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Original article

Effect of over-wintering and incubation temperatures on adult emergence in *Osmia cornuta* Latr (Hymenoptera, Megachilidae)

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Summary — Cocoons of the potential orchard pollinator *Osmia cornuta* were exposed to different over-wintering and incubation temperatures to see if adult emergence could be manipulated and bees could be induced to emerge in synchrony with almond bloom. Bees over-wintered in a warehouse in an almond-growing area emerged over a period of time longer than the blooming period of commercial almond orchards. Conversely, bees over-wintered in refrigerators completed emergence in less than a week. Longer over-wintering periods yielded shorter emergence periods, but lower over-wintering temperatures did not. Incubation of over-wintered cocoons for 24 h failed to consistently accelerate emergence. The shortest emergence periods were obtained when bees were over-wintered at 3°C for 120 d.

Osmia cornuta* / over-wintering-temperature / emergence / pollination / *Prunus amygdalus

INTRODUCTION

Studies on *Osmia cornuta* as a potential orchard pollinator (Asensio, 1984; Torchio and Asensio, 1985; Torchio *et al,* 1987; Krunic *et al,* 1989, 1990; Bosch, 1994) have demonstrated that this species readily visits orchard crops, accepts man-made nesting domiciles, and large populations can be trap-nested in certain areas of Europe. Field-trapped populations can be over-wintered at cool temperatures and subsequently released in orchards, where they successfully nest and increase their numbers utilizing *Prunus* pollen as a primary source for cell provision (Márquez *et al,* 1994). Pollen collecting behaviour by *O cornuta* also permits this bee to be an effective pollinator of almond (Bosch, 1994).

Almond trees bloom very early in the year, often during inclement weather conditions, when insect flight and pollen germination is restricted (Griggs and Iwakiri, 1975). In addition, almond flowering periods in northeastern Spain are relatively short (around 20 days (Socias and Felipe, 1979; Vargas, 1984)). Also, the receptivity of stigmas rapidly declines as almond flowers become older and, as a result, flowers should be pollinated on the day they open or the day after to increase the chances for fruit set (Griggs and Iwakiri, 1964). It is very important, therefore, to provide adequate numbers of pollinators to ensure pollination of the highest possible number of flowers during the short bloom period. This is especially so in almond, where fruit set approaches 40% (Kester and Griggs, 1959). These are the reasons why great care should be taken to synchronize bee activity with almond bloom.

O cornuta over-winter as adults inside their cocoons and emerge from February to April (depending on the area) as the temperatures warm. Males emerge first, followed a few days later by females with which they mate soon after the latter emerge from natal nests. Mated females search for suitable cavities to start nesting activities. Nests are arranged as linear series of cells, with female eggs being laid in the innermost cells and male eggs in the outermost cells.

O cornuta emergence coincides with almond bloom in the Mediterranean climate areas where this crop is cultivated. To better exploit the pollinating potential of managed populations of this pollinator, it is necessary to determine the patterns of adult emergence. This would allow growers and/or beekeepers to predict the dates of bee emergence, to advance or delay it in order to improve synchronization with blooming time of early-to-late-flowering cultivars and shorten the period of emergence, so that most bees emerge over as short a time as possible.

The present study was designed to investigate effects of different over-wintering temperature regimes and different incubation treatments on *O cornuta* patterns of emergence. The following questions were addressed. How late do bees emerge after they are taken out of the over-wintering cooler? Is it possible to advance or shorten the emergence period by modifying over-wintering temperatures or by changing the length of the over-wintering period? Is it possible to accelerate bee emergence by incubating cocoons?

MATERIALS AND METHODS

Two different populations of *O cornuta* were used in this study. The first (A) came from trap-nests placed in Ribera d'Ebre (northeastern Spain) in February 1990. The second population (B) came from nests placed in a cooler area near Belgrade (Serbia) where *O cornuta* flies later, in March–April.

All bees were kept at room temperature for the spring and summer, and on November 3 groups of 20 female and 40 male cocoons were separated in Petri dishes and placed in refrigerators at different temperatures and 60% relative humidity. In January to March (depending on the treatment), the Petri dishes were taken out of the coolers and the cocoons were individually placed in glass vials that were marked according to sex, experiment and treatment. Different groups of bees were incubated at different temperatures. Glass vials were checked every morning to record the day of emergence.

Experiment 1

This experiment was designed to determine if bees over-wintered near natural conditions would emerge in synchrony with almond bloom. A first group (1.1) of bees from population A was kept in a warehouse adjacent to several almond orchards at the Experimental Station of Mas Bové (IRTA) in Reus (northeastern Spain). Another 2 groups of bees (1.2 and 1.3) were kept at 3°C for 90 and 105 d, respectively, and then placed in the same warehouse as the first group.

Experiment 2

To study the effect of the length of the over-wintering period on emergence, 4 groups of bees (2.1 to 2.4) from population A were kept at 3°C for 75, 90, 105 and 120 d, respectively, at which time they were placed in a 20°C-temperature cabinet until emergence. Three groups of bees (2.5–2.7) from population B were exposed to 3°C for 90, 105 and 120 d, respectively, and incubated as the bees from population A.

Experiment 3

To determine how over-wintering temperatures affected emergence, bees from population A were kept for 105 d at different temperature regimes. One group (3.1) of bees was kept for 65 d at 10°C, followed by 10 d at 7°C, and 30 d at 3°C. A second group (3.2) was kept for 75 d at 7°C, followed by 30 d at 3°C. Finally, a third group (3.3) was kept for 105 d at 3°C. All groups were incubated at 20°C.

