File SF1.

Analysis of the nest structure, and pollen loads from *M. parietina* abdominal brushes and contribution of *M. parietina* to fruit development of Sulla (*Hedysarum coronarium* L.)

1. Material and methods.

Structure of isolated nests

During spring 2017, 16 isolated nests, with already closed cells, were carefully collected from vertical walls by inserting a metal spatula between the wall and the nest and hitting the spatula handle with a hammer. All the nests we observed in the area, including the isolated ones, were built on flat surfaces (mostly on iron beams, as described in the main text, or on walls), while we did not find nests attached to twigs as reported for some exceptional nests of this same species by Schulten and Wiering (2001).
For 8 of the collected nests (fig. S1) which were better preserved, we measured the weight (scale KERN KB, 1 mg resolution), their length and width, counted the number of cells, and for four of the most external cells, we measured the thickness of the external coverage.
-During spring 2018, building material was collected from 10 females returning to their nests, inserted into Eppendorf vials, and taken to the laboratory. The building material collected from each female was also weighted to evaluate the number of flights necessary to build an entire nest.

Pollen analysis

Sainfoin (*Onobrychis viciifolia* Scop.), *Lotus corniculatus* L. (Fabaceae), and sage (*Salvia pratensis*; Lamiaceae) have been reported among the favoured foraging plants for *M. parietina* in central Europe, and the abundance of sainfoin seemed fundamental for the success of this bee reproduction (Ulrich, 2012). However, none of these plants was abundant in our experimental site, while Sulla (Italian sainfoin, *Hedysarum coronarium* L.), was widespread and was often visited by these bees. Therefore, we decided to analyze the pollen they collect to understand the main food source for *M. parietina* larvae.
In May and June 2018, pollen was removed from the abdominal brushes (fig. 2C) of 9 bees returning to the nests and identified through the comparison with pollen gathered from other plants present around the nests at a distance of about 200 meters. Pollen was transferred into 1.5 ml Eppendorf. Samples were subjected to acetolysis (Erdtman, 1960), included in a water/glycerol solution 50% v/v, and observed under a light microscope operating at 400-630 X. Identification of Poaceae pollen was performed according to Andersen (1979). Pollen percentages were calculated on the total of pollen grains. The diagrams reported in fig S2 were drawn using TILIA 2.0 (Grimm, 1994, 2004).

Contribution of *M. parietina* and other large bees to *Hedysarum coronarium* L. pollination

M. parietina was the main bee species present on *H. coronarium* inflorescence at a distance of about 200 m from the nests. In 2019, we selected 18 *H. coronarium* plants, to form six triads of close plants at a very similar development stage. In each triad, one plant was left unscreened (Control) while the inflorescences of the other two were screened either with a 7 mm grid tissue mesh to exclude large insects (LIex, Large Insects excluded) or with a 2mm grid tissue mesh to exclude most insects (MIex, Most Insects excluded).

Each triad was collected when fruits on control plants were mature. For each plant, we calculated and compared the number of fruits over the number of flowers.

2. Results and Discussion

Structure of isolated nests. Since cells and nests built on the beam could not be isolated, measurements were performed on isolated nests (N=8). Nests have an ellipsoidal section (fig.S1); cells can be oriented parallel or perpendicular to the nest substrate and, within the same nest, are generally oriented in the same direction. On average, solitary nests were made of 13 cells (minimum 7, maximum 17). The major and minor axis were respectively 7.2±2.0 and 5.1±0.8 cm, while the height from the background from which the nest was removed was 2.6±0.5 cm. The thickness of the layer covering the most external cells was 6.2 ± 2.3 mm (measured on 4 cells for each of the 8 nests). Based on the observation of the behaviour of several marked females, we estimate that bees use the latter days of their flying period to build this layer. The layer is likely a protection against closed-cell cleptoparasites and parasitoids, i.e. those parasites inserting their eggs in the cells after these have been sealed by the adult females, such as *Leucospid* wasps.

The average weight of the nests was 59.7 ± 28.2 g, and since the weight of the soil material brought by each female was 0.018 ± 0.007 g (N=10), it corresponds to more than 3,000 building flights (fig. 2C main text), which over the flight season (about two months) of one single female would correspond to more than 50 flight/day. However, since most nests are reused and enlarged year after year, the nests we measured are probably the result of the activity of more females. Although no specific observations were performed, we did notice that collecting soil and building were among the most performed activities and that dozens of bees collected material at the same time on the nearby unpaved road.

