

# Critical floral resource levels and nesting biology of the mining bee *Andrena marginata* (Hymenoptera: Andrenidae)

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As a result of changes in farming practices, the mining bee *Andrena marginata* (Andrenidae) is declining over most of its distribution in Europe, including Sweden. The species is specialized on the pollen of Dipsacaceae. The present study explored the role of pollen availability in relation to the nesting biology of this bee at a site in Sweden. The food-plant at the site was *Succisa pratensis*. An average nest (11.2 cells) was provisioned in ca. 20 days and contained about 1,300,000 pollen grains. Male cells were produced late in the season toward the lower parts of the nests that were progressive (i.e. constructed top-downwards). An average cell contained approximately 116,000 pollen grains. The bee population of *A. marginata* utilized 44.0% of the pollen production of the food-plant. Each nest required the whole-season production of ca. 6.9 pollen-producing plants (53.8 inflorescences). A pollen budget was modelled to predict changes in the bee population size during a hypothetical decrease or increase of the food-plant population. Accordingly, a very small bee population ( $\leq 20$  female individuals) was found to require up to 184 food-plant individuals. This pollen budget has potential to predict carrying capacity of bee populations in other habitats.

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The mining bee *Andrena marginata* is a rare and charismatic species. The bee requires open sandy areas for nesting and the aggregations can reach several hundred nests. The species is oligolectic on Dipsacaceae, and most known Swedish populations are utilizing *Succisa pratensis* for pollen. In at least two areas in Sweden the bee population tend to favour *Knautia arvensis* for pollen-collecting and on these sites the population has adapted the flight season to the earlier phenology of *Knautia arvensis*. In one site near Lund in the province of Skåne the bee *Andrena marginata* population has adapted to collect pollen from *Scabiosa canescens*.

The amount of nectar or pollen available limits the population of many insects (Price 1984), in-

cluding butterflies (Dempster *et al.* 1995, Gutiérrez & Thomas 2000) and solitary bees (Minckley *et al.* 1994). A correlation between resource availability and reproductive success has been shown for several solitary bee species (Rosenheim *et al.* 1996). Moreover, this insect group exhibits adaptations to food limitation such as a facultative bias towards the offspring sex that requires fewer resources (Torchio & Tepedino 1980, Frohlich & Tepedino 1986, Kim 1999, Paini & Bailey 2002).

The pollen removal in plants with generalized pollination systems can be traced to a number of abiotic and biotic factors. Abiotic factors can be pollen lost through wind and rain and biotic factors are e.g. contamination from flower-visitors, pollen-feeding and pollen-collecting by flower-



Figure 1. A female of *Andrena marginata* with pollen load of *Succisa pratensis*.

En hona av guldsandbi med fyllda skopor med pollen av ängsvädd.

visitors such as female solitary bees that collect pollen for provisioning brood cells.

A few studies have explored specialized bees in terms of the degree of pollen utilization, defined as the proportion of the total pollen production that is packed in brood cells to feed offspring. Müller *et al.* (2006) studied a number of specialized solitary bee species and suggested a general degree of pollen utilization of ca. 40%. Other solitary bee species have been reported to utilize about 68% of the pollen produced by *Helianthus annuus* (Minckley *et al.* 1994), 96% of the pollen in *Campanula rapunculus* (Schlindwein *et al.* 2005), and 33% of the pollen production in *Knautia arvensis* (Larsson 2005). These studies have based their estimates on brood cell pollen requirements compared to the total pollen produced by the local plant population. So far, no studies have estimated the degree of utilization and incorporated it into calculations of bee population size.



Figure 2. A typical nest aggregation of *Andrena marginata*, below on the right side a nest entrance equipped with a tumulus. En typisk aggregation av bon som hör till guldsandbi. Det infällda fotot visar en boingång med den typiska tumulus.

Among hymenopterans, the allocation of parental investment to offspring is a decision by the female: she either uses stored sperm to fertilize the egg (for female offspring) or does not (for male offspring). Because offspring show a sexual size dimorphism in most wasps and bees (i.e. females are considerably larger than males), the provision mass per brood cell is larger for female offspring. As predicted by Fisher (1930), the investment sex ratio, (i.e. the relative resource required for the total male vs. female offspring), has been shown to be 1:1 in several bee species (Tepedino & Torchio 1982, Frohlich & Tepedino 1986, Johnson 1988, Helms 1994). In these cases, the numerical sex ratio, i.e. the relative number of male vs. female individuals, is biased towards the smaller-investment sex (e.g. Visscher & Danforth 1993).

Most bee taxa utilize pollen as resource for their progeny. Specialized bees utilize pollen from a few or even a single species of plant. Among solitary bees, each female constructs her own nest, gathering pollen and distributing it in separate brood cells for each offspring. Thus, the resources required for bee progeny may be measured by quantifying pollen provisions in nests, and the available resources may be measured by quantifying the number of pollen grains produced over one bee generation within the flight area of the target bee population.