Experiment 4

To study the effect of incubation on emergence, bees from population A were kept at 3°C for 120 d, and then either (i) transferred to the above-mentioned warehouse (4.1), (ii) incubated at 20°C for 1 d and then transferred to the warehouse (4.2) or (iii) incubated for 1 d at 26°C and then transferred to the warehouse (4.3).

Experiment 5

As in *Experiment 4*, different incubation regimes were tested in this experiment. Three groups of bees from population B were kept at 3°C for 128 d. The first group (5.1) was then transferred to a bee shelter (protected from direct sunlight) in an almond orchard. The second group (5.2) was transferred to a warehouse for 1 d, then incubated for another 24 h at 20°C, and finally placed in the same bee shelter as the first group. The third group (5.3) was incubated for 1 d at 20°C and then transferred to the shelter in the orchard.

RESULTS

Experiment 1. Effect of non-refrigerated over-wintering

The bees that were not placed in the cooler (1.1) initiated emergence on January 20 (around 10 d ahead of wild populations in Ribera d'Ebre), and the last female emerged 42 d after (fig 1.1). Bees from groups 1.2 and 1.3 were taken out of the cooler on February 3 and 18, respectively, and males from both groups began to emerge the day after. The mean emergence time of groups 1.2 and 1.3 was significantly shorter than the mean emergence time of group 1.1 (fig 1) (males: ANOVA, $F = 80.38$, $p < 0.00001$; females: ANOVA, $F = 177.69$, $p < 0.00001$).

Experiment 2. Effect of length of over-wintering and origin of bees

The results of experiment 2 are summarized in figure 2. A few males from group 2.4 that had already chewed their way out of cocoons prior to their removal from the cooler were considered as having emerged on the first day of incubation.

The emergence periods were advanced and shortened as over-wintering periods were extended (males of population A: ANOVA, $F = 28.50$, $p < 0.00001$; females of population A: ANOVA, $F = 22.44$, $p < 0.0001$; males of population B: ANOVA, $F = 62.93$, $p < 0.000\,01$; females of population B: ANOVA, $F = 48.67$, $p < 0.000\,1$). Nevertheless, bees of population A emerged sooner than bees of population B exposed to the same over-wintering regimes (groups 2.2 and 2.5, 2.3 and 2.6, and 2.4 and 2.7, respectively) (fig 2). The differences in mean times of emergence between the 3 above-mentioned pairs were highly significant (*t*-Student's test, $p < 0.0001$, for males and females of all pairs).

Experiment 3. Effect of over-wintering temperatures

The bees in this experiment were exposed to different over-wintering temperatures, from warmer to cooler (fig 3). No significant differences were found between mean emergence times of the 3 groups (males: ANOVA, $F = 0.81$, $p > 0.4$; females: ANOVA, $F = 0.21$, $p > 0.8$).

