the broadest sense: Hymenoptera Apoidea) generally provide the best "service", thanks to their abundance as well as their behaviour, often "virtuous" (constancy, loyalty, efficiency).

Why do bees visit flowers?

Like all pollinating animals, hymenoptera are attracted by flowers on which they usually find a "reward", commonly represented by food sources such as nectar and pollen. As the pollinator takes its reward, it accidently gets loaded with pollen and involuntarily "reciprocates" by transporting and depositing the pollen on another flower.

This is a fully fledged exchange of goods and services between two organisms that are thus very closely dependent on each other, in a way that can be more or less marked, depending on the degree of specialization of the relationship.

Consequently, when we plan management work to protect rare and threatened entomophilous plants it is essential to consider not only the plant species per se, but also the pollinator species connected with them. Both the quantity and quality of the progeny of the "partner" plant, and thus its possibility to propagate, as well as the genetic variability of the populations depend on the abundance and visiting behaviour of these wild pollinators, as well as their interactions with all the flowering species in the habitat.

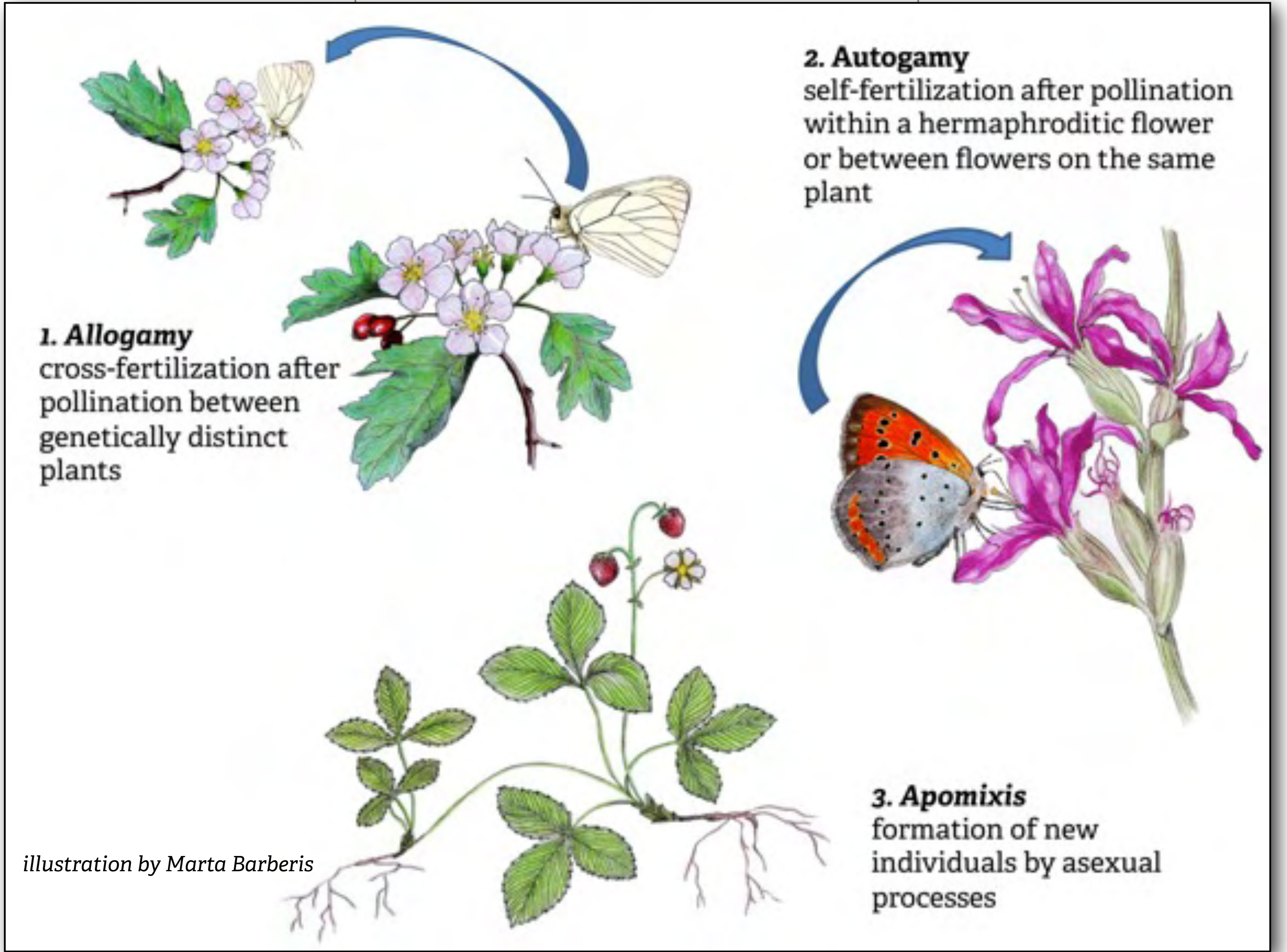


illustration by Marta Barberis

CHAPTER 1

Conservation

Analysis techniques for natural populations and conservation strategies

Monitoring population structure, plant demography and reproductive fitness

Population of dittany at Parco Regionale dei Gessi Bolognesi e Calanchi dell'Abbadessa, SCI/SPA IT4050001.

The life cycle of flowering plants typically consists of many consecutive steps: flowering, ovule fertilization and seed production, seed germination, seedling establishment and growth, and so on. However, each phase might become a limiting factor compromising population performance and persistence. It is therefore necessary to identify, when present, the limiting stages to the development and survival of a population. To do so, it is useful to study plant development and change over time in natural populations and to consider different parameters that can define their trends. The most valuable tool to highlight population dynamics is demographic monitoring, which entails the collection and analysis of repeated observations or measurements. Species and population monitoring may help to determine which factors impact the populations, positively or negatively, and to predict the possible effects of management activities. Consequently, these findings may be useful to validate or redirect specific actions when it comes to making management plans.

Before starting a monitoring plan it is important to choose the traits that are going to be estimated. The presence or absence of the species is a simple qualitative attribute, while common quantitative measurable attributes include spatial cover, the density of individuals, and the frequency and abundance of age classes. For prolonged monitoring, it is more useful to consider traits that depend on life history and permit quantitative analyses. First of all, it is important to collect relevant information on the species and/or populations that are going to be considered. Specifically, when doing a demographic survey with repeated observations through time, the life stages of single plant individuals must be defined and characterized. Typical categories may refer to class ages, such as i) seedlings (just emerged from seeds), ii) juveniles (with no reproductive structures), iii) sterile adults (non-flowering individuals), and iv) fertile adults (flowering plants). To determine the features that make it possible to distinguish the different classes (e.g., plant height, leaf or leaflet number), it is helpful to consult the existing bibliography regarding the studied species. If no information is available, specific studies for this aim must be carried out before this monitoring can be implemented.

Once plant demographic stages have been identified, the sampling method must be delineated. The random or fixed transect is a simple method that can be useful when there is an ecological gradient in the population, but also with sparse plants within a large population. Data can be collected at fixed intervals along the line or by using observation quadrats – not necessarily adjacent – throughout the transect. The biggest disadvantage is that transects can be very time-consuming over long distances if plant cover is dense; moreover, data collected from random transects may not be suitable for statistical analysis. The permanent plot is a more relevant method. Data from several years are needed, but this can be more informative about changes in density and the trend of shifts in age classes, especially for perennial plants. Moreover, it makes it possible to overcome problems of seed dormancy and to evaluate the presence of soil seed banks. The number and measurements of the plots should be defined in advance, depending on the size of the populations and on the monitoring effort that can be conducted. Furthermore, the position of the quadrats must also be decided, depending on the ecological context present in a population. For example, if a population grows in different

***The example of* Dictamnus albus**

***Study species**: Dictamnus albus, a long-lived perennial herb that reproduces sexually by seed.*

***Optimal habitat**: Edges between woods and grasslands and clearings in xerothermic woodlands.*

***Limiting factor**: Forest closure, low incident light at ground level that limits adult flowering and seedling establishment.*

***Negative consequences**: In the long term, the absence of plant turnover from seed will lead to local extinction of the population.*

***Management actions**: We cut trees and shrubs in two permanent plots of 16 m2 to create artificial clearings, one in a younger portion of the wood and the other in a more mature part with more dense wood canopy. Two additional plots were chosen as controls.*

***Sampling method**: We divided the plots in 16 quadrats of 1x1 m2 and used fixed quadrats. We counted the number of plants within quadrats divided by age class: seedling (a), plantlet (b), juvenile (c), sterile adult (d), flowering adult (e).*

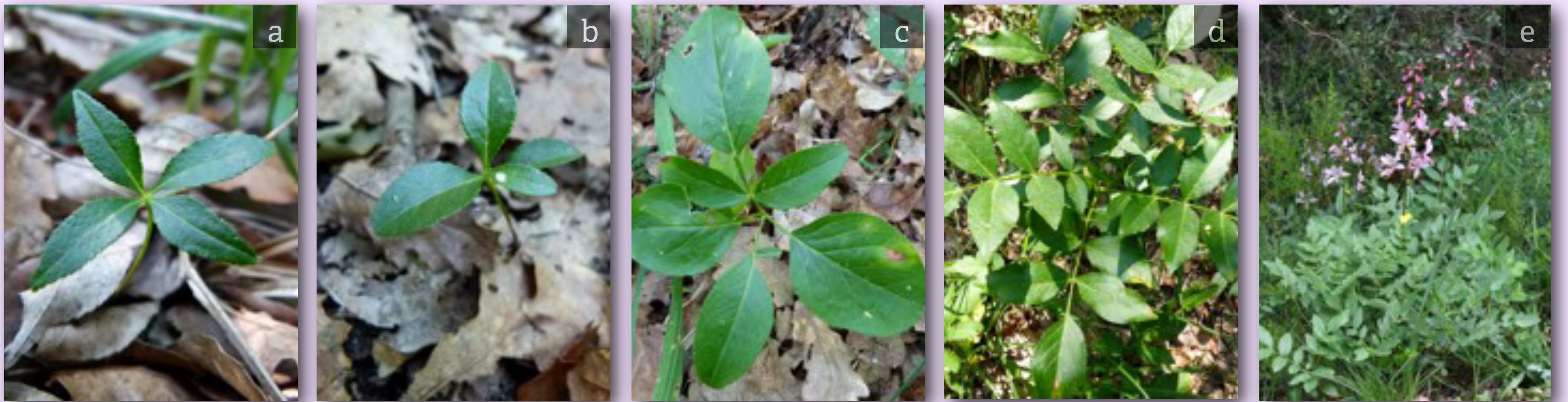
***Results 1**: Increased ground illuminance in both managed plots, of up to 8 times with respect to control plots in the young wood and more than 3 times in the mature wood.*

***Results 2**: Increased number of flowering plants and seedling germination in the managed plot in the young wood with respect to control.*

***Results 3**: First flowering plants observed in the managed area in the mature wood; no flowering plants were observed in the control plot.*

***Outcomes**: Inexpensive actions such as selective cutting of trees to create small artificial clearings may be very effective to help the development of local populations of plants typical of prairie-woodland ecotones. In a species like* D. albus*, it is better to act at early stages of wood closure to have higher and more immediate benefits.*

***Future activities**: Managed plots must be maintained through the periodic cutting of shrubs. A lapse of 3–5 years may be sufficient depending on growth rate of the species involved.*



habitats (e.g., at the wood edge/inside the wood, in humid/dry soils), monitoring should be performed in each context. Sampling period and effort through time must also be defined. These should depend on the life cycle of the species or specific changes of conditions during the vegetative season. For example, many herbaceous species pass the winter in different vegetative forms (roots, leaf rosettes) and only towards spring do they start to grow aerial or fertile parts. If possible, it is better to perform the observations when every life stage can be observed at the same time: it often coincides with flowering time, when it is possible not only to discriminate fertile plants but also to determine plantlets that have just germinated by seed as well as later stages of plant development. If this is not possible, samplings should be repeated at different times to cover all life stages.