The aim of the present study was to develop the concept of a "pollen budget analysis": a tool that potentially can be used for predicting, maintaining and restoring viable wild-bee populations. To explore the relationship between the population sizes of an obligate pollen-feeder and its food-plant, I chose a system involving the plant *Succisa pratensis* and its specialized bee *Andrena marginata*. I first present the hitherto undescribed nesting biology of the species *A. marginata*, then estimate the number of pollen grains and food-plant individuals required to meet the needs of one brood cell, one nest and the bee population as a whole. Finally, I explore how the pollen budget analysis can be used to predict how a bee population size is affected by changes in the population of the pollen-producing plant.

## Materials and Methods

### Study system

*Andrena marginata* Fabricius (Fig. 1) is one of several solitary bee species highly specialized in the collection of pollen from Dipsacaceae (Westrich 1989). The nests of *A. marginata* are constructed as burrows and the females often prefer exposed sandy patches such as river embankments, disturbed patches created by cattle, paths or road banks (Kocourek 1966, Westrich 1989). The nests entrance is surrounded by a tumulus (Fig. 2), which at the study site contained sand of a slightly different colour than the soil surface, a feature which makes nests easy to locate and count, even if they have been abandoned. The nest architecture, brood cell construction, larval development and hibernation have hitherto not been described. The flight range of *A. marginata* is unknown, but other specialized mining bee species of about the same size tend to have a home range up to 500 m (Eickwort & Ginsberg 1980, Gebhardt & Röhr 1987, Gathmann & Tscharrntke 2002).

The main pollen source for *A. marginata* in NW and C Europe is *Succisa pratensis* and *Knautia arvensis*, but *A. marginata* individuals may also visit *Scabiosa columbaria*, *S. ochroleuca* and *S. canescens* (Westrich 1989, Pekkarinen 1997). Among these potential food-plants of *A. marginata*, only *S. pratensis* was present in the study area. The inflorescences of *S. pratensis* are nectariferous, and attract a broad spectrum of nectar-feeding, pollen-feeding and pollen-collecting flower-visitor taxa (Knuth 1898). The herb *S. pratensis* is gynodioecious (i.e., populations are composed of females and hermaphrodites). A variable (0-30%) proportion of female individuals have been reported from different areas in Europe (Müller 1883, Knuth 1898, Kay 1982). Only hermaphroditic individuals produce pollen.

*Succisa pratensis* is found in calcareous open grasslands, moist meadows and pastures, and has suffered a notable decline due to changes in land management (Ekstam *et al.* 1998, Vergeer *et al.* 2004). As a result, the specialist bee *A. marginata* has declined in most areas in C and NW Europe (Westrich 1989, Falk 1991, Pekkarinen 1999), and is currently red-listed in a number of European countries.



Figure 3. The site Stridsvagnshöjden in the county of Halland hosts one of the largest nest aggregations in Sweden of *Andrena marginata*. The site consists of large open sandy areas surrounded by wetlands that deliver numerous flowers of *Succisa pratensis* – the host plant.

Lokalen Stridvagnshöjden vid Tönnersjömålet utanför Mästocka hyser en av Sveriges största boaggregationer av guldsandbi. Här finns stora öppna sandtytor som omges av våtmarker som producerar mycket ängsvädd.

### Study site

The study was performed in August and September 2003–2004 in an open heathland landscape in SW Sweden (56°40'29"N, 13°15'28"E, Veinge-Tjärby parish). The heath is named Tönnersjömålet and the specific site is usually referred to as Stridsvagnshöjden (Fig. 3). This heath has historically been used by farmers as a pasture, but since 1940 the area has been managed by controlled burning annually and used as a military training area. This activity regularly creates patches of exposed sand. The vegetation is dominated by *Calluna vulgaris* and with presence of several herbs favoured by the management regime, e.g. *Arnica montana*, *Succisa pratensis* and the shrub *Genista pilosa*.

The study population of the food-plant *S. pratensis* was distributed in patches on and around

a hill, covering ca. 27 ha. The plant population was isolated by wetlands from other flowering individuals of *S. pratensis* by >550 m. The nests of *A. marginata* were scattered and in small aggregations in the well-drained sandy soil on the top and on the slopes of the hill; thus the nests were located in a central area in relation to the food-plant population. The greatest distance between an *A. marginata* nest and the most peripheral food-plant was ca. 290 m.

### Nest excavation

To quantify pollen provisions and to document the nest architecture, nests of *Andrena marginata* were excavated. For excavation (Fig. 4), nests in complete or almost complete structure were chosen on the basis of the behaviour and shape of the female. Prior to excavation, melted tin was



Figure 4. The procedure of excavating solitary bee nest requires patience and accuracy. A single nest usually demands 1-3 hours. It is important to carefully choose site and spot for excavation in order to minimize the negative effect on bee the population.