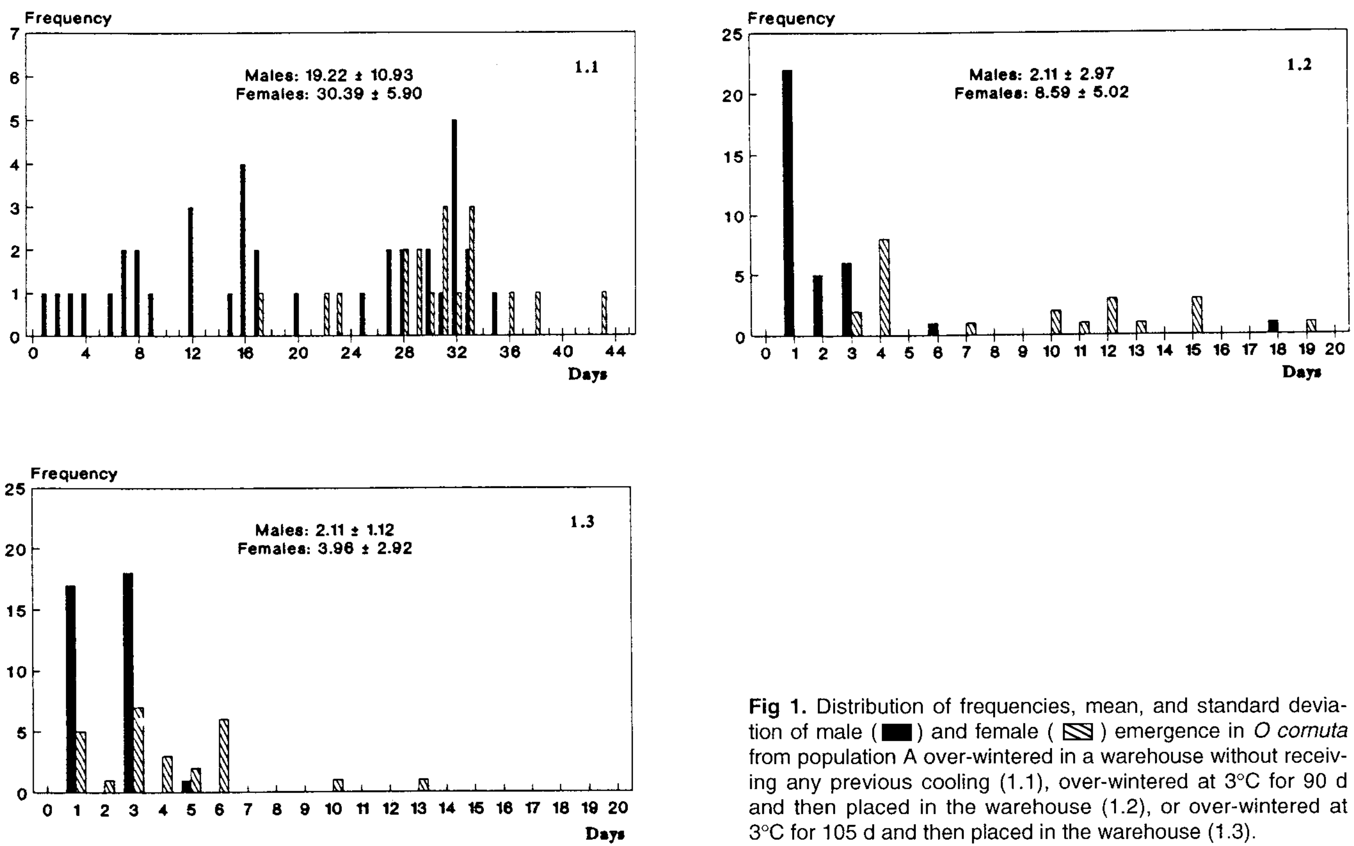


Fig 1. Distribution of frequencies, mean, and standard deviation of male (■) and female (▨) emergence in *O cornuta* from population A over-wintered in a warehouse without receiving any previous cooling (1.1), over-wintered at 3°C for 90 d and then placed in the warehouse (1.2), or over-wintered at 3°C for 105 d and then placed in the warehouse (1.3).

Experiment 4. Effect of incubation temperatures

The temperature in the warehouse in which bees were placed after over-wintering fluctuated between 10 and 17°C. Incubation at 20 or 26°C for 1 d failed to accelerate bee emergence (fig 4) (males: ANOVA, $F = 0.73$, $p > 0.4$; females: ANOVA, $F = 0.75$, $p > 0.4$). The emergence of bees of group 4.2 was quick and short (97.2% of the males emerged in 3 d, and 81% of the females emerged in 4 d) (fig 4.2), but not as quick as emergence of group 2.4 (see figure 2.A) which received the same over-wintering treatment but a continuous incubation at 20°C (males: t-Student's = 3.45, $p < 0.001$; females: t-Student's = 5.14, $p < 0.001$).

Experiment 5. Effect of incubation temperatures

The air temperature in the almond orchard in which the bees were placed after incubation fluctuated from 6 to 23°C (mean = 13°C). The different incubation regimes tested did not influence female emergence (fig 5) (ANOVA, $F = 0.51$, $p > 0.6$), but males that received 1 d incubation at 20°C (fig 5.3) emerged earlier than those incubated 1 d in the warehouse (10–17°C) followed by 1 d at 20°C (fig 5.2), and those not incubated prior to placement in the field (fig 5.1) (ANOVA, $F = 6.24$, $p < 0.005$).