Before starting field surveys, it is important to prepare complete data collection forms. These field data sheets must contain all the information need to univocally define the population and plot being observed, and the plant characteristics that are being monitored. A good heading might report the date, site and number of the plot observed, with GPS coordinates if possible, the name of the investigator(s), the type of unit under survey (e.g., control plot, managed plot – wood cut, weeded), and reference number of any photographs that have been taken.

The table with field data should include a plant ID number, the life stage of the observed plant, the number of vegetative or flowering stem(s), plant height or other important morphological or morphometric characters, the number of flowers, the number of fruits and the number of seeds that are produced. Moreover, it is useful to report traits that can define species biology, such as life history traits (annual or perennial), life expectancy (short or long), reproductive ecology (flowering and seed maturation period, seed production and availability) and seedling ecology (germination and establishment requirements). It is also helpful to report main population/species features (population size, number and distribution of populations), habitat characteristics concerning soil and disturbance, types of natural threats (e.g., herbivores, invasive species) and anthropogenic threats (e.g., logging, grazing).

Monitoring in the field requires simple but specific material to define observation plots and follow plant development. The most common and helpful elements needed during sampling are rigid pre-assembled quadrats; if possible, quadrats should have a grid to help plant counts (e.g., a quadrat of 1x1 m2 divided in 100 cells of 10x10 cm2). Bands and stripes are often needed to delimit observation plots, and yardsticks or tape measures may be helpful to delineate the position of individuals within a quadrat. Tags and nails may also be useful to univocally mark single plants and to follow their development through time.

Additional analyses that can be performed together with demographic sampling concern plant reproductive fitness monitoring. Different parameters may be used to assess plant fitness and might be chosen before planning the monitoring campaigns: for instance, the total or mean number of flowers, fruits or seeds produced by plants or in a standard plot (e.g., the permanent quadrat) may be considered. The proportions of fruit:flower and seed:ovules may give more clear and comparable results. Further studies may then focus on seed features: seed weight, seed vitality, and germination and plantlets characteristics may be assessed in the laboratory to understand resource allocation and post-zygotic effects. Like demographic monitoring, specific data sheets and appropriate material must be prepared in advance. Field data sheets should contain information regarding the fitness components that are being observed, as well as data on the site(s) and plants being monitored. Usually, simple materials are required on the field, such as gloves and bags to sample plant material (fruit, seeds) or for seed germination in controlled pots, while more professional equipment is required for laboratory analysis. The monitoring period should also be decided depending on plant development in order to guarantee the best evaluation of fitness components.

Pollination deficit: field techniques for a practical approach

Monitoring of visitors and pollinators of Dictamnus albus

Pollination limitation occurs when plants produce fewer fruits and/or seeds than they would with adequate pollen receipt. It is often the consequence of insufficient visits by pollen vectors or of low efficiency in pollen transfer, though other pre- and post-zygotic factors may influence reproductive success. Pollination deficit may have two components that are not mutually exclusive. Quantitative limitation is a result of insufficient pollen receipt after insufficient visits by pollen-bearing animals; qualitative limitation occurs: i) when pollen with reduced viability reaches the stigma, ii) after self or geitonogamous pollination mainly in self-incompatible species, or iii) after heterospecific pollen transfer.

The occurrence and magnitude of pollination limitation varies over years within populations and among populations. Different ecological perturbations may alter the plant-pollinator relationships and culminate in pollination limitation. One of the most common threats to natural species is habitat fragmentation (e.g., deforestation, urbanization, increase of intensive cultivation regimes): this can directly or indirectly modify the abundance and composition of animal and plant species, leading to an increase of pollination limitation. The decrease of plant population density or dimension and pollinator loss may contribute to modifying ecological interactions with negative effects on plant reproductive success. Reduced seed production may hamper the persistence of natural populations, especially in short-lived species that rely on seed for population persistence; it can also promote the evolution of self-compatibility mechanisms and have a role in the selection of secondary flower traits.

A relatively simple and inexpensive method to detect the presence of pollination limitation in the field is the supplementation of flowers with cross-pollen, which consists of replacing the natural vector that would transport pollen from the anthers of a flower to the stigma of a different compatible flower. The reproductive success of pollen-augmented plants will be then compared to that of control plants (not manipulated). First of all, two preliminary conditions must be fulfilled to succeed with hand pollination: pollen collected from the donor flower must be viable and the receiving stigma must be receptive. Morphological and phenological traits may be directly observed in order to estimate pollen viability and stigmatic receptivity, and/or more precise biochemical analyses may be performed: specific techniques allow these assessments both in the field and in the laboratory. It is helpful to consult specific literature and/or refer to experts' knowledge. Generally,

illustration by Marta Barberis

it is necessary to sample freshly dehisced anthers that present good amounts of pollen, usually easily detectable by eye, in order to increase the probability to collect viable pollen. It is also useful to mix up or simply use pollen taken from different flowers and plants, and to perform multiple pollinations on a same flower on consecutive days.

In order to avoid genetic similarities, with consequent problems that could reduce seed production and fitness of the progeny, we recommend choosing donor and receiving plants that are sufficiently far apart from one another. It is not possible to define a minimum distance good for all plant species, since the population genetic structure is influenced by mating systems and dissemination strategies. However, an average distance of 20–30 metres should be adequate within a population, even though lower distances do not necessarily imply reduced fecundity. It is important not to sample individuals too far apart from each other or belonging to different populations, since local adaptations may imply lower fitness of outcrossed progeny.

Entire flowers or single stamens with dehiscent anthers may be collected as pollen donors. Fine forceps should be used and samples can be put in vials or small containers, which are easy to carry and handle. Once a sufficient number of donor anthers have been gathered one can proceed with hand pollination. Depending on their size, stamens may be held with fine forceps and anthers can be gently brushed directly on receptive stigmas. It is usually possible to observe the pollen deposited on the stigma, depending on the size and amount of pollen grains.

Alternatively, a paintbrush with fine tip can be used to collect the pollen and touch the stigma. Once the flower has been handled, it is important to mark the plants and single flowers univocally, by means of progressively numbered tags (a handy and inexpensive method is to cut a straw crosswise and lengthwise to make small cylindrical markers for floral pedicels).

Control flowers are not manipulated and are naturally pollinated by insects. Like hand pollinations, control plants and flowers must be marked, preferably with tags of different colours. The number of plants and flowers considered for each test will then be recorded on specific sheets for data handling and analysis.

A significant sample size must be chosen in order to obtain results representative of the entire population. The number of selected plants can vary according to population size and the type of inflorescence. A sample of 20–30 plants per treatment can be considered adequate: only one flower can be chosen for single-flowered plants, while the number of test-flowers will change in multiple inflorescences. All the flowers on a plant – or most if this is not possible – should be assigned to a single test.

In the case of dehiscent fruits, it is important to prevent seeds from dispersing before countings. At the end of the flowering, plants or single flowers should be isolated using lightweight and breathable netting, such as tulle or nonwoven fabric. The number of fruits and viable seeds will be counted at ripening. Seed viability may be assessed with specific analyses, such as germination techniques at different stages of development.

One must then calculate the proportion of fruit produced on the number of flowers considered (fruit:flower) in order to obtain the fruiting percentage. Similarly, the proportion of seeds produced on initial ovules (seed:ovule) must be calculated. Non-fecundated or aborted ovules are usually similar in shape and dimension to those present in the flower before fruiting, even though their number may vary considerably among species.

The last step to assess the presence of pollination limitation involves comparing the productivity of hand-pollinated flowers and open pollinated controls: if the proportion of fruit and seed produced after artificial pollination is significantly higher than controls, then we can infer that the studied population is suffering from pollination deficit. Simple statistical analyses, such as chi-square or t-tests, may be used to estimate if the differences recorded in fruit and/or seed production may be considered random or a consequence of manipulations.

Pollination glossary

Allogamy: *cross-fertilization after pollination between genetically distinct plants; a pollen vector is needed.*

Anther: *terminal portion of the stamen that produces pollen, released at dehiscence.*

Autogamy: *self-fertilization after pollination within a hermaphroditic flower or between flowers on the same plant (see geitonogamy).*

Dehiscence: *spontaneous opening of plant structure (e.g., anther d., fruit d.).*

Dichogamy: *temporal separation of anther dehiscence and stigmatic receptivity within a same flower; prevents or limits self-fertilization within a flower.*

Geitonogamy: *pollination between different flowers of the same plant; it has the same genetic characteristics as self-pollination (see autogamy).*

Herkogamy: *spatial separation of anthers and stigma within a same flower; morphologic mechanism that prevents or reduces self-fertilization.*

Ovary: *portion of the pistil that contains the ovules; it develops into fruit after fertilization.*

Pistil: *female reproductive structure of the flower; composed of the ovary, style and stigma.*

Pollen: *single cell representing the male gametophyte; it contains male gametes. Formed within the anther and released at dehiscence. Primary reward for pollinators.*

Pollen vector: *means by which pollen in transferred from anther to stigma. The main pollen vectors are animals (zoophily), wind (anemophily) and water (hydrophily).*

Self-incompatibility: *genetic mechanism that prevents self-fertilization. Pollen produced by a flower cannot germinate or fertilize ovules within a same flower, in flowers on a same plant or in genetically similar plants.*

Stamen: *male organ composed of sterile filament and terminal anther.*

Stigma: *upper or terminal portion of the style on which pollen grains are received.*

Style: *sterile part of the pistil that, if present, connects stigma and ovary*

Habitat restoration and land management

The Apennines around Bologna

The Bologna region has a diverse and naturally rich range of ecosystems – wetlands, prairies, woodlands – that have been changed over time by human activities such as landscaping. In urban environments, landscaping has fragmented natural areas, making them disconnected and causing natural processes to break down. Active habitat restoration and land management can protect biodiversity and help conserve and restore the health of natural habitats for the people, plants and animals that depend on them.

Habitat loss is currently the leading cause of species endangerment and is predicted to be so in the future; at the same time, conservation biology is undergoing a paradigmatic shift away from single-species conservation efforts and toward habitat, ecosystem and regional efforts.

The broad context of habitat- or ecosystem-level conservation efforts is especially appropriate for the PP-ICON project because of the web of interactions linking plant species via pollinators. The habitat-management actions have been performed in a protected area, the Parco Regionale dei Gessi Bolognesi e Calanchi dell'Abbadessa, SCI/SPA IT4050001.

Surveys were conducted on site to identify four portions of woods with similar ecological conditions, two in young and more dynamic woods and two in older and more mature woods. The paired sites had the same exposure and wood cover.

At the beginning of the project the habitat was managed to establish the best environmental condition for the future persistence of the target plant population. Four areas representative of the population of *Dictamnus albus* were identified and marked; subsequently, selective cuts of trees and shrubs were performed, opening two clearings that contrast the wood closure to assure optimal light incidence for the growth of dittany plants.

At the same time the effective pollinators of the target plant were identified and collected, in order to artificially rear and introduce them into the target area over the following years. As yet no experimental restoration has evaluated the relative effectiveness of restoring floral and nesting resources. Nevertheless, a number of studies suggest that either floral or nest site availability can limit bee reproduction or population size.

For effective restoration, the maintenance of the introduced pollinators was ensured by growing autochthonous nectariferous plant species, which were planted in the target area. The fitness of the plant and the presence of its effective pollinators were monitored for the duration of the project; pollinator insects in the target area were also monitored, to evaluate the impact of the intervention on the pollinating fauna.