Utgrävning av solitärbibon är en aktivitet som kräver både tålamod och noggrannhet. Utgrävning av ett bibo kräver vanligen 1-3 timmar. Det är viktigt att välja lokal och angreppssätt med omsorg för att minimera den negativa påverkan som en utgrävning har för en population.

poured in each nest. Each nest was carefully excavated during 1-3 h. The tin structure served as a central reference point for those branches that were already filled up with soil and therefore not reached by the melted tin. The brood cells were classified based on the contained offspring as: *large* containing a postdefecating larva where feces were present in the cell and the larva had a distinctly wrinkled segmentation and there were no (remaining) pollen provision; *medium* containing a predefecating larva where no feces were present and the larva had no wrinkled segmentation and there were no (remaining) pollen provision; *small* still containing some pollen provision with a feeding larva that was longer than the diameter of the pollen provision; *initial* containing a larva feeding, or close to the commencement of feeding, on the pollen ball, and with the length of the larva being smaller

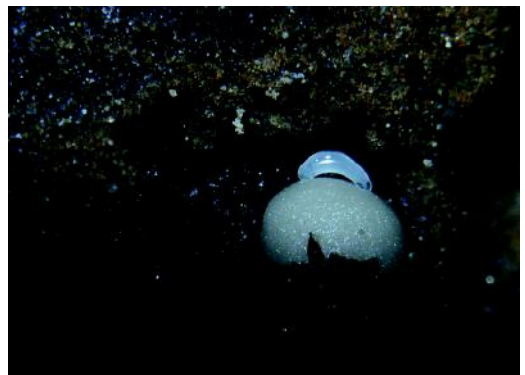


Figure 5. A small larva, just a few days old, of *Andrena marginata*. The larva remains on the top of the pollen load where it was deposited as an egg by the female bee.

En larv av guldsandbi som alldeles nyligt kläckts från sitt ägg. Larven sitter på toppen av pollenbollen på samma plats där dess ägg deponerades av honan.

than the diameter of the pollen provision; *egg* containing an egg; and *unclosed* representing a cell which not yet had been sealed by the female bee and still physically connected to the main shaft. The brood cell angle of inclination, depth from soil surface and distance to main shaft of the tunnel were measured.

To quantify the brood cell pollen requirements, cells that contained pollen provisions with unhatched eggs were collected in 70% ethanol. In the laboratory, the provision samples were sonicated (KS101, Kerry Ultrasonics Ltd.) for 35 min and quantified by counting a known volume proportion of the sample under a binocular microscope (modified from Kearns & Inouye 1993). I used the average of 3 independent volume proportions from each provision sample.

To determine brood cell sex distribution within nests, a number of larvae were collected for subsequent artificial rearing. These reared larvae differed in male vs. female sex significantly in 3 characteristics: age order in nest, distance to main shaft and cell angle of inclination. The age order was given during excavation as a within-nest relative measure of when the cells were constructed. The age order is easy to see during excavation as cells usually can be ordered from unclosed, egg, initial (Fig. 5), medium and large representing cells with increasing age. This order was set relative to the total number of cells. For example, the second constructed cell in a nest containing 20 cells was given the relative order 0.10. Distance to main shaft was measured during excavation and is illustrated in figure 8. The cell inclination was also measured in the field and represents the cell inclination compared to a horizontal line. The average values of these three characteristics were used to estimate the sex of brood cells containing pollen provisions, as most of those cells only were measured and not object to rearing. A cell was estimated as male (female) if it showed  $>0.52$  ( $<0.38$ ) (relative order 0-1),  $<2.06$  ( $>5.23$ ) (cm) and  $<11.28$  ( $>17.31$ ) ( $^{\circ}$ ) for each of the 3 characteristics, respectively. Brood cells with  $\geq 2$  male (or female) characteristics were considered as male (or female), while brood cells lacking  $\geq 2$  characteristics or with any conflicting characteristic were not estimated to sex.

The pollen requirements for an average nest,  $P_{nest}$ , was calculated as:

$$P_{nest} = n_f \times p_f + n_m \times p_m$$

where,

$n_f$  = the average number of female cells per nest,

$p_f$  = the average number of pollen grains per female cell,

$n_m$  = the average number of male cells per nest,

$p_m$  = the average number of pollen grains per male cell.

#### *Pollen-collecting bouts*

To estimate the per-day foraging efficiency, the foraging time of a group of adult females were recorded during one full day (on 13 Aug 2004, between 08.10 a.m. and 18.15 p.m.). The pollen amount gathered during a pollen-collecting bout was measured by sampling female bees at nest site during arrival from collecting pollen. These bees were stored in 70% ethanol and sonicated for pollen release from the bee's scopae. The bees were then moved to a new jar of 70% ethanol and sonicated again. This process was repeated 3 times and the bees were then put under a binocular microscope for visual counting of any remaining pollen grains. The pollen content in the jars was quantified as described for the pollen provision samples.