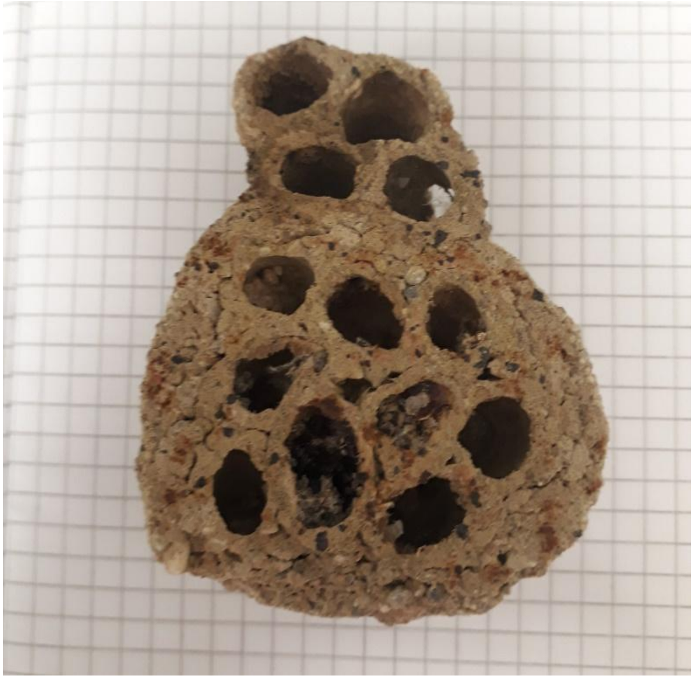

Fig.S1. A nest of *Megachile parietina* removed from the vertical surface.

Pollen identification

Pollen analysis revealed that in one single foraging flight, *M. parietina* females collected 90.7-99.9% of pollen grains from one single taxon (fig. S2). The most represented taxa, *Hedysarum* cf. *coronarium*, and *Lotus* sp., belong to the Fabaceae family. In all the samples, other pollen grains were recorded in a far lower amount. *Spartium*, Rosaceae cf. *Rosa* and *Castanea* occurred in a low but not negligible quantity, suggesting that these plants were also visited by the bee, perhaps in previous foraging flights. Other grains were sporadically recorded, with percentages <0.5, and are possible contaminants, perhaps gathered on flowers where they had been carried by biotic or abiotic vectors.