Pollinator monitoring programs and long-term data series are essential for understanding if we are in the midst of a global pollinator decline. The issue has received great attention in the media as well as academic literature and the need to establish pollinator-monitoring programmes was recognized internationally in 1993 when pollinators were incorporated into the Convention on Biological Diversity. Pollinator monitoring is also an important goal of various EU programmes, which are now collecting monitoring data in several countries.

Restoring Habitats

Think twice before you plant flowers, especially in a natural habitat! Are they a ***native*** *species that will promote healthy habits for plants and animals, or could they be* ***invasive*** *species?*

Native plants are those flowers, grasses, shrubs and trees indigenous to our geographical region. Invasive species are those that, when introduced to a new location, either intentionally or accidentally spread prolifically, competing with native species for resources and eventually ***dominating the landscape****. Some invasive species were, or still are, popular ornamental plants used in landscaping.*
Because plants form the base of our food web, these invasive species can potentially wipe out not only native plants, but also the animals that rely on them. Restoration work must focus on getting rid of these invaders, studying how habitat degradation, invasive species and other disturbances change plant and animal ***diversity and processes.***

We can help restore native habitats by choosing to landscape with native plants best suited to our region. Landscaping in the likeness of nature can bring many benefits. Being naturally suited for an area, native plants require ***minimal maintenance and watering*** *once established.*

The restoration strategy comprised the collection of seeds and/or adult plants (depending on species lifespan), seed germination, seed propagation, transplantation of juveniles and/or adults in abandoned pastures close to the target population of dittany or at wood fringes. Seeds and/or adult individuals were collected from local (regional) wild populations (except for *Lamium* spp. and *Pulmonaria vallarsae*, transplanted from botanic garden populations with a regional provenance) by technicians and expert botanists. Seeds of each species were directly dispersed in the target area in order to increase the possibility of propagation and plants obtained from seeds germinated at the botanic garden were transplanted in the target area.

A critical element of restoration plantings for pollinators is the choice of plant species to include in the mixes. Mixes should include plant species that, in combination, provide a long period of bloom and are preferred by a diverse pollinator community. Another important finding emerging from studies of floral restorations is that often only a few plant species are responsible for the great majority of pollinator visits. This suggests that restorations can be made more

List of plant species transplanted in the area

Species	**Material**	**Provenance**
Helleborus viridis L.	*Plants*	*Natural population*
Lamium purpureum L.	*Plants*	*Botanic Garden*
Lamium maculatum L.	*Plants*	*Botanic Garden*
Pulmonaria vallarsae Kerner	*Plants*	*Botanic Garden, natural population*
Trifolium pratense L.	*Plants*	*Botanic Garden, natural population*
Veronica barrellieri Schott ex R. et S.	*Seeds, plants*	*Natural population*
Cephalaria transsylvanica (L.) Schrader	*Seeds*	*Natural population*
Lathyrus latifolius L.	*Seeds*	*Natural population*
Vicia sativa L.	*Seeds*	*Natural population*
Trifolium repens L.	*Seeds*	*Natural population*
Securigera varia (L.) Lassen	*Seeds, plants*	*Natural population*
Melilotus officinalis L.	*Plants*	*Natural population*
Scorpiurus muricatus L.	*Seeds*	*Natural population*
Vicia cracca L.	*Seeds, plants*	*Natural population*
Clinopodium nepeta (L.) Kuntze	*plants*	*Natural population*
Prunella laciniata L.	*Seeds*	*Natural population*
Hedysarum coronarium L.	*Seeds, plants*	*Natural population*

efficient and cost-effective by focusing on a subset of highly attractive species rather than simply increasing floral diversity.

In our project, nectariferous plant species have been selected taking into account the attractiveness to main pollinators of Dictamnus albus. In addition, the transplanted species have an asynchronous period of flowering to support the pollinators throughout their life cycle, with large periods of overlap to guarantee the insects more food resources.

During the monitoring of pollinators in the target area, flowers of some of these species were visited by insects, highlighting the success of the action. Moreover, the fact that a few species produced plantlets after natural germination in the field supports the positive results.

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CHAPTER 2

Pollination

Techniques for taking censuses, rearing and reintroducing pollinator insects

Census of pollinators in the field: methods of collection and identification

Wild pollinators sampling

The path to arrive at a model of sampling pollinating insects to be shared by the scientific community was long and can be broadly divided into two distinct historical moments, corresponding to two different information needs.

From the mid-eighteenth to the late twentieth century entomologists were chiefly committed to cataloguing all the species present in nature. The sampling technique was to walk through the most diverse habitats, haphazardly collecting material with a handnet.

By improving their personal experience, entomologists sampled more selectively, aiming to collect different-looking individuals to increase the chance of capturing different species.

Sampling methods for measuring and monitoring pollinator biodiversity

Surface areas and measurements indicated by way of example refer to one-hectare sampling areas

Observation plots
10 rectangular plots of 1x2 m
Observation and collection periods of 6 min.
10 observation rounds per growing season

Standardized transect walks
A permanent vegetated corridor 200x2 m (or 250x4) divided into 10 equal subunits
Specimens collected and counted during a regular walk of 5 minutes for each subunit (45–50 minutes total)
10 observation rounds per growing season

Variable transect walks
Within an area of 1 hectare, the observer is free to visit all the species in bloom during a thirty-minute walk at a slow pace
10 observation rounds per growing season

Pan traps
Triplets of pitfall traps are created with bowls with a capacity of 400 ml and a diameter of 20 cm, of which one painted yellow, one white and one blue with special UV-bright coatings that are very attractive to pollinators. Five triplets are filled with water and a few drops of detergent for a total of 15 traps. The triplets, set 15 metres apart from each other, are left on site for 48h.
6 observation rounds per growing season

Trap nests with reed internodes
10 poles, each formed by bundles of about 150 reeds of Phragmites australis, *with a diameter varying between 2 and 10 mm and a length of 15–20 cm*
Each pole of 2 trap nests is left in the field for the entire season

Trap nests with paper tubes of different sizes
10 poles, each formed by bundles of about 150 paper tunnels with a diameter of 6.5, 8 and 10 mm
Each pole of 3 trap nests is left in the field for the entire growing season

This methodology is called survey and, over time, it made it possible to set up national catalogues of species, which now constitute a valuable basis for wildlife checklists on a continental level.

Europe and North America are the best-surveyed areas in the world, where the discovery of pollinators new to science has slowed down considerably in recent years. However, this method is still valid in the less-explored areas of the world, where there is still a good chance of finding new species.

Nevertheless, today in countries with developed economies, the exploitation of natural resources has reached a critical point and there is a widespread risk of a loss of biodiversity. Consequently, the goals of scientific investigation on pollinators have changed.

The diversity and abundance of

Advantages and disadvantages of two of the most widely used methods

Pan traps and standard transects have been the most efficient methods in comparative tests and are therefore the most widely used today.

PAN TRAPS

Advantages

- *negligible collector bias*
- *highest sample coverage*
- *collected the highest number of species*
- *detected species similar to those of the transect methods*
- *inexpensive, reliable and simple to use*
- *can be used to attract pollinators in the absence of bloom*
- *provide data from sampling effort that is easy to quantify*

Disadvantages

- *provide no information on floral associations*
- *do not measure bee abundance*
- *might undersample larger bees*
- *do not only collect bees*
- *have a colour-dependent taxonomic bias*
- *high post sampling processing time (in preparing the bees)*

TRANSECT METHODS (standardized or variable)

Advantages

- *principal method for detailed studies on plant-pollinator associations*
- *good sample coverage in different habitat types*
- *avoid killing abundant and easy-to-identify bee species*
- *efficient method with respect to numbers of collected bee species*
- *efficient method with respect to number of individuals*

Disadvantages

- *strong collector bias*
- *large amount of time invested in fieldwork*

pollinators appear to be declining, but at different rates in different regions. Therefore, it is unclear what the result of these changes can be globally.

There has thus arisen the need to standardize the sampling protocols in order to make data from monitoring in long-term and large-scale programmes directly comparable.

Ultimately, it became necessary to develop a new set of standardized methods capable of providing uniform data in time and space, which can yield population and diversity trends. In other words, we moved from population surveys to monitoring.

A creative way to host bees in your garden: artificial nests

Pollinator nest made of bamboo stems

For people who like to observe different bee species on their own terrace or in their garden and at the same time want increase the population of wild pollinators, building artificial nests can be a creative way to accomplish these purposes. There are different kind of artificial nests for bees, the form of which depends on the intended use, from the simpler do-it-yourself models to put on a terrace or in the garden, to the more complicated type requiring carpentry work, to be put in natural parks and public gardens. There are types of artificial nests designed for research purposes, with transparent parts permitting observation of the internal development, and highly professional structures intended for crop-pollination services, suited to the mass release of pollinators. In this chapter we describe types of artificial nests that are simple to construct and put into place, which can meet both the "artistic" and the functional needs to increment the biodiversity of pollinators.

Making nests for bumblebees

Most bumblebee species nest underground, usually in pre-existing cavities such as abandoned mouse or vole nests. The nest entrance is usually hidden by the vegetation.
Artificial nests for bumblebees can be assembled starting from wooden boxes or any other container differing in size or material (plastic, metal), with enough internal space for the development of the colony and equipped with ventilation holes measuring at least 4 mm to avoid moisture. The box should measure at least 15x15x15 cm, but larger sizes (up to 20–30 cm) are preferable since they permit the development of larger colonies. These containers should be fitted with a hidden entrance, such as a small hole covered by vegetation or, better yet, an opening located a sufficient distance from the nest, e.g., using a long tube connecting the entrance hole to the outside, to keep predators from penetrating the nest.
A feasible and attractive nest can be built using an upside-down terracotta flower pot and a few other simple materials. The flower pot should have a diameter of about 20 cm and must have ventilation holes, two on the side and one on top (i.e., the bottom of the pot), to avoid overheating and moisture; the holes must be covered with mosquito netting to keep other insects such as ants from entering into the nest. The inner part of the nest must be filled with nesting material such as padding or wadding, as long as it is natural and not synthetic, or dried moss, grass or straw, chopped into small pieces. It can be mixed with something that can attract bumblebees, like bedding for caged hamsters or other rodents, readily available at pet shops. The nesting material should be placed above the ground so it doesn't get wet. For this purpose you can use a piece of netting stiff enough to support it, but enough soft to be shaped into a cradle for the nesting material. The nesting material is then placed on the net to form a round bed, and the net is placed on a layer of pebbles, to use as drainage and keep the nesting materials from getting wet when it rains.
The nest entrance can be made using a piece of garden hose with an internal diameter of at least 18 mm and a length of about 25–30 cm. To make sure the nest and the entrance tunnel are kept dry when it rains, the hose must have drainage holes along the outside curve. The hose must be placed with one end on the net and the drainage holes facing downwards.
Everything is covered by the pot placed upside down, leaving the inner end of the hose under the pot. At the end, the base of the pot and the hose must be covered with soil and leaves, leaving only the outer end of the hole free. Existing cavities, like

the ones formed by the roots at the bottom of a tree, can also be used. In this case, pebbles are not necessary for drainage and the net cradle can be put directly on the edges of the cavity, closing the openings between the cavity and the pot rim with clay soil.
To keep rain and water from entering the pot through the upper hole, you must cover the nest with a lid. You can use a flower pot dish of the right size, putting a stone on it to prevent wind or animals from uncovering the nest. The nest entrance can be protected and kept pervious by putting stones under and all around the distal part of the hose.
To encourage colonization by bumblebees, it is crucial to find a good location for the nest. The place should get little or no sun, and must have good water drainage and be sheltered from the wind. The best places are raised banks at the bottom of trees or hedges, as long as they are covered and protected from weather, but near open spaces with plants and flowers.