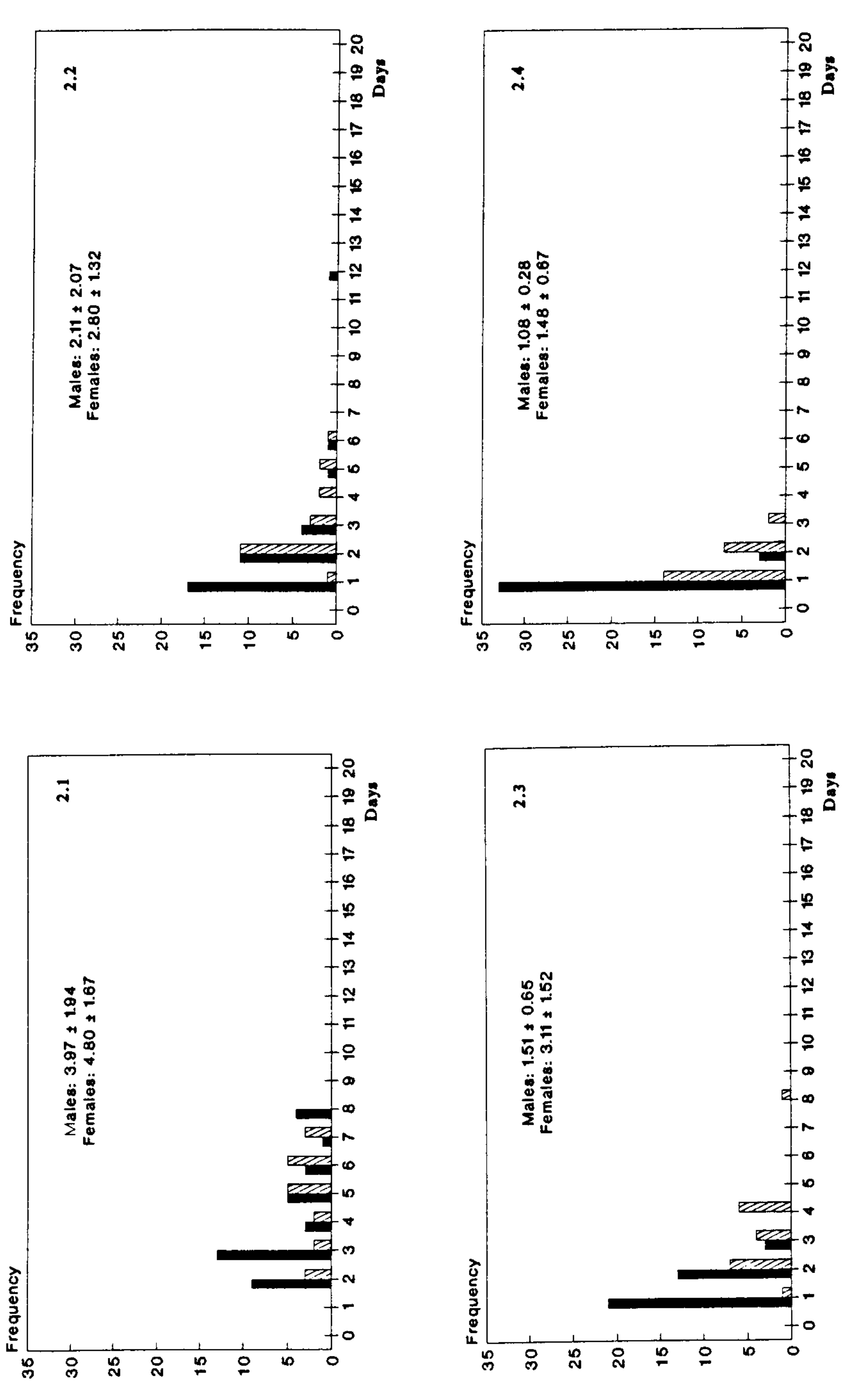
DISCUSSION

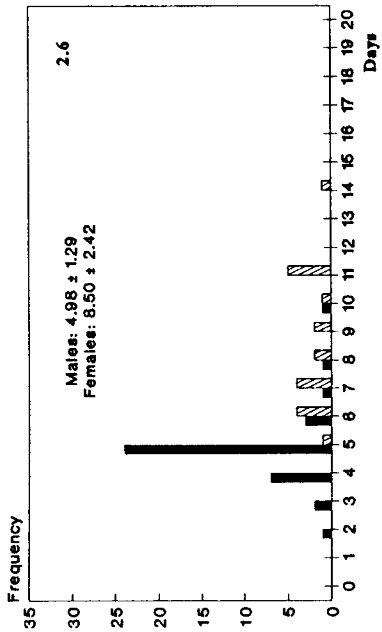
Like other *Osmia (Osmia)* species studied for crop pollination, *O cornuta* is univoltine, it over-winters in the adult stage inside the cocoon, and a cold winter period is necessary to trigger emergence (bees that are not exposed to low temperatures do not emerge the following spring). If *O cornuta* is to be established as a commercial pollinator of orchard crops, it will be necessary to synchronize emergence of bees with flower initiation of those crops. This study attempts to quantify the influence of over-wintering and incubation temperatures on spring emergence patterns of *O cornuta* for subsequent incorporation into viable management programmes. An interpretation of results obtained is described below.

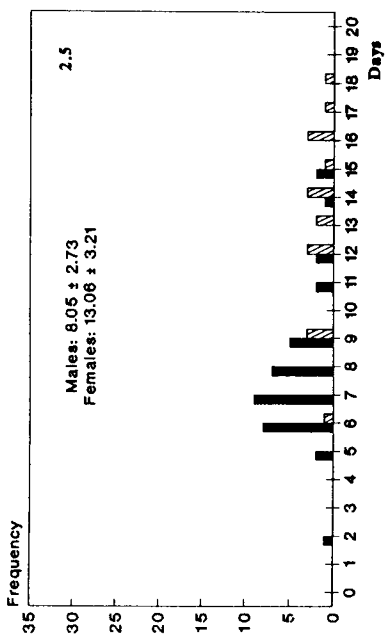
Treatment 1 of *Experiment 1* was designed to determine expected emergence patterns if a grower reared his own bees without over-wintering or incubation facilities. The experimental design also resembled natural conditions, although daily temperature fluctuations were smaller in the warehouse than outdoors. The results of *Experiment 1* demonstrated that much longer emergence periods are expressed when *O cornuta* bees are wintered in open environments *versus* those refrigerated. Male bees that were kept in a refrigerator for only part of the winter and then placed in the warehouse (groups 1.2 and 1.3) emerged in synchrony with bloom of early-flowering almond cultivars, but female emergence (especially in 1.2) was extended compared with bees of group 4.1, which were over-wintered in a refrigerator for 120 d and incubated in the warehouse.

These results suggest that populations of *O cornuta* reserved for almond pollination should be refrigerated during winter months, so they can be induced to emerge over a short period of time in synchrony with bloom initiation. The pattern of emergence expressed by bees from group 1.1 would be unsuitable in almond pollination operations, since the emergence period is much longer than the flowering period of an almond orchard.

