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## Laboratory Rearing of *Osmia cornuta* Latreille (Hymenoptera Megachilidae) on Artificial Diet (\*)

### INTRODUCTION

In the last fifty years agricultural practices and farming methods based on specialized and intensive single-crop systems, the indiscriminate use of pesticides, the destruction of self-sown and undisturbed vegetation and the environmental pollution determined a consistent decrease of wild pollinating insects (Torchio, 1991; Rondinini and Pinzauti, 1994; Radeghieri *et al.*, 1998; Porrini *et al.*, 1999). At the same time, the number of agricultural productions that depend on optimal flower pollination in order to gain fruits and/or seeds (plants cultivated under confinement, plants with unisexual flowers, self-unfertilized crops) increased. Also self-fertile crops frequently set less fruits due to adverse climatic conditions and/or physiological problems, connected to the floral biology (Felicoli and Pinzauti, 1994a).

In Italy, pollination service is usually provided by honey bee colonies in the open field, that are rented purposely, and by nucleus colonies of honey bees or bumblebees in confined environment (Celli *et al.*, 1992; Rondinini and Pinzauti, 1994). Nevertheless, several species of Apoidea on certain crops and under particular environmental conditions might be more effective (Holm, 1973; Torchio, 1991; Bosch, 1994; Seidelmann, 1995a; Wilson *et al.*, 1999). In 1997, a National Project called A.M.A. (B.H.E. = Bee, Honey, Environment) was started up in Italy which includes studies on the biology and pollination efficiency of solitary bees.

At the Institute of Entomology "G. Grandi" (University of Bologna) the research was focused on *Osmia cornuta* Latreille (Hymenoptera Megachilidae) due to its ecological and biological features. This obligate univoltine species (Tasei, 1973; Torchio, 1989) performs flying and pollen gathering activities also at quite low temperatures (Bosch, 1994) within a restricted area (Krunic *et al.*, 1995). These cavity-nesting bees move from tree to tree more readily than honey bees without orienting along rows, thus enhancing cross-pollination (Bosch and Blas, 1994a). They have gregarious nesting behaviour and are active also in confinements (Lepore and Pinzauti, 1994; Pinzauti *et al.*, 1997; Ladurner *et al.*, in press).

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Furthermore, the adult emergency pattern can be modulated and synchronised with blooming (Torchio and Asensio, 1985; Bosch and Blas, 1994b; Felicioli and Pinzauti, 1994a; Felicioli, 1995).

In southern Europe adults of *O.cornuta* may appear from February to April depending on weather conditions. Males emerge first, followed a few days later by females. Mated females nest in suitable cavities, disposing brood cells in a linear order, separated by mud partitions. Female eggs are laid on the pollen provisions in the innermost cells of the nest, whereas male eggs are deposited on the outermost ones. Females' flying and pollen gathering activity lasts up to one month. Hatching takes place a few days after eggs are laid and adult state is reached by September. Bees overwinter within cocoons as imago and emerge the next spring as the temperature warms.

In Italy, *O.cornuta* populations are multiplied in the open field, using the technique of "releasing and rearing" (Felicioli and Pinzauti, 1994b). This management has some disadvantages: big losses due to parasitism and high dispersal rates due to bad weather may occur. Laboratory rearing of *O.cornuta* is therefore a necessary condition for year-round rearing and would enable to overcome these problems (Steen, v. d., 1997). This, in turn, requires the setting-up of an artificial diet, with pollen as basic nourishment.

The pollen preferences of different *Osmia* species were studied by Tasei (1973), Torchio and Asensio (1985), Ricciardelli D'Albore and Pinzauti (1993) and others, analysing pollen samples gathered and stored in the nests, but few data are available regarding the presence of other substances, as nectar or glandular secretions.

Regarding the presence of nectar, Seidelmann (1995b) asserts that in pollen loafs collected by *O.rufa* L., a European species closely related to *O.cornuta*, pollen/nectar ratio is equal to 0.98. Torchio (1985) argued that, when nectar resources are inadequate, provisions made by *O.lignaria propinqua* Cresson are not sufficiently wetted on their surfaces to assure a strong bonding of the egg. Subsequently the strong movements of the developing embryo during late embryogenesis can easily cause a weakly attached egg to dislodge from its anchor, ecdysis can not be completed, the embryo dies and the egg collapses. Concerning *O.cornuta*, Tasei (1973) describes the pollen loafs as uniformly damp. Torchio *et al.* (1987) and Bosch (1994) suggest that these bees, as their relatives, store nectar and pollen in the brood cells.