Sequential steps for the creation of an artificial nest for bumblebees

Making nests for solitary bees

Beside bumblebees, which live in societies, there are many species of wild bees (superfamily Apoidea) that are called “solitary” because their females make individual nest cells for their larvae. These bees build their nests in many different ways: in holes in walls, old snail shells, old nests of other insects, hollow canes, pieces of dried branches and plant stems. Some of them have also been found in electric plug-holes and other kinds of cavities.
Depending on the nesting habits, solitary bees can be divided in two main groups. Those that use hollow plant stems or tunnels previously bored into dead wood or stones are cavity-nesting bees; those that nest in small tunnels or holes in the ground or in sandy banks are called ground-nesting bees.
Among solitary bees, many species like to group their nest cells together in nest aggregations, and a few of them show a kind of social behaviour.

Example of an artificial nest for bumblebees

A house on a tree

Among the almost 80 bumblebee species present in Europe, only one does not like to nest underground. Bombus hypnorum *is the only bumblebee species that prefers to build its nest above ground, usually in tree cavities or abandoned bird's nests, and as a result it is called "tree bumblebee". The species often lives near human settlements and can be found in nest boxes, placed by people on trees or house walls for birds, especially when the box has hosted a bird's nest for at least one year. In this case, the remains of the nest can serve as optimal nesting material.*

Bumblebees prefer nest boxes with a small entrance hole, like the ones for blue tits (Cyanistes caeruleus). *If you want to further encourage a* B. hypnorum *family to occupy a bird's nest that has never been used by birds, you can fill the box with the same nesting material described for nest of underground-nesting species.*

Cavity-nesting bees

Many species of the family Megachilidae, but also some small Colletidae, genus *Hylaeus*, are cavity-nesting bees, building their nests in pre-existing cavities like hollow stems and holes in wood or rocks. Mason bees (genus *Osmia*), leafcutter bees (genus *Megachile*) and bees of the genus *Anthidium* are among the most common Megachilidae nesting in cavities.

It is quite easy to encourage these bees to occupy artificial nests, which can be made by drilling holes varying in diameter (between 2 mm and 10 mm) and about 10 cm long, closed at one end, in logs or wooden blocks. The open ends of these holes should be smooth and free of splinters, as the bees will not enter holes with rough splintered wood around them.

Alternatively, you can collect stems from plants of *Arundo donax* or *Phragmites* spp., cut them into pieces about 20 cm long and assemble them into bundles, bound with ropes or metal ties. An important precaution is that the pieces contain at least one node, because when they are open at both ends bees will not nest in them, but the space left by nodes should be long enough for cell construction. Very fast and easy cane bundles can be made by cutting up dried reeds sold as portable screens at garden centres.

Wooden blocks with holes and cane bundles can be fastened to the wall of the house, hung on branches or laid on a shelf, in a protected place and far from the ground to avoid nest intruders.

Bee species with different body sizes, from the smallest Colletidae bees to the biggest mason bees, will occupy different diameters of tunnels. Here they will construct a series of "cells" in each tunnel, each containing a mass of pollen and nectar mixed together, on which they will lay an egg. The egg soon hatches and the larva develops rapidly by eating the mixture of pollen and nectar, and then it pupates. Some species spend the cold season in a dormant state as pupae, until they are ready to emerge as adult bees the following spring or summer. Other species, like osmia, spend most of this period as fully formed but dormant adults and emerge in spring. If you want to follow bee

development inside the tunnels, you can insert a transparent straw into the hole and then extract it for observation, or you can even build the whole tunnel with a transparent tube; in this case the tubes should be contained in a structure that can be opened and closed.
You can check nest occupation by inspecting the entrance to the holes: if it is closed by a cap of mud or other material, then it has been occupied. Bees of the genus *Osmia* cap the holes with mud patches (hence the name "mason bees"), while *Megachile rotundata* close them with round pieces of leaves cut from plants and tied together (hence the name "leafcutter bee"). Other species use resin collected from plants kneaded with small pebbles, like the megachilid *Heriades truncorum*, but also flower petals, or chewed wood, leaves or plant fibres, as is the case with *Osmia cerulescens*. The megachilid bee *Anthidium manicatum* wraps each larval cell and closes the hole entrance with cotton wool made from hairs collected from hairy plants, such as lamb's ears (*Stachys byzantina*) or others; due to this behaviour it is called the "wool carder bee".

Carpenter bees

Carpenter bees mainly belong to *Xylocopa* sp., of which there are over 750 species worldwide, mainly tropical and subtropical. At our latitude the most common species are *X. violacea, X. valga* and *X. iris*. *Xylocopa* species nest in dead and live wood, or

Mason bees flying in front of an artificial nest

in canes and bamboo stems. They may use pre-existing cavities, reuse the tunnels from a previous generation or dig themselves new tunnels with their powerful mandibles. *X. violacea*, one of the bigger species (up to 3 cm long), nests in dead wood and digs a tunnel up to 30 cm long containing 10–15 cells.
Xylocopa females guard their nests against predators but also against other females, which may attempt to clear out a ready-made tunnel and lay their own eggs. Male and female emergence occurs around February-March and is followed by mating. In this period males set up territories near where the females are emerging and defend them from other males so they can mate. By May all the females will have emerged and mated, and start to look for a nest, while the males all die.

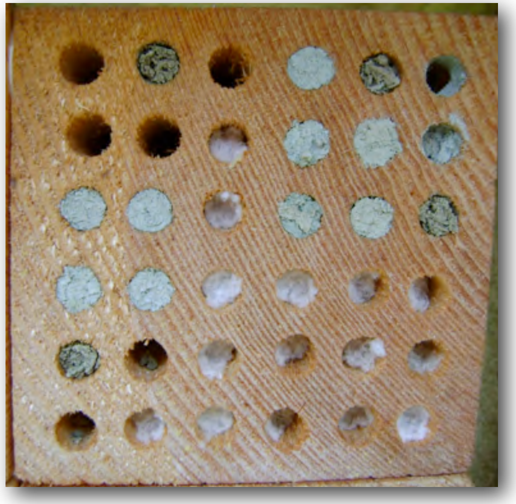
Female of *Xylocopa* often display social behaviour, sharing the nest with other females or cohabiting with their own offspring. Often 2 or 3 females occupy the same tunnel, although only one lays eggs and provisions the cells, while the others behave as helpers and guard the nest. The helpers can be the daughters or nieces of the reproductive female, and they will lay their own eggs in the following season. *Xylocopa* females overwinter as adults, sometimes in gregarious groups within the same tunnel. To promote *Xylocopa* nesting, bamboo canes with a minimum diameter of 10–12 mm should be used; the length should be at least 40 cm, regardless of whether or not there are nodes, because the females are able to break them down with their mandibles.

Ground-nesting bees

Most solitary bee species nest underground. Ground-nesting bees include the so-called mining bees or digging bees and belong to the family Andrenidae, Halictidae (sweat bees), Colletidae (cellophane bees) and some species of Apidae, subfamily Anthophorinae.

Mining bees

The female of these ground-nesting bees typically digs cylindrical tunnels from 0.5 to 1 cm wide in areas where vegetation is sparse and the soil is loose, sandy and dry. Nests are easy recognizable above ground as conical piles of soil with a hole in the middle that serves as the entrance to the bee burrows. The tunnels dug by females are 20–30 cm long, at the end of which they construct a round chamber where the eggs are laid and the larvae will grow. There can be a single tunnel and a single chamber, or the tunnels can fork to create more than one brood chamber. The larvae in the chamber are provisioned with nectar and pollen by the females.



Nest holes closed with different materials: mud patches above and left, cotton wool below and right

The females of ground-nesting bees can sometimes display social behaviour. Mining and cellophane bees are always solitary, although they often show gregarious habits, since many females build their nests close to each other. Sweat bees may be solitary, gregarious or even social, since some species display collective behaviour, with their tunnels interconnecting underground and the females cooperating to care for offspring and defend the nests.

Xylocopa *nesting as a group*

Ground-nesting bees generally select bare and dry soil when choosing nest sites, and avoid damp areas. Some of them prefer sandy soils, like many species of *Andrena*, while others select soil with particular chemical compositions, like the American halictids *Nomia melanderi*, also called “alkali bee” due to its preference for salty soils.

To encourage the various species of ground-nesting bees it will suffice to maintain patches of bare soil by removing the covering and creating a bedding, filled with dry soil, possibly the kind preferred by the bee species. It is also very important to avoid the use of pesticides in the area, since solitary bees are very susceptible to these chemicals.

Digging bees

Some solitary bees build their nests in old mortar and cracks in walls or dig themselves cavities in exposed slopes or embankments made of dried clay or soft rock. The latter are the so-called digger bees, which mainly belong to the subfamily Anthophorinae, and the most common species at our latitudes belong to the *Anthophora* genus.

To attract these species to artificial nests, hollow bricks can be used, eventually with holes filled with clay where they can dig. Alternatively, artificial mud bricks can be made. Mud bricks can be shaped easily by filling a plastic bucket with clay soil and water, and stirring the mixture into a slurry. After the mixture has settled, excess water is drained off and the remaining sediment is poured into a container of the required shape and size. After a few days or weeks, the block will be completely dry and can be extracted from the container. Mud bricks can also serve as the equivalent of a wooden nest block, by drilling nesting holes of the recommended size.

Building a “bee hotel”

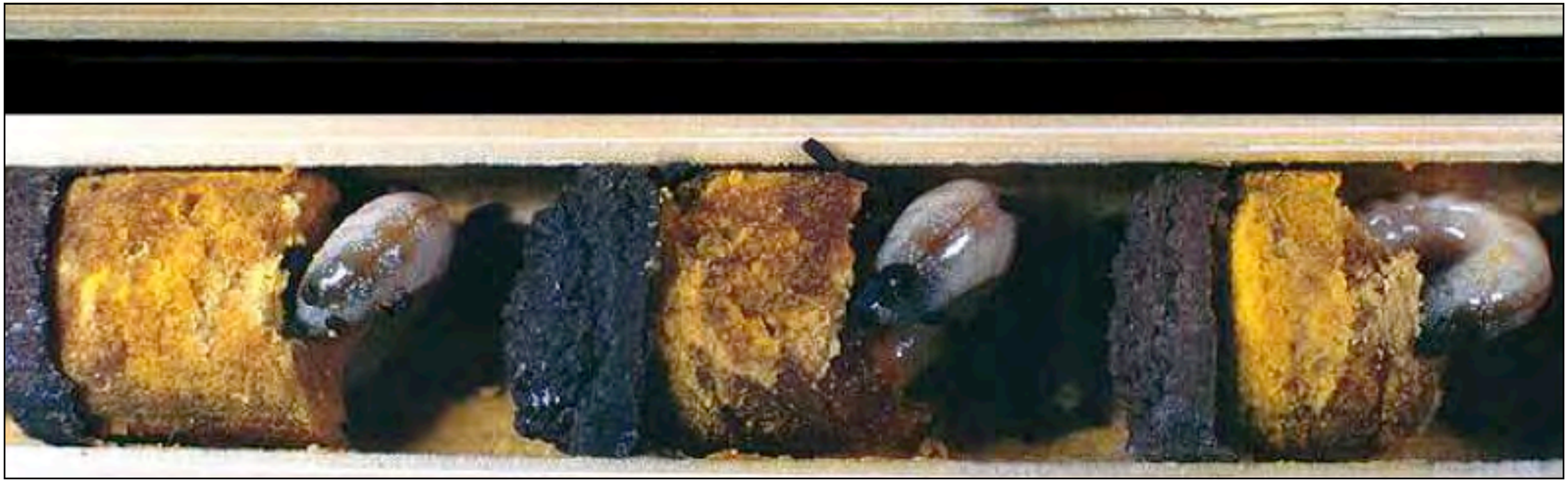
A nice and convenient way to gather all these bee varieties is to build a special structure, called “bee house”, “bee condo” or, more frequently, “bee hotel”. They can be simple wooden boxes, open on one side and fastened to a fence, wall or pole, or more complex structures with many shelves and a roof. The structure does not need to be much deeper than the blocks or cane bunches, but it is better if it has a wooden back and an overhang on top for protection from rain and wind.