#### *Food-plant population characteristics*

To estimate total pollen production, a number of food-plant characteristics were recorded. The population size of the food-plant was measured on a single day (9 Sep 2004) by counting all individuals flowering or that had flowered during the current season. For hermaphroditic morph frequency determination, I registered the sex of 260 random individuals of *S. pratensis*. Additionally, I randomly sampled hermaphroditic individuals (females were excluded as they do not produce pollen) in late-season, measuring the total number of inflorescences produced over the season and flowers per inflorescence. Anthers were collected on a single day (12 Aug 2004) and placed in 70% ethanol for pollen content quantification, as described for the pollen provisions. The pollen production in the plant population,  $P_p$ , was calculated as:

$$P_p = S \times D_{herm} \times i \times f \times a_{fl} \times a_p$$

where,

- $S$  = plant population size (number of individuals),  
 $D_{herm}$  = proportion of hermaphrodites in the population,  
 $i$  = number of inflorescences per hermaphroditic individual,  
 $f$  = number of flowers per hermaphroditic inflorescence,  
 $a_{fl}$  = number of anthers per flower (4 in *S. pratensis*),  
 $a_p$  = number of pollen grains per anther.

#### The available pollen resource

The amount of pollen remaining available for flower-visitors,  $P_a$ , was calculated as  $P_p$  corrected for number pollen grains that had not been removed from anthers. The latter pollen amount was quantified by counting pollen grains in withered anthers under a binocular microscope (sampled in the field on 9 Sep 2004).

#### The bee population size

The utilization of pollen removal by *A. marginata* may be calculated by measuring the visitation frequency by all flower-visitors (see Larsson 2005). By correcting the visitation frequency by per-visit effectiveness (for each species or for each group of species), the removal of pollen by each species is obtained. The utilization of *A. marginata* is the contribution to pollen removal relative to the total pollen removal by all flower-visitors. The bee population size, in this study defined as the number of reproducing female individuals,  $R$ , may be calculated as

$$R = \frac{P_a \times UT_{spec. bee}}{P_{nest}}$$

where,

- $UT_{spec. bee}$  = the utilization of pollen by *A. marginata*,  
 $P_{nest}$  = the pollen requirements for one average nest.

In this study,  $R$  was estimated by counting on a single day (8 Sep 2004) the number of nests that were active or had been active during the season. This count also included nests that had been abandoned before completion; however such nests were also included in the excavation and will therefore not affect the result.  $P_a$  and

$P_{nest}$  were estimated by the plant morphology analysis and the nest excavation data, respectively. In this study  $UT_{spec. bee}$  was not empirically measured but calculated from the formula.

#### Statistical analyses

Statistical analyses were performed with Minitab 14.0 (Minitab Inc. 2000). The Anderson-Darling test was used to test normal distribution. For comparing various characters of male vs. female brood cells  $t$ -tests were used. Linear regressions were used to test the effect of brood cell depth on amount of pollen provision, and the effect of cell order on cell depth, the lateral tunnel length and cell angle of inclination. LSD-test was used to test differences between brood cell categories in their relation to cell depth from soil surface. For mean values standard error ( $\pm$ SE) is provided.

## Results

### Nest architecture

A total of 32 nests were excavated. The nest-entrance was usually surrounded by a distinct tumulus (an asymmetric heap of sand). The tumulus was left open when the female was foraging, and was sometimes closed when the bee was inside the nest, and was generally closed during unfavourable weather and during the night. The main shaft of the tunnel averaged  $24 \pm 0.81$  cm in length ( $N=32$  nests), and went downwards irregularly at  $90$ - $100^\circ$  from the soil surface. The diameter of the main shaft was  $5.4 \pm 0.12$  mm ( $N=6$  nests). The cells were  $10.9 \pm 0.26$  mm ( $N=6$  cells) in length and  $6.3 \pm 0.12$  mm ( $N=10$  cells) at their broadest part, forming a non-symmetric ellipse more flattened at the bottom than at the top. The cell angle of inclination was  $15.4 \pm 0.81^\circ$  ( $N=158$  cells). The average nest had  $11.2 \pm 1.4$  cells ( $N=32$ ). The stages of the cells and their spatial distribution within the nest revealed that the nest was constructed progressively, (i.e. from the top downwards, Fig. 6, 7a). The distance from the cell to the main shaft indicated a decrease as the nest construction advanced (Fig. 7b). The nest resembled an inverted straight cone, having its base  $16.1 \pm 0.42$  cm ( $N=32$ ) below the soil surface (Fig. 8). This type of nest has been described as linear-branched (Malyshev 1926).

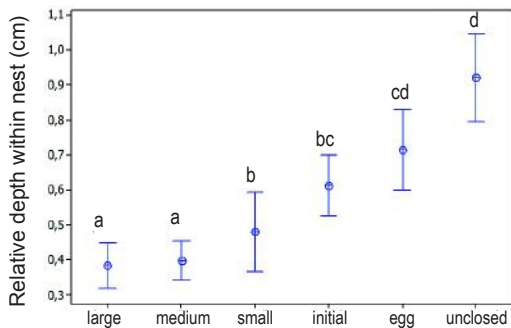


Figure 6. The variation in brood cell content (large larva, medium larva, small larva, egg or unclosed cell) (N=306) shows that the nests are constructed from top downwards. Bars not sharing a letter are significantly different at  $p < 0.05$  (LSD-test). Error bars:  $\pm$ SE.