The length of the over-wintering period has a strong effect on *O cornuta* emergence. Both early- and late-flying populations (A and B, respectively) responded similarly, by advancing emergence as


2.1
Males: 3.97 ± 1.94
Females: 4.80 ± 1.67
2.2
Males: 2.11 ± 2.07
Females: 2.80 ± 1.32
2.3
Males: 1.51 ± 0.65
Females: 3.11 ± 1.52
2.4
Males: 1.08 ± 0.28
Females: 1.48 ± 0.67
Frequency
Days





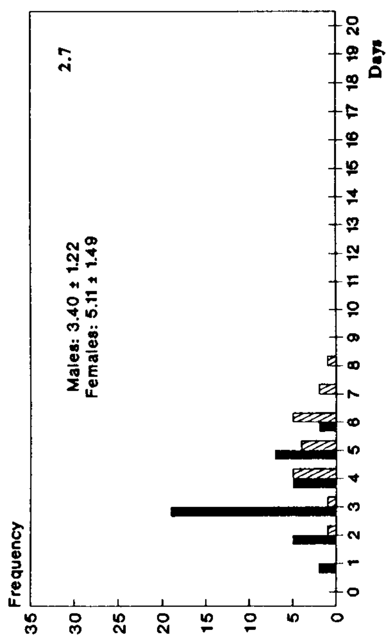


Fig 2. Distribution of frequencies, mean and standard deviation of male (■) and female (▨) emergence in *O cornuta* overwintered at 3°C for different periods of time (75 d [2.1], 90 d [2.2, 2.5], 105 d [2.3, 2.6], and 120 d [2.4, 2.7]) and incubated at 20°C. Bees from groups 2.1 to 2.4 were from an early-flying population (population A), and bees from groups 2.5 to 2.7 were from a late-flying population (population B).

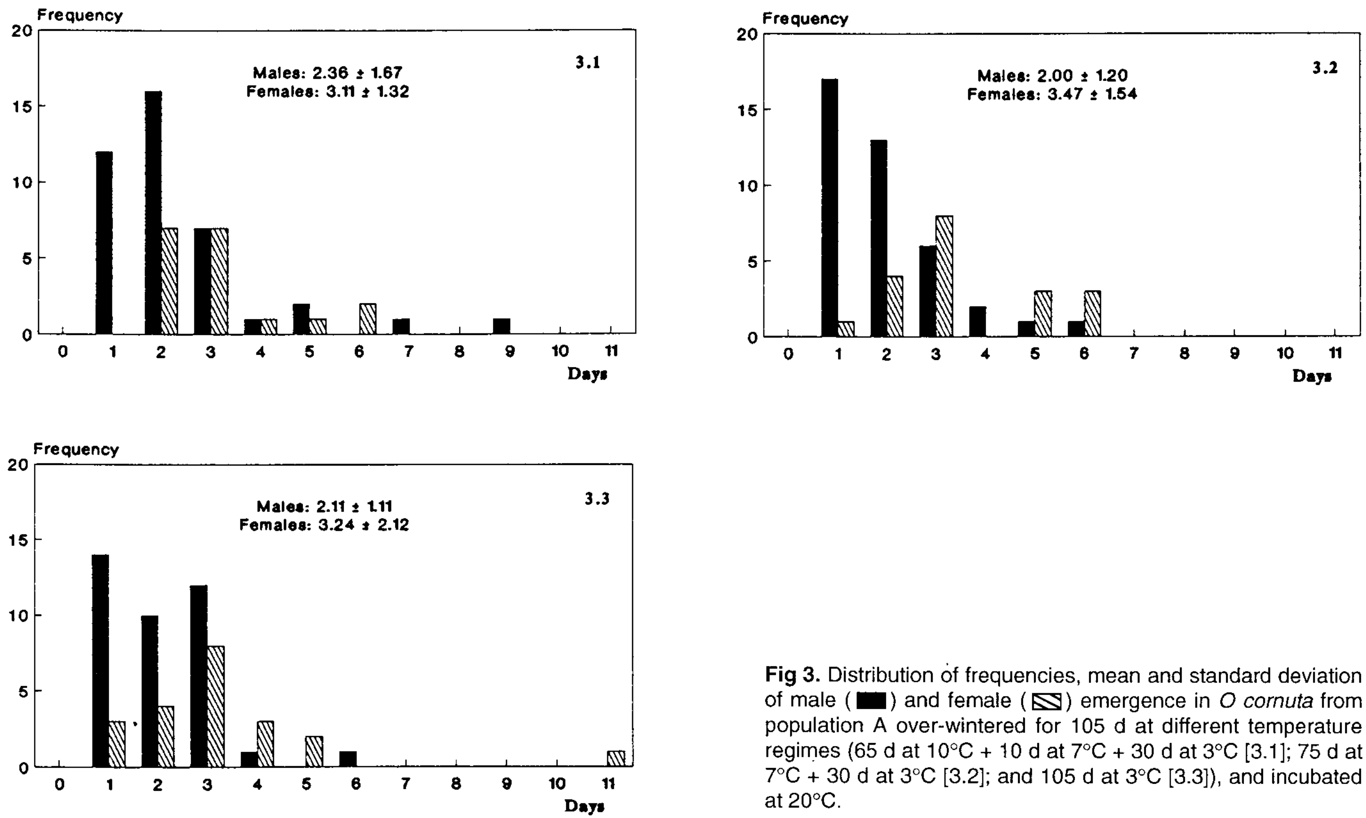


Fig 3. Distribution of frequencies, mean and standard deviation of male (■) and female (▨) emergence in *O cornuta* from population A over-wintered for 105 d at different temperature regimes (65 d at 10°C + 10 d at 7°C + 30 d at 3°C [3.1]; 75 d at 7°C + 30 d at 3°C [3.2]; and 105 d at 3°C [3.3]), and incubated at 20°C.