Solitary bees rely on the heat of the sun to get warm, especially in the morning, so the bee hotel must be positioned in full sun, facing south-east or south, and there must be no vegetation in front of it that will block the entrances to the tunnels. Nests should be positioned at a certain height from the ground, to limit access to predators and parasites. If the structure is attached to a pole or is set on legs, they can be covered with entomological glue to avoid the predation of ants or other insects in the nest.

As autumn and winter can be very rainy at our latitudes, you must ensure the nests are protected from getting too wet. If your bee hotel is not waterproof, the nest must be moved somewhere cold and dry during the autumn and winter, and then put back in the structure in early spring. From March onwards, young bees that have overwintered in a dormant state inside the tunnels will emerge and start the cycle over again. Some of them will readily select our bee hotel again for the next generation of bees.

The bee hotel placed in the PP-ICON project area

The rearing of megachilids



Nido pedotrofico di Osmia cornuta *ricavato in una canna di* Arundo donax

Among the ground-nester and cavity-nester solitary bees of the family Megachilidae, only some of the cavity-nester species of the genera *Megachile, Osmia, Heriades* and *Anthidium* are reared for commercial, experimental and gardening purposes.

Food, lack of nesting sites and parasites are the main limiting factors in the increase of the bee populations, so knowledge and management of these three parameters are crucial in rearing these bees successfully. On the other hand, these bees tend to have a gregarious nesting behaviour and readily colonize artificial nests (bundle of cane segments of *Arundo donax* or *Phragmites communis*, drilled wooden blocks, or stacked grooved boards of wood or polystyrene) intentionally placed within their areas of action. Many studies were carried out in the last century on solitary bees using the artificial nest-trapping procedure. Furthermore, these species spend winter in their cocoons as imago or larvae in diapause. Each cocoon is found inside a pedotrophic cell where the insect spends the time needed to complete the whole pre-imaginal development. Diapause operates on the bee metabolism by changing the temperature of the diapausing bees so that the emergence of bees can be synchronized with blooming.

Within the genera of megachilids that are currently reared or are candidates to be reared, there are monovoltine, parsivoltine and bivoltine species.

In the diapause state these bees can tolerate temperatures even lower than 20°C below zero. The time of exposure also seems to play an important role.

Often the cells are placed in rows inside the nest. However, nesting always occurs in pre-existent cavities and in an odal or dependent pattern (well-ordered rows of cells). In some cases, like in *Osmia caerulescens*, nesting can be anodal or independent.

For these reasons the choice of artificial nest is very important. The diameter of the holes has to be carefully chosen, as does the material of the nest. The possibility of recycling must be

Coppia di maschi di Osmia cornuta *al mattino presto e appena affacciatisi alla imboccatura di un nido vuoto e pronti a riprendere la propria attività di "patrolling" dopo la inattività notturna*

considered as well as the handling if special tools for stripping the cocoons are used.

Plasticity in food collection, nest acceptation and diapause are the main reason these bees are the ones that are the most successfully reared.

Up to now some species of *Osmia* (*Osmia cornifrons, Osmia cornuta, Osmia bicornis, Osmia lignaria*) and of *Megachile*

Fascio di canne di Arundo donax *allestito ad arte per permettere la nidificazione di apoidei megachilidi. Sono visibili alcune canne già sigillate con fango, indice di avvenuta colonizzazione*

(*Megachile rotundata*) are also reared for commercial purposes, while other species of the genus *Osmia, Megachile, Heriades* and *Anthidium* are reared mainly for experimental purposes.

General procedures and recommendations are described here in order to successfully rear and increase a bee population: adaptation to the species; the life cycle; voltinism type; diapausing state; commercial, experimental or leisure purposes.

Knowledge of the life cycle, ethology, anatomy, physiology and biochemistry of the megachilid bees makes it possible to successfully set up rearing and production methods as well as to organize protocols for their utilization in pollination campaigns. Up to now production and utilization are quite separate and independent except for a few cases (*Megachile rotundata*).

Rearing methods can involve two strategies: the open-field strategy and the confined-environment strategy (tunnels, greenhouses). In both cases the start-up of rearing strongly depends on the availability of an initial population. In this case, the Releasing and Rearing (RR) method is the most common; otherwise it is necessary to start with a Nest-Trapping (NT) method and then return to RR the following season.

In the NT campaign, the assembled artificial nest is very useful. This type of nest can be built with different types of material, such as various woods, hardboard, recycled plastic, pressed polystyrene and terracotta. Grooved boards of the above-mentioned materials are assembled. The diameter of the grooves is chosen according to the species of bee. Once the

Femmina di Osmia cornuta *appena arrivata al nido con il proprio carico di polline ben visibile sul gastro*

boards are assembled these grooves become tunnels of the right size.

This strong modular unit is easy to handle, can be reused or opened up for inspection, and can easily be assembled and disassembled.

This kind of artificial nest trap with tunnels measuring 2 to 14 mm in diameter is particularly helpful in inducing different species of megachilids to nest (2 and 4 mm *Heriades truncorum*; 6 mm *Megachile rotundata* and *Osmia caerulescens*; 8 mm *Osmia rufa* and *Megachile centuncularis*; 10 mm *Osmia cornuta* and *Megachile centuncularis*; 12 mm *Osmia cornuta* and *Anthidium manicatum*; 14 mm *Xilocopa violacea*).

The artificial nests are located in chosen environments, at a height of about two meters from the ground, and with the tunnels placed horizontally. The best time for placing the nests is the end of winter/beginning of spring and the nests remain in loco until the following autumn. This way, the bees that fly during early or late spring, or during summer up to the end of September, can be induced to nest.

Once the artificial nests are colonized they can be collected and opened, and the contents analysed. The bees inside their cocoons (larvae or imago if their pre-imago cycle has ended) can be collected, divided into species and put in special containers.

In the RR method a certain number of cocoons with insects ready to emerge are located along with artificial nests in structures called shelters (“bee hotels”). These structures can be either mobile or fixed. The timing and placement of the shelter in the open field depends on the species of bee to be multiplied. For good production it is important to choose places with a large trophic resource (production of pollen in a unit of time –

as Freddie Mercury said, I want it all, I want it now! [Queen, 1988]).

The RR method is based on the SWOT system (S strength, W weakness, O opportunity and T threat), in which S stands for gregariousness, artificial nest acceptance and diapause; W biotic and abiotic limiting factors, dispersion and lack of technology; O diapause modularity, nest handling, management of food sources; and T pesticide and parasite proliferation.

The nest-trapping method can be performed in the wild or in specific agro-eco-systems, depending on species presence and pollen availability through time and space.

If RR is performed in a natural (wild) environment, for a good bee progeny output it is important to choose environments with a large trophic source. This makes it possible to increase the parental population from five to ten times. In agro-eco-systems (managed crops) it is quite normal for some Osmia species to obtain a progeny output of two to five times the parental population, while better results could be obtained using *Megachile rotundata* on alfalfa fields, depending on its extension and initial bee population size.

Open-field increase of bee populations has the disadvantage of being restricted in time and space (cyclical rearing). It is also affected by parasites, predators and nest destroyers.

Performing RR in confined environments is a partial solution because this makes it possible to increase the parental population from five to ten times, but only releasing a small number of cocoons.

Age of the parental generation used in RR method is very important because it affects the mating behaviour of the emerged bees and subsequently the sex-ratio of the output population. Age of the parental generation could vary depending on how the population has been managed during diapause as well as on the forced delay in synchronization of emergence with blooming.

The artificial nests built with blocks of a wide range of materials (wood, plastic, terracotta, etc.) are accepted as nesting sites by the bees according to species, but all of them seem to prefer canes. Canes that are two months to one year old are better accepted by the bees compared to newly cut ones that are fresh and still green. During the night the just-emerged males of several species (especially *Osmia* sp.) colonize the old open tunnels, but if new tunnels are available they will readily accept them first. Females also prefer new tunnels, if available.

The presence of a nesting female bee in a given area is a visual stimulus for other co-specific females to nest in the same area. It is therefore useful to leave some colonized nests in loco so the newly emerged bees will start their activity in a nesting site where some females are already performing nesting activities. In this case the old artificial nests must be replaced at least every two years in order to control parasites.

The presence of enemies – parasites, predators and nest destroyers – has a great influence on the consistency and presence of a bee population.

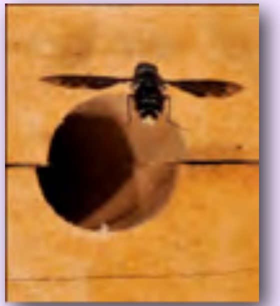
Since the number and type of enemies is itself influenced by time, place and species of reared bees, we will refer to those enemies that are commonly associated with reared bees. Following are a few biological notes about some of the natural enemies of megachilids populations that damage the numerical increase of bees obtained using the RR method.

The fungus *Ascosphaera aggregata* is always present in rearing *Megachile rotundata* and causes damage to the larvae. The spores on leaves and infected cocoons are passively collected by the megachile female at emergence then transported to the newly built pedotrophic nest. The main parasites limiting the presence of the megachilids bee in the field are calcidid wasps such as *Pteromalus apum* (*Megachile rotundata*) and *Monodontomerus obscurus* (*Megachile* sp., *Osmia* sp. and *Heriades* sp.), which can parasitize up to 100% of the population if left uncontrolled.

Trichodes alvearius *laying an egg in an* Osmia cornuta *nest*

Cacoxenus indagator *entering an* Osmia caerulescens *nest*

Male of Osmia cornuta *covered by* Chaetodactylus osmiae



Anthrax anthrax *laying eggs in a solitary bee artificial nest*

Besides the above-mentioned parasites, there are others that cause high mortality in larvae of *M. rotundata*; for example the megachilid *Coelioxys rufocaudata* normally causes heavy loss (even up to 40%). This kleptoparasite is very dangerous because, contrary to what occurs in America, in Italy it has a life cycle parallel to that of *M. rotundata*. High parasitization by *Coelioxys* can be avoided by inducing intense activity of gregarious-type nesting. In fact an intense coming and going of nesting females (entrance-exit) in front of the nest discourages the females of *Coelioxys* from entering the nest-tunnels, thereby limiting damage to the new generation.

The coleopter cleride (*Trichodes alvearius*) is one of the worst enemies of megachilid bees and if not kept under control it would destroy the bee nests. One single larva of *T. alvearius* can destroy all the small sequenced larvae in a single tunnel and continues with other tunnels when possible. At the end of summer the larvae of *T. alvearius* form a brown-coloured cocoon where they pupate. In October-November, during the stripping of the cocoons when the nests are opened it is often possible to find the larvae of this predator still active in the tunnels.

Extensive loss in bee multiplication and output production is due to the fly *Physocephala vittata* (Conopidae), a very dangerous endoparasitoid of *M. rotundata*, the fly *Cacoxenus indagator* (Drosophilidae) that is a spring kleptoparasite and especially affects bees of genus *Osmia*, and the most generalist and destructive ectoparasitoid, *Anthrax anthrax* (Bombyliidae), which is able to parasitize many female megachilid larvae, usually located at the innermost part of the nest. Once the parasite becomes an armed pupa it moves towards the exit, destroying all the cocoons on her way out.