Variationen av födocellsinnehållet (stor larv, intermediär larv, liten larv, ägg eller icke försluten cell) (N=306) visar att boet anläggs från toppen och nedåt. Kategorier som inte delar en bokstav skiljer sig signifikant med  $p < 0,05$  (LSD-test). Linjerna visar standardfelet.

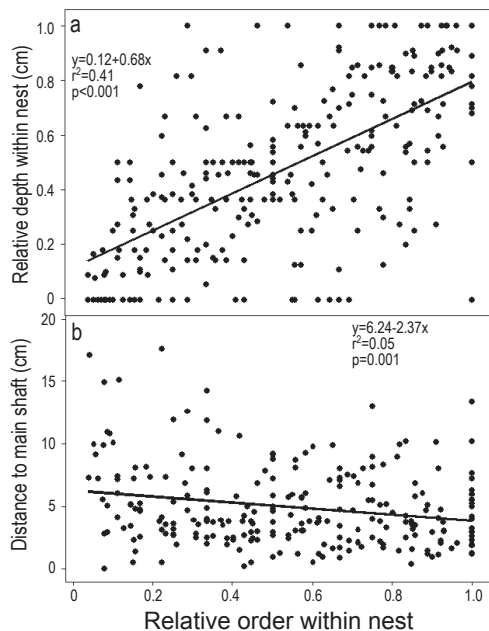


Figure 7. The relationship between brood cell order within the nest and – a) the depth of the brood cell and – b) the distance from the brood cell to the main shaft.

Kopplingen mellan födocellernas inbördes ordning – a) och djupet och – b) avståndet mellan födocellen och boets huvudschakt.

### Development

The egg of *A. marginata* is white and shiny and  $2.5 \pm 0.07$  mm (N=5 eggs from different nests) in length. The egg is laid on the top of the pollen provision and attached to the pollen mass with one end. After consumption of the pollen provision, the larva defecates and develops wrinkled segmentation and turns into a prepupa. The species hibernates as prepupae.

### Pollen provision

Cells were provisioned with  $111,910 \pm 6,091$  pollen grains (N=23) for male estimated cells and  $120,494 \pm 6,017$  pollen grains (N=26) for offspring not estimated to sex (t-test:  $t = -1.00$ ,  $p = 0.321$ ). No pollen provisions were estimated to belong to a female cell due to their characteristics in the nest – all sampled pollen provisions had male cell characteristics. All cell provisions

were therefore pooled in further analysis. An average cell contained  $116,465 \pm 4,286$  pollen grains (N=49). The amount of cell provision did not increase with increasing distance to the main shaft of the tunnel, with depth, or order of the brood cell (all  $p > 0.05$ ). This indicates that the female tend to provision the same amount to male cells regardless when or where in the nest the cell is built. However, only estimated male cells were sampled. Probably, female cells are provisioned with more pollen and as female cells were not sampled we do not know whether female cells are provisioned differently with increasing distance to the main shaft of the tunnel, with depth, or order of the brood cell. An average pollen provision represents pollen that was produced by ca. 2.0 hermaphroditic inflorescences or 0.3 individuals of *S. pratensis* during a complete season.



*Foraging and pollen load*

Females foraged between 08.10 a.m. and 18.15 p.m. No females were found to provision more than one nest simultaneously. There were no indications (e.g. rubbed, reduced wing surface of females initiating a new nest) of females constructing a second nest. Pollen-foraging females spent on average  $74 \pm 6.7$  min ( $N=8$ ) outside the nest per pollen-collecting bout. Females returning to their nests carried  $8,861 \pm 1,769$  pollen grains ( $N=14$ ). Consequently, an average cell required 14 pollen-foraging trips for its completion. An average nest (11.2 cells) consumed 1,299,029 pollen grains, thus requiring ca. 150 collecting bouts. Under favourable weather conditions, females were able to make 7-9 pollen-foraging trips during one day. Therefore, about 20 days with good weather conditions were needed to provision an average nest.

*Artificial rearing and sex ratio*

Out of the collected brood cells ( $N=111$ ), 51 individuals (10 males and 41 females) of *A. marginata* were successfully reared in the laboratory. The sex ratio was 19.6% males. The remaining cells were reared unsuccessfully as the larvae were killed by fungal or bacterial parasitism ( $N=57$ ) or starvation ( $N=3$ ) and sex determination could not be done. Female cells were located further away from the main shaft of the tunnel when compared to the male cells ( $t=3.77$ ,  $p=0.001$ ). The angle of inclination of the female cells was steeper when compared to male cells ( $t=2.37$ ,  $p=0.031$ ). Moreover, by measuring the