Referring to the presence of glandular secretions, by injecting C<sub>14</sub> into females of *O.cornuta* and later detecting it in the pollen provisions and larvae, Heroin-Delauney (1966) demonstrated that secretion is added to the provision. For analysing palinologically pollen provisions collected by *Osmia* spp., Tasei (1973) suggests to prepare pollen samples for microscopic observation as soon as possible, since glandular secretions, added by the females, digest the pollen grains' cytoplasm, thus altering their general structure. However, nothing is known about the composition of these glandular secretions.

In 1998 eggs and larvae of *O.cornuta* were reared on an oligidic diet, composed primarily of crude ingredients (Dougherty, 1959), in order to evaluate its impact on the progeny's mortality and development.

In 1999, further trials were set out in order to investigate the chemical and nutritional features of the pollen provisions collected by *O.cornuta* females and to evidence the possible presence of nectar in the pollen loafs. The research aimed also to verify the existence of a maternal factor, produced and added by the mother bee to the provision, to study its importance for the progeny's survival and development and to obtain information on its composition. Finally the impact of the manipulation, i. e. handling and displacing eggs and/or larvae, was quantified.

## MATERIALS AND METHODS

Preparation of the diet and live material.

In 1998, the oligidic diet was prepared following the indications given by van der Steen (1997). Frozen pollen, collected by honey bees, was exsiccated for 1 hour at 40 °C under infrared light and subsequently ground till it got pulverized. Gelatine capsules (type "000") were then filled with 0.4 g pollen paste, made of 75% (weight) pulverized pollen and 25% (weight) sucrose solution (50% vol.). A bit of honey was added to the sucrose solution.

At the end of April, 735 eggs and larvae were retrieved from pedotrophic nests placed in a pear orchard for a pollination trial, and divided into two groups. The individuals of the first group were carefully taken from their pollen provision and allocated on the oligidic diet inside the gelatine capsules. In the second group the individuals were left on their maternal provision, transferring altogether inside identical empty capsules, without manipulating them directly. This group acted as control. Both were incubated in complete darkness at  $t=20\pm 1^{\circ}\text{C}$ , R.H.= $70\pm 10\%$ . The distribution of the different developmental states at the moment of the displacement on the two diets is reported in table 1.

Tab. 1 - Number of eggs and larvae, reared on pollen provisions collected by the mother bee (natural provision) and on oligidic diet (artificial provision).

	Eggs (239)	Larvae (496)
Natural provision	95	236
Artificial provision	148	260

Also in 1999, the research was carried out using eggs and/or larvae recovered in artificial nests placed in a pear orchard for a pollination trial. In order to evaluate the importance of the maternal factor and the manipulation for the progeny's survival and development, on April 28, eggs/larvae retrieved from the nests were displaced inside gelatine capsules (type "000") and provided with the following three different diets:

Group 1 - without maternal factor and with manipulation - 30 eggs/larvae (one egg, twenty-one 3<sup>rd</sup> instar larvae and eight 4<sup>rd</sup> instar larvae), removed from their own pollen provision and displaced on 0.4 g artificial diet. The artificial provision was made of 75% (weight) pure vital pear pollen and 25% (weight) sucrose solution (50% vol.).

Group 2 - with maternal factor and with manipulation -25 eggs/larvae (one egg, fourteen 3<sup>rd</sup> instar larvae and ten 4<sup>th</sup> instar larvae), removed from their own pollen provision and displaced on a pollen provision, collected by another *O.cornuta* female;

Group 3 - with maternal factor and without manipulation -25 eggs/larvae (six eggs, thirteen 3<sup>rd</sup> instar larvae and six 4<sup>th</sup> instar larvae) left on their own pollen provision, thus acting as control.

The 3 groups were incubated in complete darkness at  $t=20\pm 1^{\circ}\text{C}$ ,  $\text{R.H.}=70\pm 10\%$ .

#### Mortality.

In 1998, the two groups were checked regularly during their development and dead individuals were removed. On October 2, 1998, the remaining cocoons were carefully opened and occupants activated teasing their heads, in order to evaluate mortality during development into imago. The temperature of the incubator was then turned down to  $15^{\circ}\text{C}$  for offering the insects an adequate pre-overwintering period (Torchio *et al.*, 1987). On November 6, 1998 they were transferred into a refrigerator ( $t=4\pm 1^{\circ}\text{C}$ ,  $\text{R.H.}=70\pm 10\%$ ) for overwintering (Bosch and Blas, 1994b; Felicicoli, 1995). On March 5, 1999 the adults inside the cocoons had finished diapause and were again activated, thus enabling the determination of mortality during overwintering.