The most successful ways to fight parasites are cocoon stripping and renewal of nesting material.

Exposure to predators and parasites can be also reduced by: 1) covering nest shelters with a net against birds; 2) releasing bees in emergence filtering boxes; 3) avoiding the reuse of old nests: destruction of the old nest will kill all the parasitoids (calcidids, bombyliids and drosophilids) that will emerge later than the bees. In this way, multiplication of these parasitoids will be prevented.

In laboratory conditions a black light traps kills *Monodontomerus* and other wasp enemies of *Megachile rotundata*.

Rearing megachilids in laboratory conditions using artificial light and an artificial diet, and in year-round conditions would represent a great solution for many problems such as bad weather or lack of quantity/quality of food, as well as the effects of pathogens and parasites. Up to now no laboratory condition rearing methods have been developed successfully. Several attempts were made on *Osmia* species by researchers at the Universities of Bologna and Pisa in Italy. They found that *Osmia cornuta* reared on pollen-based artificial diets showed high larval mortality. The control bees, which were allowed to consume the pollen lump provided by their mother, had lower mortality rates. The authors suggest this happens because female *O. cornuta* bees add something, perhaps an enzyme, to the pollen they provide for their larvae.

Before these experiments, trials with artificial diets were performed on *Megachile rotundata* in the Seventies, revealing that the bees reared on pollen substitutes either died or had lower pre-pupal weights than the control group.

Celle pedotrofiche di Osmia cornuta *dove sono visibili le uova deposte sulla scorta pollinica appena due giorni prima e dove è già visibile l'inizio della embriogenesi (indicata dalla presenza della parte apicale trasparente)*

Bumblebees colony rearing and release

Artificial rearing of bumblebee colonies

Colony originated from wild queens

In addition to the creation of nesting artificial sites, previously described, another way to promote the re-colonization of an environment by bumblebees is by collecting the wild queens, rearing them in captivity and then releasing the ensuing colonies.

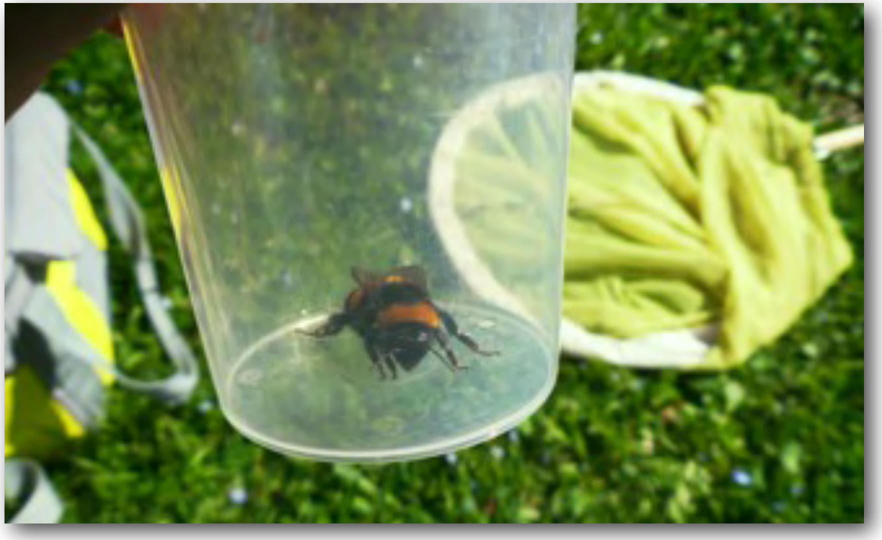
Rearing queens artificially makes it possible to increase the diapause survival rate and to establish a colony because it guarantees optimal development conditions such as temperature, humidity, food availability and protection from predators and parasites. The practices described here are applicable to small rearing situations, which are useful primarily for research and study purposes; these are only a few of the possible methods that can be used.

Wild queen collection

Queens can be collected at two different times of the year: early autumn and late winter. In autumn (between early September and late October) queens can be found on late flowerings, where they forage to accumulate energy stocks to get through diapause. In the Mediterranean area they are readily found on strawberry trees (*Arbutus unedo*), rosemary (*Rosmarinus officinalis*) and peppermint (*Mentha* sp.), but it is sufficient to find a field or garden with nectariferous flowers (wild or cultivated) where the queens can feed.

In early spring (between February and March), however, queens have just emerged from diapause and are easily found on early flowerings. One of the first plants to bloom in our latitudes is loquat (*Eriobotrya japonica*), followed by the large blossoms of *Prunus* fruit trees: almonds (*P. dulcis*), cherry plums (*P. cerasifera*), blackthorns (*P. spinosa*), apricots (*P. armeniaca*), cherries (*P. avium*). In the South, citrus trees can also be considered.

Queens can be collected using an entomological net. Collected queens must immediately be transferred to a perforated container and placed in the dark in a cooled bag, to anaesthetize



Bumblebee queen collected in the wild

them slightly to avoid additional stress due to capture and transport. It is very important not to catch queens that already have full pollen baskets, because this means they already have found a colony.

After collection, the queens are placed in flight cages for 1–2 weeks, with sucrose syrup and fresh pollen (collected by bees), to allow them to absorb the right amount of energy and protein resources for diapause (if they are captured in autumn) or deposition (spring capture). The cages must be placed in a room with a natural cycle of light and dark, and be at ambient temperature (at least 18–20°C). After the flight period, depending on capture time queens will follow two different paths: diapause

Bumblebees

Bumblebees are hymenopterans belonging to the genus Bombus *(Latreille, 1802), family Apidae. More than 250 bumblebee species have been described. Most of the species are located in the temperate regions of Europe, Asia and America, some are found in the tropics, and a few are found in South America and southern Asia. Moreover, bumblebee species can be found around the Arctic Circle and at high elevations in mountainous regions.*

Bumblebees are eusocial insects like honey bees, but they are defined as "primitively eusocial" because their life cycle shows a solitary phase. Like honey bees, they have only one reproductive individual (the queen) and many sterile workers, all sisters and daughters of the queen, who represent almost all the colony; in the summer months new queens and a certain number of males are produced and they leave the colony to mate. Finally, in late summer mated queens enter into diapause while males and the colony die. The following spring the queens who survive diapause found a new colony.

Bumblebees are haplodiploids: queens and workers are born from fertilized eggs, while males from unfertilized eggs.

Larvae develop inside wax cells that the queen and workers enlarge according to the larvae's increasing size. Bumblebee species are divided into two different categories based on the larval feeding type: pocket makers, including Bombus pascuorum, *and pollen storers, such as* B. terrestris. *In the first group the workers deposit a pollen and nectar mixture at the base of a common larval chamber from which the larvae feed themselves. In the second one pollen and nectar are stored separately in cavities made from the pupal cocoon or specially constructed wax cups*

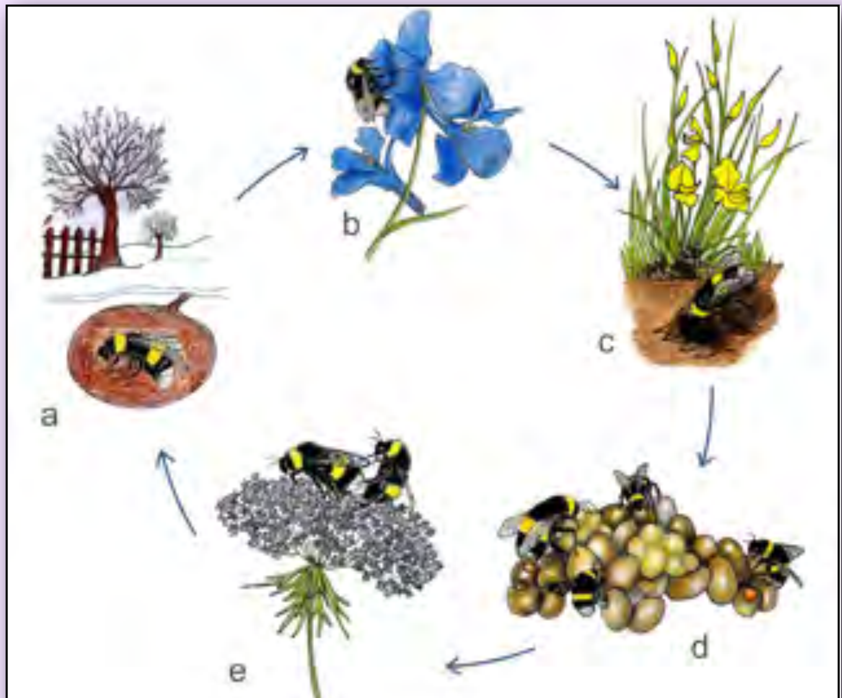


illustration by Marta Barberis

The bumblebee colony life cycle

a) Diapausing queen
b) Queen foraging on flowers to recover from diapause
c) Queen searching for a nesting site
d) Colony foundation and development
e) Bumblebee mating

Flying cages made of wood and metal mesh, placed in front of a window to allow a natural light/dark cycle

or colony production.

Diapause

In nature queens overwinter deep in the ground, so the artificial diapause must imitate natural conditions. Closed containers are ideal (wood is preferable because it is natural and breathable) filled with gardening soil to maintain the proper humidity.

Containers should initially be placed in an incubator at 15˚C (intermediate temperature between ambient and hibernation temperatures) for a week. The function of this period is to induce natural entry into diapause and avoid excessive temperature swings. At this stage it is better to put the food (sugar syrup and pollen) in the container. The food should then be removed from the container and the temperature decreased to 5˚C for about 3 months.

When they awaken, the queens surviving diapause are placed in flight cages for about a week under natural conditions of temperature and light/dark cycle. They are fed fresh pollen and sucrose syrup, essential to recuperate energy after diapause and to develop the ovaries to lay eggs.

It is normal that a certain percentage of queens (up to 50%) will not survive diapause, but this is still lower than what would occur in natural conditions.

Colony initiation

To initiate deposition starters are needed in which the queens will be individually positioned. A starter consists of a container (plastic or cardboard) that is small (a 10x5cm base is sufficient) and has perforated sides to let in air and moisture. An absorbent paper substrate and fresh pollen are placed inside it. The substrate will absorb faeces and any excessive condensation; pollen is put in a small container alone or mixed with syrup to form a ball. Starters are closed but have a hole in order to insert a syrup-filled syringe. The syringe should be blunt to allow the queen to suck the content with its tongue.

Starters can be placed into an incubator or in an air-conditioned room. The temperature must be maintained at 28–29˚C and the

Cuckoo bumblebees

The species of the subgenus Psithyrus, *now included in the genus* Bombus, *are bumblebee social parasites.*
Psithyrus *do not have a worker caste but their queens lay eggs in other species of* Bombus *nests, leaving the task of raising their offspring to host workers. Generally they resemble the host bumblebees, but the hair mantle is not as dense, revealing brilliant abdominal plates. The females do not have a pollen basket and the wing membranes are dark.*
Psithyrus *females come out of hibernation after the host species, waiting until the bumblebee nest has been founded. To locate it they follow the typical smell of the host colony. When they enter a nest, they act calmly, their legs close to their body, to be accepted from the colony. They can be attacked by the host workers but are rarely killed because they have anatomical features allowing them to withstand the attack.*
Subsequently Psithyrus *females hide in the nest material until they acquire the colony's distinctive aroma and will not elicit worker hostility.*
Usually the Psithyrus *queen kills the host colony's queen, but this does not always happen; in some cases it seems that the two queens can live together in the same colony without killing each other.*
When the parasitized nest has a sufficient number of workers, the Psithyrus *female destroys the queen's host eggs and larvae, and reuses the mixture of pollen and wax of the cells to build those in which to lay her eggs. While the host workers rear the* Psithyrus *brood, the parasite female plays no other role in the colony life. New larvae will emerge as parasite adult males and females. Males look like females but are smaller.*
Psithyrus *and* Bombus *have a common ancestor and this type of parasitism may have evolved from conflicts that commonly occur among different species of bumblebee queens (or even the same species) to occupy a nesting site.*

humidity at 60% minimum.