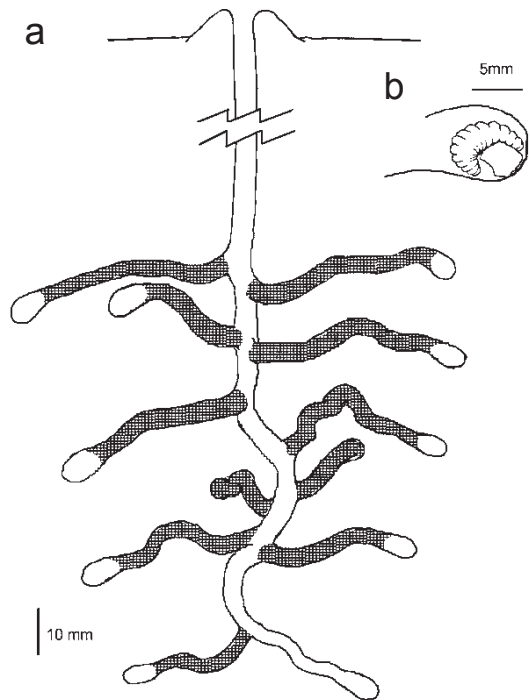
*Critical resources of a mining bee*

Figure 8. Schematic architecture of the nest (a) and one brood cell (b) of *Andrena marginata*. The number, the length and the relative position of the lateral burrows represent an average based on 32 nest excavations. A grid illustrates the lateral tunnels that have been re-filled with soil by the female bee.

Schematisk arkitektur av boet (a) och av en födocell (b) för guldsandbiet. Antalet, längden och den relativa placeringen av sidogångar representerar ett genomsnitt baserat på 32 utgrävda bon. Mörkade sidogångar indikerar att de fyllts igen av honan.

Table 1. Results of comparative analysis of male, female and parasitized cells of *A. marginata* artificially reared in the laboratory. Standard error is indicated in parenthesis.

Resultaten av en jämförande analys av hanliga, honliga och parasiterade guldsandbiceller uppfödda i lab. Inom parantes finns standardfelet.

Brood cells	Male	Female	Parasitized
N	10	41	57
Order (no.) in nest <sup>1</sup> / Ordningsnummer i boet	6.29 (1.23)	5.66 (0.65)	8.53 (0.82)
Relative order within nest <sup>2</sup> / Relativ ordning i boet	0.52 (0.04)	0.38 (0.04)	0.47 (0.04)
Depth (cm) from soil surface/Djup (cm) under markytan	19.38 (0.92)	19.89 (0.46)	21.31 (0.41)
Relative depth within nest <sup>1</sup> / Relativt djup under markytan	0.24 (0.09)	0.44 (0.04)	0.45 (0.04)
Distance (cm) to main shaft <sup>2</sup> / Avstånd (cm) till huvudgången	2.06 (0.59)	5.23 (0.60)	4.73 (0.45)
Inclination (°) of the cell <sup>2</sup> / Lutning (°) på celler	11.28 (2.00)	17.31 (1.57)	16.56 (2.06)

<sup>1</sup> parasitized vs. female+male cells significantly different with  $P<0.05$

<sup>2</sup> male vs. female cells significantly different with  $P<0.05$

Table 2. Population characteristics of the food-plant *S. pratensis* included in the pollen budget.

Populationsspecifika data för värdväxten ängsvädd som användes i pollenbudgeten.

Population characteristics	N	Mean	Min	Max	SE
Inflorescences per individual (no.)/ Antal blomhuvuden per individ	22	7.8	1	42	1.80
Flowers per inflorescence (no.)/ Antal blommor per blomhuvud	51	84	52	122	2.29
Pollen grains per anther (no.)/ Antal pollenkorn per ståndare	22	168	105	236	7.82
Pollen left in withered anthers (grains per anther)/ Pollen kvar i vissna ståndare (pollenkorn per ståndare)	100	6.0	0	28	0.74

relative order within the nest (=order/number of cells in the nest) ( $t=2.57$ ,  $p=0.025$ ), female cells were found to have been constructed earlier and thus must be positioned closer to the soil surface when compared to male cells. However, the particular cells brought for artificial rearing (Table 1) did not show that female cells were closer to soil surface when compared to male cells. This is thought to be an effect of the limited numbers of sampled cells ( $N=51$  of non-parasitized cells) and the fact that these sampled cells were brought from late season. When comparing the successfully reared cells (male and female in one group) with parasitized cells (Table 1), I found that the parasitized brood cells were produced later ( $t=-2.77$ ,  $p=0.007$ ), and were placed deeper below the soil surface ( $t=-2.62$ ,  $p=0.010$ ).

#### Pollen utilization

The food-plant population characteristics (Table 2) were used to calculate total pollen production. The plant population consisted of 4,014 individuals and had a hermaphroditic morph frequency of 99.6%. The number of anthers in *S. pratensis* flowers is fixed and includes 4 anthers per flower (Knuth 1898). The solitary bee *A. marginata* ♀ was found to utilize 44.0% of all pollen produced, 52.4% was removed by other flower-visitors and 3.6% of the pollen remained in anthers. When considering only pollen available for flower-visitors, *A. marginata* utilized 45.6%.