In 1999, the 3 groups were checked twice a day (at 10.00 a.m. pre-pupal and 6.00 p.m.) for a more precise determination of the mortality of the different developmental stages. Cocoons were opened and occupants activated on October 6, 1999 in order to verify if they had completed metamorphosis into adults and if they were still alive. The temperature of the incubator was then turned down to  $15^{\circ}\text{C}$ . On November 19, 1999 they were transferred into the refrigerator ( $t=4\pm 1^{\circ}\text{C}$ ,  $\text{R.H.}=70\pm 10\%$ ) for overwintering.

#### Development time.

In both years of trials, since some larvae at the beginning of the trials had already reached the 4<sup>th</sup> larval instar, only the development time of the 5<sup>th</sup> larval instar (from the moment the larva begins to defecate till it starts spinning its cocoon) has been measured. Since metamorphosis into pups occur inside the cocoons, this development time was not evaluated.

#### Chemical and nutritional features of *O.cornuta* pollen provisions.

In 1999, samples of pollen provisions collected by *O.cornuta* in the pear orchard were sent to the Dept. of Environmental Sciences (University of Siena) in order to investigate the chemical and nutritional features in comparison to pure pear pollen. In detail, the following parameters were analysed:

Total soluble sugars (refractometrically) - 0.5 g of pollen provision collected by *O.cornuta* females and the same amount of pure vital pear pollen were dissolved in 5 g distilled water. After 5 minutes of rest, the 2 solutions were centrifuged for 10 minutes at 3000 rpm. Afterwards the pellet volume (cc), the supernatant volume (cc) and the content of total soluble sugars (%) were measured.

Water content - 0.5 g of pollen provision collected by *O.cornuta* females and

0.5 g of pure vital pear pollen were exsiccated for 6 hours at 100°C and subsequently weighted. The whole procedure was repeated 3 times.

Sucrose, Glucose and Fructose content ( $\mu\text{g}/\text{mg}$ ) – sucrose, glucose and fructose contents of both samples were measured three times, using an enzymatic kit (Boehringer Mannheim test-combination kit No. 716 260).

Microscopic observations – samples of pollen provision collected by *O. cornuta* females and of pure vital pear pollen were microscopically observed, in water as well as in immersion oil.

#### Statistical analysis.

In 1998 as well as in 1999, data regarding mortality on the different diets and for the different developmental stages have been compared by means of  $\chi^2$  in contingency tables 2x2. In 1998, also the developmental state at the moment of the displacement has been taken into account. Development times of the 5<sup>th</sup> instar larvae of the different groups were compared by the non-parametric Mann-Whitney U-Test in both years of trials.

## RESULTS

### Mortality.

In 1998, mortality rates during development into imago and overwintering were registered. Results are summarized in table 2.

Tab. 2 – Mortality (%) during development into imago and overwintering in 1998.

	Transferred as eggs/ 1 <sup>st</sup> instar larvae		Transferred as 2 <sup>nd</sup> -5 <sup>th</sup> instar larvae	
	Development into imago	Overwintering	Development into imago	Overwintering
Artificial provision (with direct manipulation)	54.7	50.0	72.7	13.3
Natural provision (without direct manipulation)	14.7	8.2	25.0	5.8

Mortality during development into imago was significantly higher for the individuals reared on the artificial diet than for those fed with their natural provision, either if the displacement on the artificial diet occurred as eggs or larvae (in both cases  $p < 0.00001$ ). On the artificial provision, mortality was significantly higher for the individuals displaced from their cell to the artificial diet as larvae than for those displaced as eggs ( $p = 0.0002$ ). In the control group, left on the natural provision, no significantly different mortality was recorded between the individuals transferred (with their provision) into the capsule as eggs or as larvae.

Referring to the mortality during overwintering, it was significantly higher for the individuals reared on the artificial diet when they were displaced in the egg state with respect to the control left on natural provision ( $p=0.00001$ ). On the other hand, no significant difference between artificial and natural diet was found for those displaced as larvae. On the artificial diet, mortality was significantly higher for the individuals transferred as eggs than for those displaced as larvae ( $p=0.0237$ ), whereas on the natural provision, no significant difference was recorded in the mortality during overwintering between the two groups of individuals.