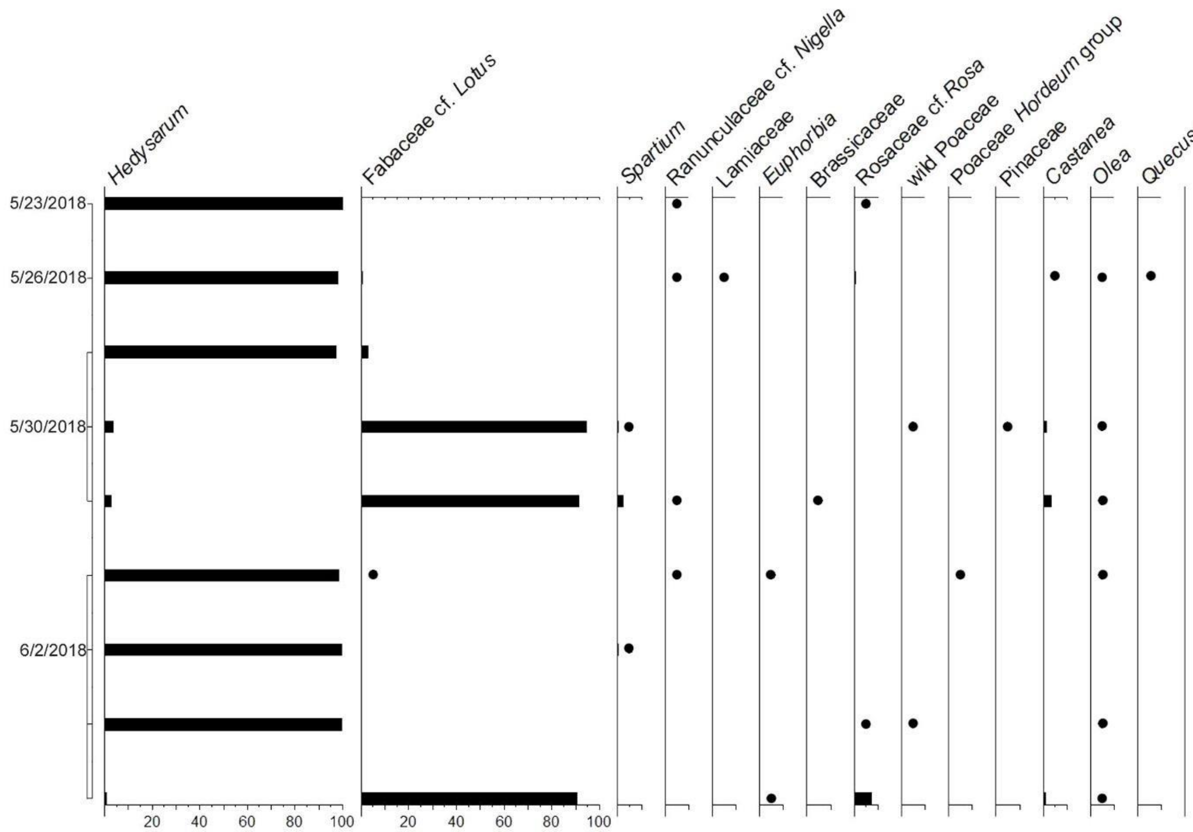

Fig. S2. Pollen grains collected from *M. parietina* abdominal brushes (percentage values) by different females on four different dates.

As a contribution to the reasons for the decline of *M. parietina* in central Europe and the possible threats to this species (see main text), we have collected the estimated data of areas of Sainfoin and Italian Sainfoin cultivated as fodder crops in Italy in the last 15 years, finding them rather stable, with a slight decline only for the first species (ISTAT, 2021) (fig. S3). Although we miss precise data on the population trend in Italy, *M. parietina* is considered widespread in most of the national territory (Quaranta and Cornalba, in prep.).

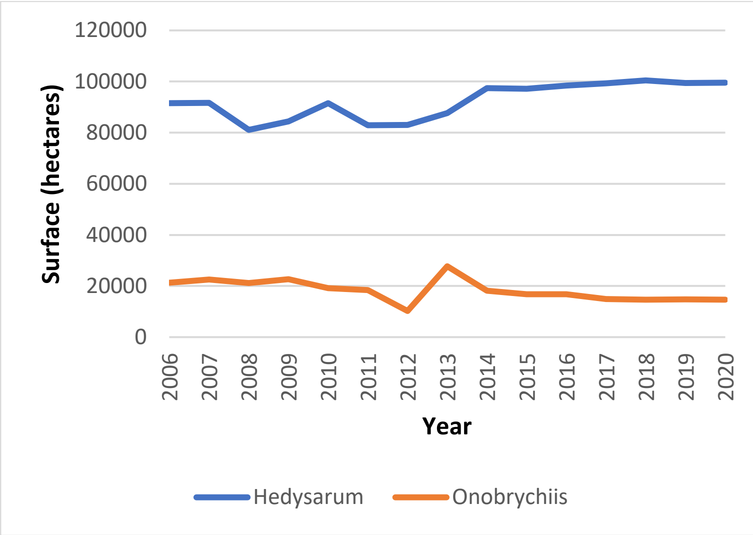

Fig. S3. Total surface of Sainfoin (*Onobrychis viciifolia*), and Italian sainfoin (*Hedysarum coronarium*) in the period 2006-2020 in Italy (ISTAT, 2021).

Contribution of *M. parietina* and other large bees to *Hedysarum coronarium* L. fruits

Inflorescence of *H. coronarium* differed considerably for the ratio between the number of fruits and flowers according to the treatment (fig. S4; one factor ANOVA gdl=2, F=22.8896, P=3.86E-05). Data show that insects excluded by the large mesh contribute about one-half to the fruit formation.

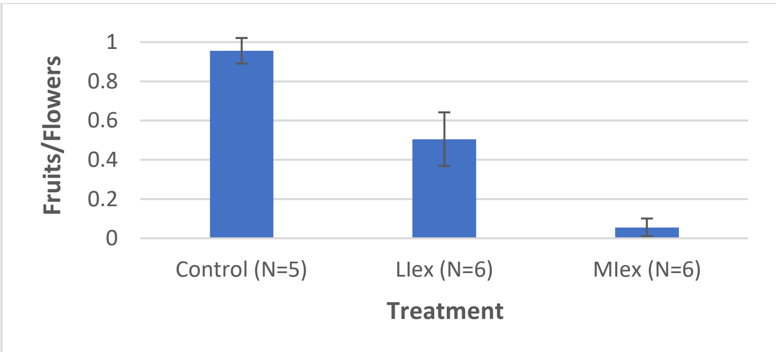

Fig. S4. Ratio between the number of fruits and that of flowers in *H. coronarium* inflorescences unscreened (Control) or screened with meshes with a large or small grid size, excluding therefore only large insects (LIex) or most insects (MIex).

3. References

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