#### Bee vs. food-plant population size

An average nest of *A. marginata* represented the whole-season pollen production of 22.7 inflorescences or 2.9 hermaphroditic *S. pratensis* individuals. However, when all other pollen removal agents were included, the average nest required 53.8 inflorescences or 6.9 hermaphroditic individuals. The entire bee population was

estimated, by counting nest entrances, to include 580 pollen-collecting females. This bee population required 3,998 hermaphroditic individuals (or 4,014 if adjusted for the presence of female individuals) of *S. pratensis*. This empirical bee and food-plant relationship was extrapolated over hypothetical sizes of the bee population. By assuming a similar degree of utilization (44.0%), the respective required resource was calculated for a range of sizes of the bee population (Table 3). A small bee population numbering only 20 females were found to require up to 184 individuals of the food-plant *S. pratensis* (Table 3).

#### Discussion

The results of the present study provide an estimation of the critical pollen resources required for one nest and for an entire bee population as a whole. The model corrects for pollen removed by other flower-visitors. Thus, such pollen budget analysis may open a new tool-oriented approach in conservation, where the relationship between specialized solitary bees and their food-plants is expressed in the currency of easy-counted plant individuals. Further variables of the food-plant (e.g. density) can be easily explored and included in the model.

#### Pollen budget

Pollen budget data (Table 3) allow the prediction of bee population size in response to a change in the food-plant population. For example, the pollen budget in this study predicted that 346 individuals of the food-plant can support 50 nesting female bees. Most of the current populations of *A. marginata* in Sweden are small and probably include less than 50 reproducing females (L. A. Nilsson and M. Stenmark, *personal observations*). For these already small bee populations, a small reduction in food-plant numbers or en-

Table 3. The hypothetical size of plant populations needed to support *A. marginata* populations of different sizes (as number of pollen-collecting females) at 99.6%, 90.0% and 75.0 % hermaphroditic morph frequency. The bee population size 580 and food-plant population size 4,014 represent empirical data; the other results are predictions from the pollen budget.

Den hypotetiska storleken på växtpopulationer som behövs för att föda guldsandbipopulationer av olika storlekar (mätt som pollensamlade honor) vid 99,6%, 90,0% och 75,0% hermafroditfrekvens (bara hermafroditer producerar pollen). Bipopulationen 580 honor och värdväxtpopulationen 4 014 baseras på data insamlade i fält; övriga data är beräkningar från pollenbudgeten.

Bee population size	% hermaphroditic morph frequency		
	99.6%	90.0%	75.0%
5	35	38	46
20	138	153	184
50	346	383	460
100	692	766	919
580	4,014	4,442	5,330
1,000	6,920	7,658	9,190

hanced pollen removal by other flower-visitors may have serious consequences, including local extinction. In areas where local bee populations form a metapopulation, plant populations that lose their specialist bees may be recolonized in subsequent years (cf. Ronce & Olivieri 2004). However, as a result of a general decrease of suitable nesting sites and food-plant populations (Pekkarinen 1999), *A. marginata* seems unlikely to maintain a metapopulation (M. Stenmark, *personal observations*). In such areas, in order to restore populations of *A. marginata* it is important to identify and turn a network of low-resource plant populations into populations that can host viable bee populations.

#### Pollen-foraging time

In the present study, *A. marginata* was found to spend ca. 74 min on a pollen-foraging trip. One brood cell required approximately 14 pollen-foraging trips. An average nest required about 150 collecting bouts and ca. 20 days with good weather conditions for complete provisioning. Other specialized mining bee species have been reported to spend an average of 38 min (*A. nycthemera*) (Schönitzer & Klinskik 1990), 95

min (*A. clarkella*) (Gebhardt & Röhr 1987), 98 min (*A. erythronii*) (Michener & Rettenmeyer, 1956), or 170 min (*A. crataegi*) (Osgood 1989) on each pollen-foraging trip. However, other specialized bees have also been reported to have very short pollen-collecting trips (Linsley et al. 1955). For example, the mason bee *Chelostoma rapunculi* was found spending on average 20 min on collecting bouts to *Campanula* (Münster-Swendsen & Calabuig 2000). Soil-nesting solitary bees, such as *Andrena*, usually spend several days (Stephen 1966) constructing the main shaft and one lateral tunnel of the nest before the female starts provisioning the first cell with pollen. Therefore, assuming 3 days for constructing the nest and that 20% of the next days will be unsuitable because of weather, almost 4 weeks, or 27 days, are needed to finish one average nest of *A. marginata*.

#### Female- to male-biased cells over the season

The cells that were successfully reared in the laboratory were mainly those that contained mature or nearly mature larvae (Table 1). In the laboratory, larvae in cells with remaining provision are apparently more likely to suffer from fungal or bacterial parasitism. Because reared cells were collected relatively late in the season, most mature larvae were females and only these were likely to survive. Evidently, *A. marginata* produces female cells early and male cells late in the season. Similarly, in a number of bee species with dimorphic sexes, the large-resource sex tends to be produced early and the small-resource sex late in the season (Rosenheim et al. 1996, Martins et al. 1999, Paini & Bailey 2002, Alcock et al. 2005). This phenomenon has been suggested to be an evolutionary response to an increased resource-limitation (Myers 1978) or to increased stress (Trivers & Willard 1973) throughout the season. Indeed, pollen scarcity is linked to the production of the smaller sex in both experimental (Kim 1999) and in natural habitats (Paini & Bailey 2002). Alcock et al. (2005) suggested that a seasonal reduction in pollen availability is a widespread phenomenon, and that most bees have, through convergent evolution, adopted the same 'large offspring first, small offspring later' tactic. Another explanation for producing male-biased brood cells

over the season is linked to changes in the nesting females' condition. As bees age throughout the season they lose hairs and their wing area decreases, features that reduce the foraging efficiency gradually over season. Therefore, with or without a seasonal decline in pollen availability, it is likely that selection favours the observed pattern of provisioning large offspring first.