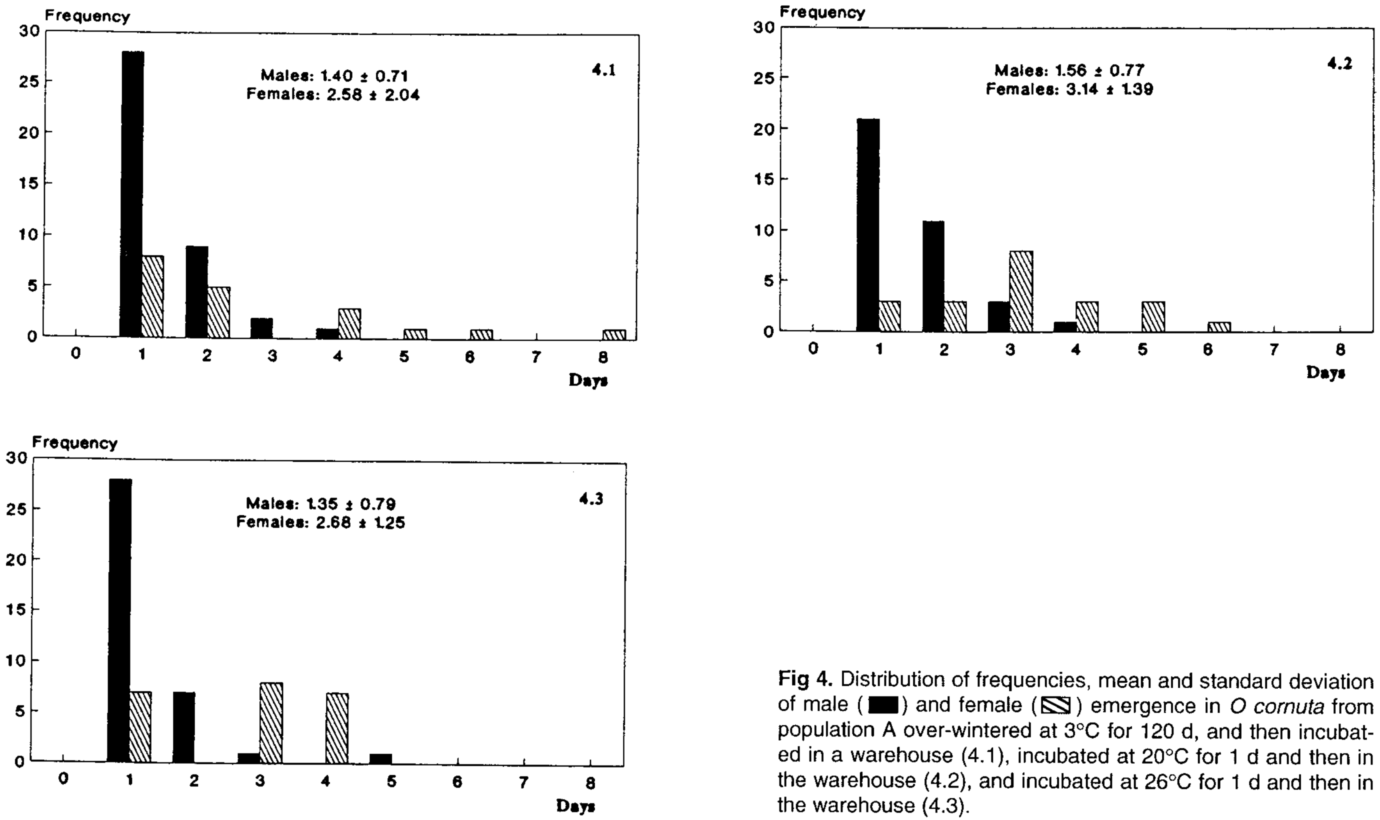


Fig 4. Distribution of frequencies, mean and standard deviation of male (■) and female (▨) emergence in *O cornuta* from population A over-wintered at 3°C for 120 d, and then incubated in a warehouse (4.1), incubated at 20°C for 1 d and then in the warehouse (4.2), and incubated at 26°C for 1 d and then in the warehouse (4.3).