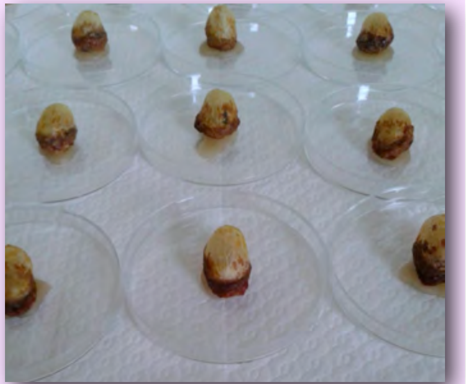
To handle bumblebees safely, lab tweezers (15 cm long) are used, working in a room with red lights. The queens cannot perceive light with a wavelength corresponding to red, seeing it as darkness, and consequently they will not fly.

There are several techniques to stimulate queens to lay eggs and they can be used individually or combined.

1. A young pupa, preferably a male pupa, is hot and thus stimulates the queen to lay egg on it. Pupae are taken from already initiated laboratory colonies, then separated from each other, cleaned of excess wax or impurities, and fixed on a support. Pupae must be replaced once a week in case of no deposition.

2. 1–5 newly emerged workers, always taken from initiated colonies.

3. 1–5 newly emerged Apis mellifera



Pupal cocoon fixed with honey bee wax to a Petri dish, ready to be put in a starter as a stimulus for egg laying

workers.

4. Two queens placed in the same starter. The strongest queen becomes dominant over the other and will be the only one to lay eggs; the other can be used later to stimulate another queen.

If methods 2, 3 and 4 are adopted, a ball of pollen mixed with syrup should be positioned in the container as substrate for the deposition.

Typically, after a month queens that have not laid eggs are discarded. Once the queens lay eggs, they are monitored and once a week the food must be changed and the container must be cleaned. Over time the colony develops and when the first workers emerge it is best to transfer the colony to a larger box (capacity of 6–7 litres) that has been perforated to permit air circulation.

The colonies are given sugar syrup, composed of mineral water, uncontaminated by chlorine and carbonates, and commercial sucrose in a 1:1 ratio. The protein nourishment consists of fresh pollen collected by bees, and then frozen or dried pollen. The first type of pollen is generally given to queens and colonies under development (higher quality); dried pollen is distributed to the colonies that have already started.

Colonies of pocket-maker species

Rearing colonies of pocket-maker species is more difficult and time-consuming. As described above, these species deposit small food masses into pockets located below the common larval chamber and the larvae feed themselves. The stages of farming remain the same as described above, but the pollen administration mode changes. Pollen must be placed in the pockets at least once a day until the first workers emerge; then they accept the pollen and put it into the pockets themselves.

Queen of B. terrestris *with a pupa (above) and two queens of* B. pascuorum *within the same starter (below)*

Bumblebee colony at the first stage of development: the queen and the workers of the first brood, pupae and, on them, some egg cups

Queen of B. pascuorum; *the pocket for larval feeding is visible under the pupa*

Possible options are to add some newly emerged B. terrestris workers to the brood, which will act as nurses to feed the larvae or allow the queen to go foraging, although this increases the chances of founder predation and death.

Colony release

When releasing colonies in the wild, a certain size is advisable, with at least a dozen workers so they effectively can defend themselves from predators and keep the brood temperature constant. Colony release is carried out in spring, possibly on a sunny day. It is best to place them in an elevated position to impede access by predators and parasites. If they are placed on a pole base, ant glue can also be applied. Before they are released, it is preferable to cover the brood with a layer of cotton wool as insulation.

Colony status can be monitored by observing the adult flight in and out of the nest, or by placing a transparent cover over it to make it possible to observe inside the colony. At the end of the season, nests can be recovered and analysed to look for possible parasites or other clues on colony development to find optimal solutions for the following year.

Bombus terrestris *colony released in the wild*

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CHAPTER 3

Dissemination

Dissemination strategies and educational activities

Dissemination and awareness: winning dissemination strategies

Information panel in the Botanic Garden

Urbanization is presenting a growing problem for biodiversity conservation, notably by increasingly isolating over half of the world's population from the experience of nature. This separation (physical, geographical and emotional) of people from nature is an important environmental issue, as it could fundamentally influence the way people value nature and their willingness to conserve it.

As has been well established in psychological literature, providing information and involving the public in the process of increasing biodiversity could enhance awareness and perception of biodiversity, reconnecting people to nature

For these reasons, dissemination plays an important role in the project, as does awareness among local inhabitants. Dissemination events, consisting of scientific workshops, meetings and walks, contribute to public awareness on the relationship between plants and pollinators, and on biodiversity conservation.
To explain the project and reach the greatest number of people, several strategies have been used. Dittany and other nectar plants are grown in the Botanic Garden of the University of Bologna and an area for disseminating the project has been set up. In the Villa Ghigi Park, a short distance from the headquarters of the Foundation that manages this large public green area, a demonstration station for dittany was set up in 2011, taking about fifteen sods from Farneto. After three years, many plants have taken root and flourished, and have produced fruits and seeds, arousing the interest of park visitors. Meetings and excursions were organized, and there was good participation by ordinary citizens and "personnel involved in the project," who for various reasons are concerned with these issues. Educational and publicity events, some for citizens and others reserved for university students, were also held at the Botanical Garden and other places, such as the headquarters of the Gessi Bolognesi e Calanchi dell'Abbadessa Regional Park in Farneto. The project has been one of the major focuses of an international scientific workshop at the University of Bologna and has been presented on several occasions in Italy and abroad at conferences and scientific seminars. Lastly, information on the project has been posted on panels situated in the various project locations, and there have been articles in the local press as well as a dedicated website.
Beyond sheer exposure to information, efforts to involve citizens directly in conservation actions have been planned. People have poor biodiversity identification skills, and are not consciously aware of their local biodiversity, but participating in a conservation activity may stimulate long-lasting interest. For this purpose, on several occasions collateral and complimentary materials (seeds for nectar plants, nests for Apoidea, etc.) were distributed to the participants, encouraging them to explore a range of methods to increase the diversity of plants and pollinators in their own gardens.

The Experimental Station of the Villa Ghigi Park

As part of the strategies to disseminate the project, the dittany botanic station specially set up in the Villa Ghigi Park in Bologna has played a key role. This green area is well known and beloved by the people of Bologna, but is also visited by many tourists and school groups of all ages and levels. The decision to set up this station in a public area that enjoys great visibility and popularity such as the park has made it possible to create a strategic showcase for the project, augmenting the

Located in Via Irnerio 42, within the nucleus of the University area, ***the Botanic Garden and the Herbarium of Bologna University*** *are amongst the oldest in Europe.*

Overall the botanic Garden covers an area of around two hectares, extending from via Irnerio to the old city walls, on which over 5,000 of local and exotic plants are grown. It includes thematic collections, reconstruction of natural habitats, two greenhouses with tropical and succulent plants. Another small greenhouse hosts a collection of carnivorous plants.

The Herbarium holds collections of dried plants dating from the 16th century onward. Today it contains more than 100,000 dried plants. In the Botanical Museum some of the most significant specimens from the various collections are on display.

The functions of the Botanic Garden and Herbarium are mainly educational, and therefore directed to school children and University students, but also to the public. In recent years, however, they have been increasingly involved in research activities, which encompass the entire spectrum of plant biology, from phenology to systematics, and from physiology to ecology.

opportunities to talk about problems tied to the conservation of a rare and threatened species such as dittany in relation to its pollinators. Furthermore, the park has permitted great resonance not only to illustrate the project but also to introduce those who use the green area to European programmes to protect the environment and natural resources, through a tangible example of active policies enacted on a local level to protect biodiversity.

From an operative standpoint, the dittany station was set up at the beginning of the programme of activities, in spring 2011, and in the years that followed it underwent care and maintenance operations aimed at its upkeep, which will continue once the project is finished. It represents an effective increase in the flora of the Villa Ghigi Park and an enrichment of the elements of interest offered by this public green area. To set up the station, 15 plants with clods of soil around them

The Villa Ghigi Park

The park covers about thirty hectares in the first hills visible from Bologna's historic district, in a particularly interesting context from a historical, landscape and environmental standpoint. The green area, opened to the public in 1975, occupies most of an ancient farming estate boasting a noble villa built in the seventeenth century. It is directly managed by the Villa Ghigi Foundation, which is headquartered in a farm structure in the green area, on behalf of the Municipality of Bologna. For over thirty years the Foundation has constantly contributed on a local level to developing a culture sensitive to the area's environmental and landscape values. It is one of the centres for education in the sustainability of excellence of the Region of Emilia-Romagna and, as part of the regional system, it participates in BAC – Bologna Ambiente Comune, the multicentre for education in sustainability of the Municipality of Bologna. Since 2012, the Foundation has also been an accredited structure as conservators ex situ of the agrobiodiversity of Emilia-Romagna. As a result, for decades the park has been one of the city's most popular and best-known green areas, a point of reference in the system of public parks in the city and the outlying metropolitan area of Bologna, and it has hosted numerous activities and events devoted to school groups and all citizens.

were obtained from the nearby area of Farneto (in the Gessi Bolognesi e Calanchi dell'Abbadessa Regional Park and the Natura 2000 SCI/SPA IT4050001 site) and transplanted in an area in the park with similar ecological and stational condition the clods of soil were manually watered frequently due to the enduring drought that has characterized the climate in recent years. In agreement with researchers from CRA-API Bologna, artificial nests for Apoidea and bumblebee colonies were placed in the station, raising the interest and curiosity of park visitors regarding the project. Lastly, an educational panel illustrating the general outlines of the project was set up at the station.

Four years after the station was set up, this action can be considered quite successful, not only with regard to the health of the plants but also the informative role it plays with regard to the project. In the years after they were planted, the plants that were brought in sprouted, and most of them bloomed, bore fruit and produced seeds. With regard to the function of informing the public, the fact that the headquarters of the Villa Ghigi Foundation is just a few metres away from the station allowed the numerous park visitors intrigued by the new set-up and sensitive to environmental issues to come directly in contact with the Foundation's staff, who thus had the chance to talk about the project, distribute informative material, and reflect together on the value of safeguarding nature and biodiversity. Furthermore, the selected site is in a strategic position in the park because a hiking trail (CAI no. 904) goes past it. The trail, which goes from the city centre to the heart of the Bologna hillsides, was inaugurated the year after the dittany station was set up and it is always very popular. Lastly, as illustrated ahead, the station has supported the main dissemination and educational activities promoted by the Foundation in the park.

Dissemination and educational activities

As already mentioned, the Botanic Garden of the University of Bologna, the Gessi Bolognesi e Calanchi dell'Abbadessa Regional Park and the Villa Ghigi Park were the main areas in which many of the project's informative and educational activities were concentrated. As a whole these activities attracted a large number of people of all kinds.