#### *Protandry and cell order*

*A. marginata* is protandrous, a widespread phenomenon among species of the genus *Andrena* (Robertson 1918, Friese 1923). Protandry occurs in nest types of *Andrena* that are both stationary (i.e. the side branches are initiated from one centre of the nest) (e.g. Neff & Simpson 1997) and dynamic (i.e. the side branches are spread vertically either progressively or regressively) (e.g. Matsumura 1970). It seems reasonable that protandry can favour shallow-lying male and deep-lying female brood cells. In such a scenario the males will, if triggered by the same temperature as the females, emerge earlier than the females. This pattern is found in e.g. *Andrena vibernella* (Stephen 1966) and *Andrena sublevigata* (Matsumura 1970). Both species have regressive nests, i.e. the female first excavates the whole main burrow and then builds and provisions the cells from the bottom to the top. It is not obvious why *A. marginata* shows a reverse pattern with progressive nests but produces male-biased brood cells as the season proceeds. One plausible explanation is that, as *A. marginata* is relatively a late-summer species, the distance to the soil surface for emergence order may be of minor importance.

#### *The use of the pollen budget*

Cane (2001) suggested that many bee species have the potential to persist in today's ecosystems which are more or less drastically altered by human activities. Clearly, this has a strong bearing on species conservation because most red-listed bee species depend on man-made habitats (Gärdenfors 2010). For resource-limited systems, a pollen budget can be a powerful tool for predicting the size of a bee population, and thus attention should be devoted to future refining of this method in nature conservation. To what extent the pollen budget may be used to

predict the bee population size after a decrease or increase in the food-plant population is directly testable by experiments. For example, in large vs. small food-plant populations a more abundant and species-rich flower-visitor fauna may remove pollen relatively more abundantly. Consequently, in such large food-plant populations the specialist bee may be able to utilize a relatively smaller proportion of the total pollen production. To answer these questions future studies should aim at studying pollen removal as a function of habitat characteristics such as food-plant population size and density, flower-visitor abundance and diversity.

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### Svensk sammanfattning

Utdikning och minskad areal slätter och bete i jordbruksbygder har lett till kraftig tillbakagång för guldsandbi *Andrena marginata* (Andrenidae) i hela det europeiska utbredningsområdet, inklusive Sverige. Arten är specialiserad att samla pollen från väddväxter. Bona grävs ut i marken på vegetationsfria ytor. Målet med min studie var att beskriva hur bona ser och att resonera om hur guldsandbi är knutet till den mängd pollen som finns.

Studien gjordes på en lokal vid Tönnersjöområdet i Halland. Vid denna lokal använde biet enbart pollen från ängsvädd *Succisa pratensis* som larvföda. Ett genomsnittligt bo (se Figur 8) bestod av 11 celler och färdigställdes under ca 20 dagar. En genomsnittlig cell innehöll ungefär 116 000 pollenkor. Bon av guldsandbi visade sig anläggas progressivt, det vill säga uppifrån och ned. Varje cell försågs med endast ett ägg. I de celler som är något större och som laddas med mer pollen lägger honan ett befruktat ägg som därmed utvecklas till en hona. I de celler som byggs något snävare och som laddas med mindre pollen lägger honan vanligen ett obefruktat ägg som därför utvecklas till en hanne. Hanceller för guldsandbi produceras sent på säsongen och läggs därför i de nedre delarna av bokonstruktionen.

Guldsandbipopulationen på den undersökta lokalen utnyttjade uppskattningsvis 44 % av ängsväddens pollenproduktion. Varje enskilt bo fylldes med pollen som motsvarar hela produktionen på 6,9 plantor (53,8 enskilda blomställningar). För att kunna förutsäga förändringar i bipopulationens storlek om ängsväddsplantornas pollenproduktion minskar eller ökar gjordes en pollenbudget. Den visade att en mycket liten hypotetisk bipopulation ( $\leq 20$  honor av biet) kräver upp till 184 pollenproducerande plantor av ängsvädd. Denna pollenbudget kan utvecklas och användas för andra pollensamlare arter av bin – men den kräver artspecifika och ibland lokalspecifika mätningar. Resonemanget om pollenbudget för hotade arter av bin bör kunna förutsäga bärkraft bisamhällen i andra livsmiljöer och därmed bli en viktig metod inom svensk naturvård.