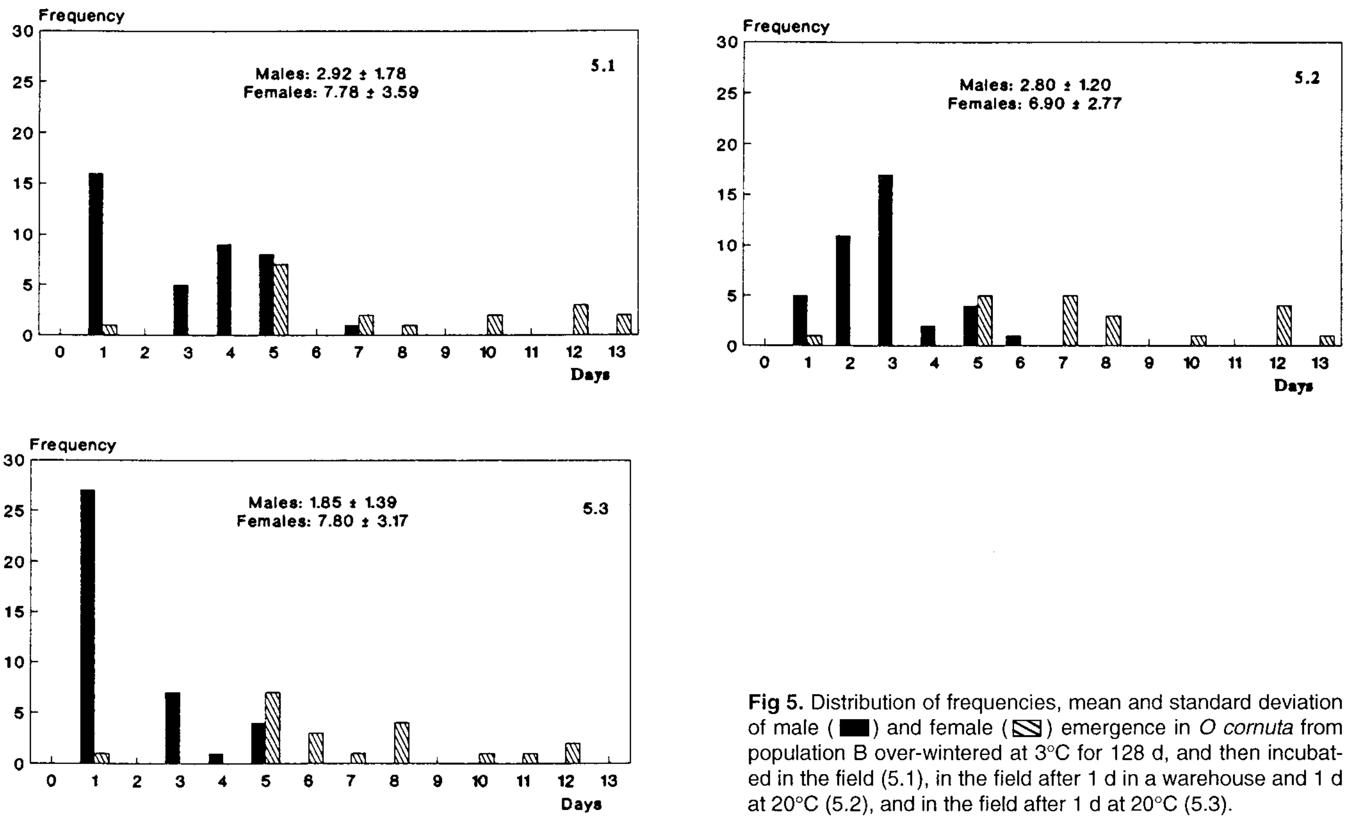


Fig 5. Distribution of frequencies, mean and standard deviation of male (■) and female (▨) emergence in *O cornuta* from population B over-wintered at 3°C for 128 d, and then incubated in the field (5.1), in the field after 1 d in a warehouse and 1 d at 20°C (5.2), and in the field after 1 d at 20°C (5.3).

over-wintering periods were extended. Similar results have been reported for *Megachile rotundata*, *O cornuta*, *O rufa*, and *O tricornis* (Johansen and Eves, 1973; Taséi, 1973; Taséi and Masure, 1978; Kristjansson, 1989; Vicens, 1991). The pattern of emergence of bees from group 2.4 is very encouraging, since 100% of males and 91.3% of females emerged over 2 d. Under such conditions, synchronization with first orchard bloom could be easily achieved.

The fact that a few males from group 2.4 emerged while they were still in the cooler indicates that 120 d is near the upper limit for which *O cornuta* from population A can be over-wintered. However, results obtained with population B indicate that late-flying populations require longer over-wintering periods. Taséi (1973) indicated that *O cornuta* from southern France emerged sooner than *O cornuta* from northern France after receiving the same over-wintering treatment, and similar differences were obtained when European and Canadian strains of *M rotundata* were compared (Taséi and Masure, 1978; Rank and Rank, 1989), thus suggesting a physiological polymorphism between ecotypes. It is, therefore, important to adjust temperature treatments to each population in order to synchronize bee emergence with orchard bloom. Emergence of part of the male population within the cooler does not represent a difficulty when populations of *O cornuta* are managed for pollination, since populations are, ideally, transported to the orchards at night or at dawn, when temperatures are too cold for bees to fly and disperse.

Over-wintering temperatures do not seem to greatly affect emergence patterns when they are applied over the same period of time. These results suggest that the length of the over-wintering period is more important than the actual over-wintering temperature in determining adult emergence in *O cornuta*. However, winter temperature does affect mortality in this species: bees exposed to milder temperatures express higher mortality rates and lower reproductive success (fewer cells produced per emerged female) than bees exposed to lower temperatures (Torchio *et al*, 1987). Mortality in the present study was too low to yield any conclusions in this regard, but 3°C seems to be an ideal temperature for over-wintering *O cornuta* populations. Other *Osmia (Osmia)* species used as orchard pollinators were successfully wintered at 5°C for 3 months *(O cornifrons)* (Maeta, 1978), or at 3°C for about 200 d *(O lignaria propinqua)* (Torchio, 1984).

Incubation treatments did not have a consistent effect on bee emergence. However, longer incubation periods at the temperatures used herein, probably have a positive effect on advancing emergence. This is supported by the fact that bees incubated continuously emerged earlier than bees incubated for just 1 d (comparison of group 2.4 and group 4.2). *O cornifrons* (Maeta, 1978) and *O rufa* (Kristjansson, 1989) have been reported to emerge earlier when incubated at higher temperatures, than when incubated at lower temperatures. However, in both studies, bees were exposed to continuous incubation, whereas *O cornuta* in *Experiments 4* and *5* of the present study were incubated for only 24 h. It is important to note that continuous incubation is not feasible in pollination operations, since too many bees would emerge within the incubation cabinet.