Data concerning mortality in 1999 of the different immature developmental stages for the three groups are summarized in table 3.

Tab. 3 – Mortality during development into the adult state in 1999.

N° of dead individuals						
	Eggs/ 1 <sup>st</sup> instar larvae	2 <sup>nd</sup> instar larvae	3 <sup>rd</sup> instar larvae	4 <sup>th</sup> instar larvae	5 <sup>th</sup> instar larvae	Pupae
Group 1	0	0	8	18	4	/
Group 2	1	0	6	1	0	0
Group 3	1	0	0	0	0	0

Only 4 individuals of Group 1, exposed to manipulation and deprived of maternal factor, developed into 5<sup>th</sup> instar larvae. They started defecating, but they all died after a few days (1, 3, 5 and 7 days, respectively). All the individuals provided with the maternal factor (Group 2 and Group 3) that completed development into 5<sup>th</sup> instar larvae did also develop into adults. Nevertheless, mortality before metamorphosis into adults in Group 2, exposed to manipulation, was significantly higher than in Group 3, whose individuals did not undergo manipulation ( $p=0.01$ ). Therefore, the following mortality rates were recorded: 100.0% in Group 1, 32.0% in Group 2 and only 4.0% in Group 3 (fig. I).

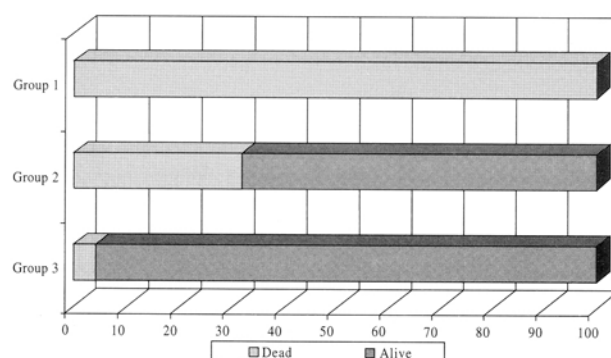


Fig. I – Mortality (%) before metamorphosis into adults.

Development time.

In 1998, the development times of the 5<sup>th</sup> instar larvae lasted significantly longer for the individuals displaced from their provision to the artificial diet in comparison to those left on their own maternal diet, both, if the displacement occurred as egg ( $Z=-5.0766$ ,  $p<0.00001$ ) or as larva ( $Z=-5.915$ ,  $p<0.00001$ ) (figures II and III).

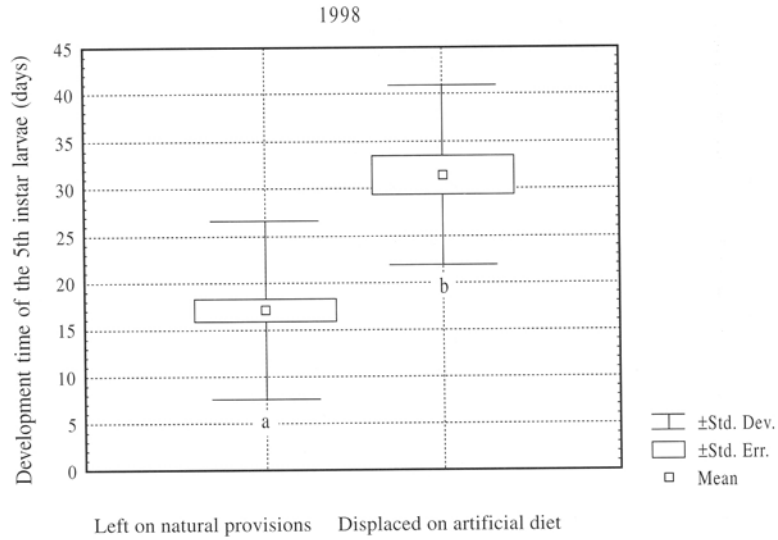


Fig. II – Development times of the 5<sup>th</sup> instar larvae in individuals transferred as egg (different letters indicate statistically significant differences).