Thanks also to the dittany demonstration station, the Villa Ghigi Park in particular was the venue for numerous actions, some of which already envisaged in the planning phase and others that were not programmed but arose spontaneously over the course of the project. Among the former, there were four annual events devoted specifically to stakeholders and to those who, for various reasons, deal with the topics of this project; these events always took place in May, when the dittany plants in the station at the park are in bloom. These events were organized as walks led by project researchers, during which various botanists and entomologists illustrated the actions undertaken and the results achieved, using easy-to-understand but scientifically correct language. At the end of each walk, the participants were invited to the Foundation headquarters for a more detailed examination of the topics, to receive informative material about the project and share a light snack made with local products. To involve as many people as possible, the four events were included on a calendar of walks and excursions that have now become traditional for the city of Bologna, known as “Le colline fuori della porta”, overseen by the Villa Ghigi Foundation and other hiking and nature associations active in the area. The programme of the initiative, eagerly awaited by the people of Bologna and often attracting numerous participants, is advertised through specific printed materials distributed in many strategic points in the city and

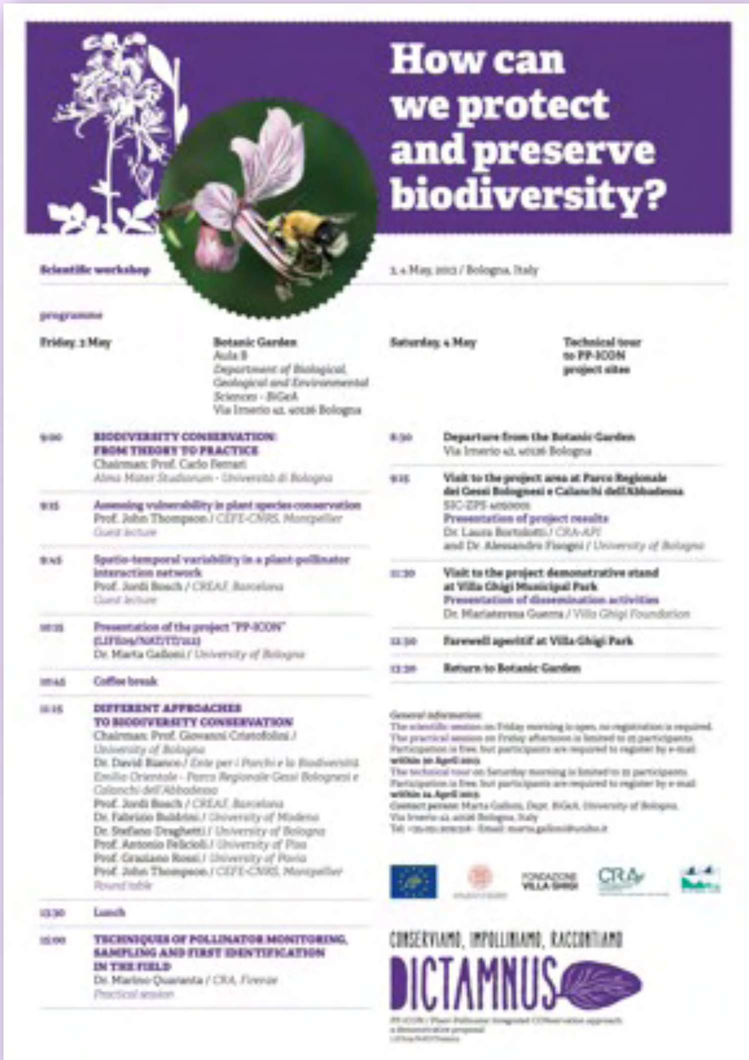
Suggestions for the effective dissemination of scientific information

Participate in national and international conferences and congresses.

- *Preferably make oral contributions, if possible.*
- *Take advantage of the opportunity to distribution informative material about the project.*
- *Consolidate relationships with other parties and make new contacts.*

Organize specific seminars and workshops.

- *Give the event international relevance by inviting foreign experts.*
- *Involve different professionals interested in the management and conservation of nature (researchers, scholars, technical experts, politicians, etc.). Devote time to practical aspects and discussions (hands-on sessions, roundtables) also regarding any criticalities that have been encountered.*
- *Plan surveys and excursions at the project sites.*
- *Propose specific informative material distributed free of charge (flyers, profiles or other publications, including technical and specialized ones like this manual!).*
- *Encourage broader participation of the scientific community, strategically choosing the date of the event so it will be before or after meetings devoted to similar topics.*
- *Encourage the participation of young naturalists, extending an invitation to graduate students studying the natural sciences.*
- *The location is also important: don't underestimate logistical aspects! Botanic gardens are the ideal venue for projects about safeguarding nature.*
- *Encourage the interaction of participants, proposing social encounters outside classic coffee and lunch breaks ("locally sourced" dinners, happy hours).*
- *In the choice of caterers, place a premium on quality, showing consistency with the topics being examined.*
- *Publicize the event through invitations and flyers printed and circulated on the Internet (mailing lists, newsletters, social networks, etc.)*



the Bologna metropolitan area, but also sent online to lists of interested parties. The walks devoted specifically to the project were also promoted individually through specific posters and invitations emailed to stakeholders, as well as special mailing lists created over the years of activity of the project through contacts that have been developed (after their first experience, numerous participants in the educational initiatives asked to be informed of subsequent events). The success of these events organized in the park, where there was an average of about one hundred participants for each walk, suggested that the Farneto area also be included in the programme of excursions tied to "Le colline fuori della porta", thereby expanding the project's informative initiatives to another strategic area.
The informative and educational initiatives promoted by the Villa Ghigi Park that were not envisaged in the planning phase but arose to respond to requests include several events targeting groups of people and school groups visiting the park, who were offered the chance to take a more detailed look at the project. Thus, various participants in courses, conferences, activities and educational visits – also from other Italian regions – had the opportunity to learn about the project. In addition to these occasional initiatives there were also other informative moments targeting park visitors intrigued by the presence of the demonstration station or tied to events organized by the Foundation, during which there was the chance to speak informally about the project, hand out brochures and extend an invitation to visit the dittany station in the park as well as other places of interest that are part of the project.

Informative material

Regarding the products designed to inform people and promote the project, in addition to the aforesaid website, in the first phase three information panels were set up in the main areas of intervention to offer a visible sign that the activities had commenced. The panels were set up in the Casa Fantini Visitor Centre of the Gessi Bolognesi e Calanchi dell'Abbadessa Regional Park (a very popular venue close to the study area of the project), in the Villa Ghigi Park at the dittany demonstration station and in the Botanic Garden of the University of Bologna, in order to inform people about the equipped space devoted to the project. To ensure the visibility of the panels, we chose a medium-large format (125x50 cm and 100x80 cm), adapting the construction materials and types of support to the different locations: in aluminium on a self-supporting structure for Casa Fantini, in aluminium on wooden posts for the Villa Ghigi Park, and in FOREX plastic on a wooden noticeboard for the Botanic Garden. The panels, which have plenty of room for pictures, are written in both Italian and English, and they describe the general outlines and actions of the project. Each panel also has an inset with a more detailed presentation of the site in which it

PPICON – the graphic project

by Marcello Signorile - muschi&licheni | design network

Given the importance of communication tools, the graphic project envisages a useful strategy that can be interpreted and applied to different media and supports.
First phase. Creation of basic elements to establish an identity: choice of fonts, the main colours and iconographic treatment (images and illustrations). Together, all these elements give communication a tone and language that are consistent and recognizable.
Second phase. Communication target: the specialized sector of researchers, but also all stakeholders. The project choice emphasizes a name-logotype (DICTAMNUS) that replaces the technical specification of the project (PPICON), which is less explanatory and has fewer possibilities for narration in communication. To make the intentions of the project even more explicit, a payoff was created to go together with the logotype: "let's conserve, let's pollinate, let's talk about it", with a direct and colloquial tone.
Third phase. In the main instruments (website, brochures) the project generates an aura of suggestion and involvement thanks to the use of images. The scientific aspect was handled through texts that were expanded on by more in-depth examinations not directly tied to research (excerpts from literature and poetry). Colour was used to underscore text categories (titles and notes) and to mark inserts with special contents. The documentation is engaging and interesting.

CONSERVIAMO, IMPOLLINIAMO, RACCONTIAMO

DICTAMNUS

PP-ICON / Plant-Pollinator Integrated CONservation approach: a demonstrative proposal / LIFE09/NAT/IT000212

is located. The signage is completed by a small informative poster (25x25 cm), set up in Farneto, that offers a short presentation of the site of the project study area.
To communicate the project, the two brochures, published in 2012 and 2014, were very effective. They were distributed as printed matter at the various events connected with the project and were also circulated on the project venues and in various strategic areas in Bologna. Furthermore, the digital version of both is part of the material that can be downloaded from the website. In keeping with other informative materials, the two brochures illustrated the project in a simple but rigorous manner from a scientific standpoint, and they are integrated with additional information intended to intrigue and interest even non-specialized readers, thanks also to their numerous illustrations. The first brochure, of which 18,000 copies were

printed, takes a detailed look at botanical aspects in particular, with numerous notes – both scientific and literary – on the plant (dittany has been cited extensively by famous authors over the centuries). Instead, the second brochure provided information on the initial results of the project, but also focused on the world of pollinators and the problems linked with their decline, involving readers by suggesting simple actions to contribute to safeguarding pollinating insects. Although it was not envisaged by the project, the second brochure was published in both Italian (15,000 copies) and English (5,000 copies), in order to circulate the project during periodic international events attended by researchers, and to cater to the foreign students and tourists who, in growing numbers, choose to visit the city of Bologna.

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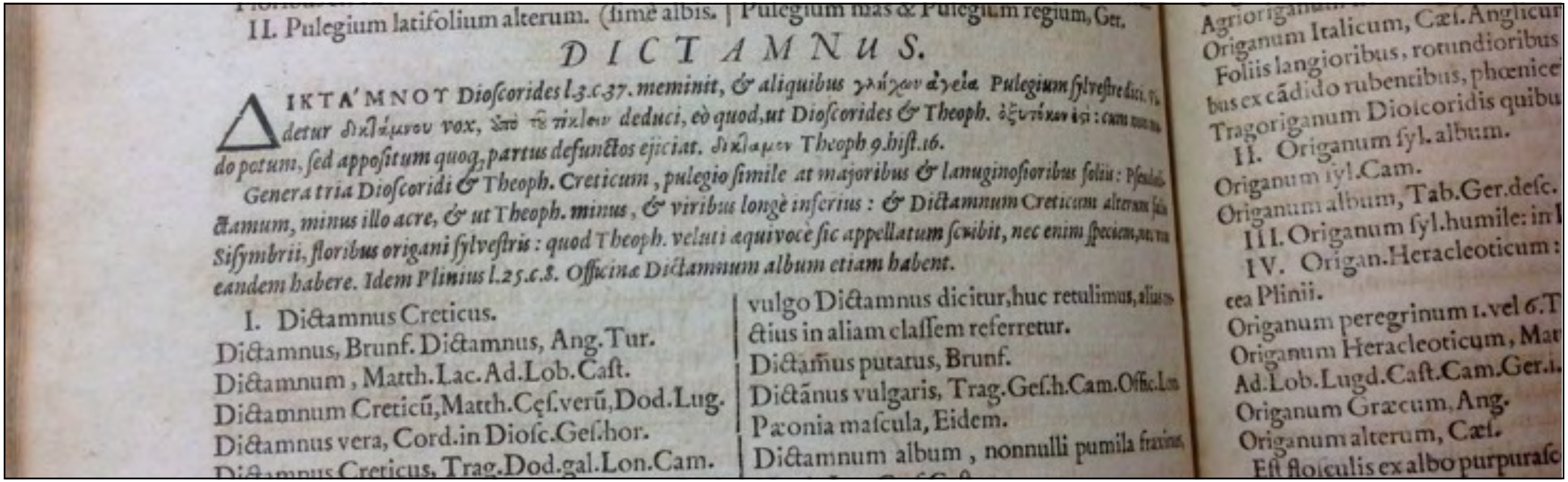

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illustration by Lucio Filippucci