The results of this study demonstrate that managed populations of *C cornuta* can be induced to emerge over a relatively short period (most females can emerge from their cocoons in as little as 4 days *(Experiment 4)*) through adequate over-wintering treatments. According to these results which should now be confirmed on undisturbed nests, *O cornuta* populations should be taken to orchards at the very beginning of, or even a few days prior to, bloom initiation, to allow emergence, mating and establishment of nesting activities. This would favour both flower pollination and bee reproduction.

ACKNOWLEDGMENTS

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Résumé — Influence des températures d'hivernage et d'incubation sur l'émergence d'*Osmia cornuta* Latr (Hymenoptera, Megachilidae). On étudie actuellement en Espagne l'utilisation d'*Osmia cornuta* comme pollinisateur alternatif des vergers. La synchronisation de l'émergence des adultes avec la floraison de la culture cible est essentielle pour utiliser au mieux le potentiel pollinisateur de cette abeille solitaire, principalement lorsque les floraisons sont courtes. Dans cette étude, des cocons mâles et femelles d'*O cornuta* ont été placés dans des récipients en verre et exposés à différents régimes de températures d'hivernage et à diverses modalités d'incubation. Les abeilles qui ont partiellement ou totalement hiverné dans un entrepôt d'une région de vergers d'amandiers ont émergé sur une période beaucoup plus longue (fig 1) que celles qui avaient hiverné dans des réfrigérateurs (figs 2 à 5). Lorsque les abeilles ont hiverné en totalité dans l'entrepôt, la période d'émergence a dépassé la période de floraison des vergers d'amandiers (fig 1.1) et cette méthode d'hivernage a donc été considérée comme convenant moins bien aux opérations de pollinisation. Les abeilles ont émergé plus tôt et sur une période plus courte en cas d'allongement des périodes d'hivernage (fig 2), mais pas en cas de baisse des températures d'hivernage (fig 3). Une incubation pendant 24 h suivant l'hivernage n'a pas réussi à accélérer notablement l'émergence (figs 4 et 5). Les résultats de cette étude montrent que les populations d'*O cornuta* qui ont hiverné dans des réfrigérateurs peuvent être amenées à émerger en moins de 1 sem, ce qui facilite la synchronisation avec la floraison de la culture cible. Bien que les besoins en traitements d'hivernage varient en fonction de l'origine des populations (fig 2), celles des régions les plus chaudes du nord-est de l'Espagne ont présenté les périodes d'émergence les plus courtes lorsque les cocons avaient été hiverné à 3°C pendant 120 j (fig 2.4).

Osmia cornuta* / température d'hivernage / émergence / pollinisation / *Prunus amygdalus

Zusammenfassung — Einfluß von Überwinterungs- und Inkubationstemperaturen auf das Ausschlüpfen von *Osmia cornuta* Latr (Hym, Megachilidae). *Osmia cornuta* wird gegenwärtig als alternativer Bestäuber in spanischen Obstgärten studiert. Synchronisation des Ausschlüpfens und Blütezeit der entsprechenden Frucht sind wesentliche Voraussetzungen für die Bestäubungsleistungen dieser solitären Biene, besonders dann, wenn die Blütezeit nur kurz ist. In dieser Untersuchung wurden männliche und weibliche Kokons von *O cornuta* in Glasgefäßen isoliert und unterschiedlichen Winter-Temperaturverhältnissen und Schlüpfbedingungen unterworfen. Bienen, die ganz oder teilweise in einem Vorratshaus in einem Gebiet mit Mandelpflanzungen überwintert wurden, schlüpften über einen viel längeren Zeitraum (Abb 1) als solche, deren Überwinterung in einem Kühlraum erfolgte (Abb 2–5). Wurden Bienen zur Gänze in dem Vorratshaus überwintert, so erstreckte sich die Schlüpfperiode über einen längeren Zeitraum als die Blühdauer der Bäume in kommerziellen Anlagen (Abb 1.1). Deshalb wurde diese Überwinterungsmethode für die Bestäubungspraxis als wenig geeignet befunden. Die Bienen

schlüpften früher und über eine kürzere Zeitspanne, wenn die Überwinterungsperiode verlängert wurde (Abb 2); dies war aber nicht der Fall, wenn man die Überwinterungstemperatur senkte (Abb 3). Eine Inkubation für 24 Stunden nach der Überwinterung führte zu keiner merklichen Beschleunigung des Ausschlüpfens (Abb 4, 5). Die Ergebnisse dieser Untersuchung zeigen, daß Populationen von *O cornuta,* die in einem Kühlraum überwintert wurden, innerhalb einer Woche zum Schlüpfen gebracht werden können, wodurch die Synchronisierung mit der Blühzeit der in Aussicht genommenen Frucht erleichtert wird. Populationen verschiedener Herkunft verlangen verschiedene Überwinterungs-Behandlungen (Abb 2); Populationen aus den wärmsten Gebieten Nordostspaniens zeigten die kürzesten Schlüpfperioden, wenn sie für 120 Tage bei 3°C überwintert wurden (Abb 2.4).

Osmia cornuta* / Überwinterung-Temperatur / Schlüpfen / Bestäubung / *Prunus amygdalus

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