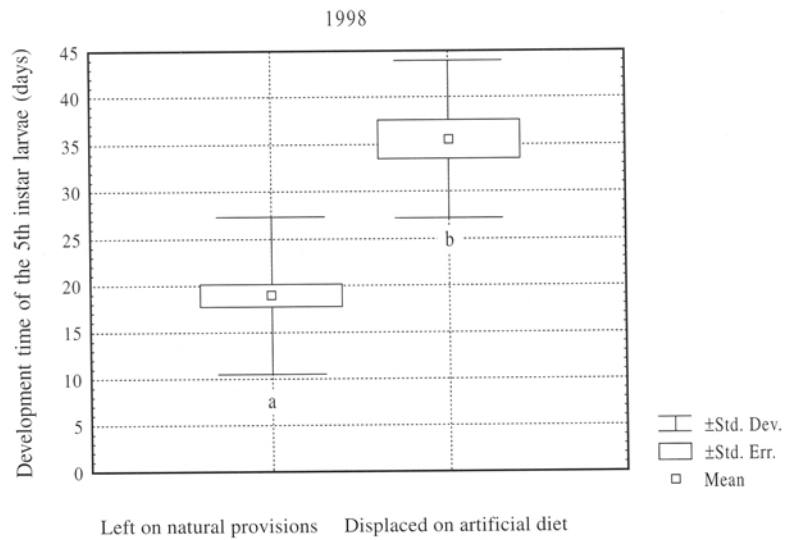


Fig. III – Development times of the 5<sup>th</sup> instar larvae in individuals transferred as larva (different letters indicate statistically significant differences).

In 1999, regarding the development times, a significant difference was recorded between the two groups provided with the maternal factor (Group 2 and Group 3). Development times of 5<sup>th</sup> instar larvae lasted significantly longer in Group 2, exposed to manipulation, than in Group 3, not manipulated ( $Z=3.5328$ ,  $p=0.0004$ ) (fig. IV).

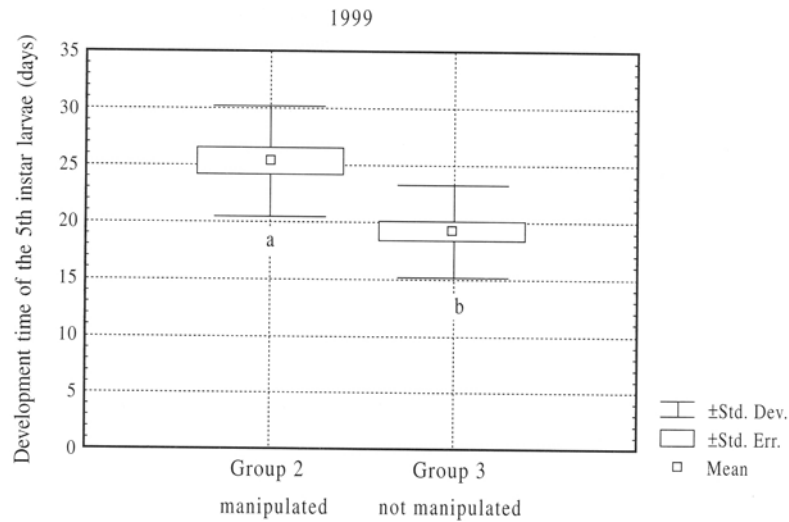


Fig. IV – Impact of manipulation on the development times of the 5<sup>th</sup> instar larvae in Group 2 and Group 3 (different letters indicate statistically significant differences).

#### Chemical and nutritional features of *O. cornuta* pollen provisions.

Results regarding the chemical and nutritional parameters analysed in 1999 are summarized in table 4.

Tab. 4 – Chemical and nutritional features of pollen provisions collected by *O. cornuta* in comparison to pure pear pollen.

	Pollen provisions collected by <i>O. cornuta</i>	Pure pear pollen
Mean concentration of total soluble sugars (%)	50	20
(appreciable mean discard $\cong$ 5%)		
Sucrose (mean $\pm$ sd) ( $\mu$ g/mg)	2.6 $\pm$ 4.5	15.1 $\pm$ 8.4
Glucose (mean $\pm$ sd) ( $\mu$ g/mg)	55.6 $\pm$ 3.8	27.3 $\pm$ 17.0
Fructose (mean $\pm$ sd) ( $\mu$ g/mg)	51.8 $\pm$ 85.0	14.7 $\pm$ 9.3
Pellet volume (cc)	0.3	1.0
Supernatant volume (cc)	4.7	4.0
Dry weight (mean $\pm$ sd) (g)	0.3796 $\pm$ 0.0252	0.4603 $\pm$ 0.0358
Dry weight variation (%)	24	8
Microscopic observation	Pollen aggregations bonded by round yellow-brownish particles (in immersion oil)	No



In the pollen provisions collected by *O. cornuta*, a higher mean concentration of soluble sugars was measured with respect to pure pear pollen. Moreover, in the former, the quantitative analysis of the different components showed a remarkable decrease in the concentration of sucrose and, at the same time, a much higher increase in glucose and fructose, with respect to the latter. Lower pellet volume and dry weight and consequently higher supernatant volume and higher dry weight variation were recorded for the pollen provision in comparison to the pure pear pollen. Observing the samples under the microscope in immersion oil, in the pollen provisions collected by *O. cornuta* pollen aggregations bonded by round yellow-brownish particles were noticed, which dissolved diluting the sample in water. No pollen aggregations were evidenced in the samples of pure pear pollen.

## DISCUSSIONS AND CONCLUSIONS

In 1998 mortality during development into imago is significantly higher for *O. cornuta* progeny when laboratory rearing occurs on artificial provisions rather than on natural ones (table 2), suggesting that something is missing in the artificial provision. It might be assumed that, for the individuals transferred as larvae, the initial feeding on the pollen loaf collected by the mother bee could provide an advantage, in terms of useful maternal substances, with respect to those individuals transferred as egg, that never fed on their maternal provision. On the contrary, data show that mortality during development into imago, for the individuals reared on artificial diet, is significantly higher for those displaced from their provision as larvae ( $p=0.0002$ ). This could be due to a higher sensitivity to manipulation of the larvae with respect to the eggs, that dissolves the benefit provided to the larva by feeding for some days on the maternal provision. Several hypotheses are possible. The chorion could be more resistant to mechanical solicitation than the young larva integument. Moreover, the first movements of the larva after the ecdysis are likely to be crucial to bond it to the provision. The displacement during the very first step of development could therefore severely compromise its capability to reattach itself to the provision (this is not true for defecating larvae, which do not need to be fixed to the provision, since they are capable of active movements inside the cell).

On the other hand, results concerning mortality during overwintering seem to lead to different conclusions, since the number of dead individuals registered on the artificial diet was significantly higher among those transferred as egg than for those displaced as larva ( $p=0.0263$ ). Moreover, no significant difference was found between the mortality rates of the larvae left on the maternal provision and that of the larvae displaced on the artificial diet, which should have suffered for manipulation. Further studies are needed to verify if larvae are effectively more sensitive to manipulation than eggs.

The results regarding development times of the 5<sup>th</sup> instar larvae in 1998 lead to the hypothesis that something is missing in the artificial diet. Independently if displacement on the artificial provision occurred as eggs or as larvae, it lasts significantly longer than in the control group left on the natural provision (figures II and III).

To sum up, high immature mortality rates, recorded in the laboratory rearing on artificial diet in 1998, suggest that *O.cornuta* females produce and add something to the pollen provisions, that we call maternal factor, essential for the progeny's survival. Data also indicate that manipulation, i. e. handling and displacing eggs and/or larvae, has a negative influence on the progeny's development.

In 1999, the significant differences in mortality ( $p=0.01$ ) as well as in development times of the 5<sup>th</sup> instar larvae ( $p=0.0004$ ) recorded between Group 2 and Group 3, differing for the manipulation factor, evidence the remarkable repercussions of handling and transferring eggs and/or larvae on survival and development, thus confirming the results obtained in 1998 trials. Manipulation strongly contributed even to mortality in Group 1, i. e. eggs/larvae that were handled and deprived of the maternal factor. Nevertheless, this can not be the only factor involved, since none of the individuals reached the adult state, whereas many individuals of Group 2, manipulated and with maternal factor, and nearly all individuals of Group 3, which were left on their own maternal provision, completed metamorphosis. It can not be excluded that low wetness of the artificial pollen loafs affected survival, since disposing eggs/larvae on the provision required us considerable manual dexterity. With regard to this, Torchio (1985) observed that *O.l.propinqua* adds sufficient nectar to attach the egg on the provisions, not adequately supplied with nectar during the deposition of the pollen loaf. Hatching occurs, but the developing larva soon dies because it is incapable of successful biting into the dry cell provisions. Many Authors evidenced that mortality of larvae before defecation primarily contributes to the mortality of the immature progeny (Kristjansson, 1992; Seidelmann, 1995b). Mortality of larvae after defecation and pupae rises only if there is a sudden increase in temperature (Seidelmann, 1995b). In van der Steen's indoor rearing of *O.rufa*, 37.0% of the progeny died as egg, 34.0% as larva before defecation and only 1.8% in the stages after defecation. Since in our 1999 study most of the individuals reared on the artificial diet did not reach the defecation phase, we may deduce that, in fact, manipulation and dryness account for the predominant part of mortality in Group 1 (figure I), but we also suppose that something missing in the artificial diet prevented the larvae from completing development. We presume that the mother bee kneads something to the pollen, as already evidenced by Heroin-Delauney (1966) with the C<sub>14</sub> method, which is essential for the progeny's survival and development.

A stepwise analysis of the data regarding the chemical and nutritional features of pollen provisions collected by *O.cornuta* females should help to clear up these enquiries (table 4). The higher mean concentration of soluble sugars measured in pollen provisions collected by *O.cornuta* with respect to pure pear pollen is a reliable indication for the presence of nectar. Also comparing the pellet volume and the supernatant volume of the two samples under investigation it may be deduced that the mother bee adds soluble material to the pollen provision. In fact, a lower dry weight and consequently a higher dry weight variation were recorded. Observing the samples under the microscope, pollen aggregations bonded by round yellow-brownish particles can be noticed only for pollen provisions collected by *O.cornuta* in immersion oil. These aggregations are absent if the

samples are diluted in water and in the samples of pure pear pollen, showing that water soluble material is added to the provision by the female. All these data, in addition to the high glucose content of the provisions collected by *O.cornuta* (table 4), suggest that this material is nectar. Since fluctuations recorded regarding fructose content are very broad, it may also be deduced that the bees in the pear orchard used various nectar sources with different sugar composition.

Nevertheless, our results do not enable us to assert that nectar is the only substance added. The increase in glucose and fructose and the decrease in sucrose, recorded for the provisions collected by *O.cornuta* (table 4), can not be explained only by the addition of nectar, but might be related to the presence of the enzyme saccharase in the insect's saliva, which could be the maternal factor hypothesized. Solitary bees' glandular secretion and pollen digestion mechanisms might be similar to those of honey bees (Nepi *et al.*, 1997), which actually, among other substances, produce saccharase and add it to the honey (White, 1963; Maurizio, 1968). It has to be pointed out, that in 1998 trials, where honey, and consequently the enzyme saccharase, was added to the oligidic diet, immature mortality never reached 100% (table 2).

This research therefore warrants further studies to analyse the impact of the pollen provisions' dryness on survival, to clear up the presence of the enzyme saccharase and to investigate if this is the only maternal factor involved or if a set of substances is added. The influence of the manipulation on the development time of the other larval instars might also be evaluated and a better technique of handling eggs and larvae could be studied.

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#### SUMMARY

In 1998 eggs and larvae of *O.cornuta* were reared on an oligidic diet, composed primarily of crude ingredients, in order to evaluate its impact on the progeny's mortality and development.

In 1999 further trials were set up, in order to investigate the chemical and nutritional features of pollen provisions collected by *O.cornuta* females and to evidence the possible presence of nectar in the pollen loafs. The research aimed also to verify the existence of a maternal factor, produced and added by the mother bee to the provision, to study its importance for the progeny's survival and development and to obtain information on its composition. Finally the impact of the manipulation was quantified.

In 1998, eggs and larvae of *O.cornuta* were transferred inside gelatine capsules, some left on their own pollen provision, some offered an oligidic diet. The eggs and larvae, left on the pollen provision collected by *O.cornuta* females in the open field, acted as control. For both groups, mortality during development into imago and during overwintering as well as development times of the 5<sup>th</sup> larval instar were measured.

In 1999, eggs/larvae of *O.cornuta* were displaced inside gelatine capsules and provided with the following three different diets: 1) without maternal factor and with manipulation - eggs/larvae removed from their own pollen provision and displaced on artificial diet; 2) with maternal factor and with manipulation - eggs/larvae removed from their own pollen provision and displaced on a pollen provision, collected by another *O.cornuta* female; 3) with maternal factor and without manipulation - eggs/larvae left on their own pollen provision, thus acting as control.

As in 1998, for each group of eggs and larvae, mortality during development and the development time of the 5<sup>th</sup> larval instar were determined.

In 1999, also the chemical and nutritional features of the pollen stored in pedotrophic nests of *O.cornuta*, placed in a pear orchard, were investigated in comparison to pure pear pollen. Results regarding nutritional values demonstrate that *O.cornuta* females add nectar to the pollen provisions, collected on different plant sources, and knead glandular secretions to the pollen. The increase in glucose and fructose and the decrease in sucrose, recorded for the provisions collected by *O.cornuta*, can not be explained only by the addition of nectar, but might be related to the presence of the enzyme saccharase in the insect's saliva, which could be the maternal factor hypothesized.

Data concerning development time and mortality in both years of trials demonstrate the remarkable impact of the manipulation on progeny's survival, which is also affected by low wetness of the artificial pollen loafs, and support the existence of the maternal factor. This research warrants further studies to analyse the impact of the pollen provisions' dryness on survival and to investigate if the enzyme saccharase is the only maternal factor involved.

KEY WORDS: *Osmia cornuta*, rearing, artificial diets.

### Allevamento in laboratorio di *Osmia cornuta* Latreille (Hymenoptera Megachilidae) su dieta artificiale

#### RIASSUNTO

Nel 1998 uova e larve di *O.cornuta* sono state allevate su una dieta oligidica, composta principalmente da sostanze grezze a composizione chimica non nota, al fine di valutare la sua influenza sulla mortalità e sullo sviluppo della progenie.

Nel 1999 sono state impostate ulteriori sperimentazioni, il cui scopo è stato lo studio delle caratteristiche chimiche e nutrizionali del polline impastato da femmine di *O.cornuta* e la verifica della presenza di nettare. Si è voluta valutare l'esistenza di un fattore materno, indagare la sua importanza per lo sviluppo della progenie e ottenere informazioni sulla sua composizione. Infine si è voluto quantificare l'impatto della manipolazione sulla sopravvivenza della progenie.

Nel 1998, uova e larve di *O.cornuta* sono state trasferite all'interno di capsule di gelatina, alcune lasciate sulla propria scorta di polline, altre prelevate dalla propria scorta e trasferite su una dieta oligidica. Le uova e larve, lasciate sulla scorta di polline raccolta dalle femmine di *O.cornuta* in pieno campo, fungevano da controllo. Per entrambi i gruppi sono stati misurati la mortalità durante lo sviluppo completo, la mortalità durante lo svernamento e i tempi di sviluppo del 5° stadio larvale.

Nel 1999, uova/larve di *O.cornuta* sono state trasferite all'interno di capsule di gelatina su tre diversi tipi di dieta: 1) senza fattore materno e con manipolazione - uova/larve prelevate dalla propria scorta di polline e trasferite su dieta artificiale; 2) con fattore materno e con manipolazione - uova/larve prelevate dalla propria scorta di polline e trasferite su una raccolta da un'altra femmina; 3) con fattore materno e senza manipolazione - uova/larve lasciate sulla propria scorta di polline.

Per ogni gruppo di uova/larve sono stati determinati la mortalità durante lo sviluppo e il tempo di sviluppo del 5° stadio larvale.

Nel 1999 sono state esaminate anche le caratteristiche chimiche e nutrizionali del polline prelevato da nidi pedotrofici di *O.cornuta*, collocati in un pereto, dal Dip. di Scienze Ambientali (Università di Siena), mettendole a confronto con quelle del polline di pero puro.

I risultati relativi ai valori nutrizionali dimostrano che le femmine di *O.cornuta* aggiungono nettare, raccolto su varie piante, alle loro scorte di polline e che le impastano con secrezioni salivari. L'aumento della concentrazione di glucosio e fruttosio e la diminuzione del saccarosio, rilevati per il polline prelevato dalle celle pedotrofiche, non possono essere spiegati dalla sola aggiunta di nettare, ma

potrebbero essere in relazione alla presenza dell'enzima invertasi nella saliva dell'insetto, identificabile con il fattore materno ipotizzato.

I dati inerenti ai tempi di sviluppo e alla mortalità, rilevati nei due anni di studio, dimostrano il notevole impatto della manipolazione sulla sopravvivenza della progenie, tra l'altro dipendente anche dall'umidità delle scorte di polline artificiali, e avvalorano l'ipotesi dell'esistenza di un fattore materno. La ricerca richiede ulteriori studi al fine di analizzare l'impatto delle scorte di polline troppo disidratate sulla sopravvivenza e di verificare se l'enzima invertasi sia l'unico fattore materno coinvolto.

PAROLE CHIAVE: *Osmia cornuta*, allevamento, diete artificiali.

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