



Kin Recognition in the largely Solitary Bee, *Manuelia postica* (Apidae: Xylocopinae)

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Abstract

The recognition of conspecifics is a central issue to social behaviour. In eusocial hymenopterans, kin recognition has been clearly demonstrated. *Manuelia postica* is a largely solitary bee species in which larvae develop inside individual cells within a nest and remain isolated from conspecifics until the destruction of partitions by adults. Nestmate recognition in *M. postica* has been previously demonstrated under experimental conditions. Isolation between individuals during development and nestmate recognition ability in adult females make *M. postica* an ideal species for testing the occurrence of kin recognition capacity in females. Kin recognition was demonstrated through cross-fostering field experiments involving the single transfer of recently enclosed larvae, and subsequent laboratory recognition bioassays with emerging females. Results suggest kin recognition occurs through self-referent phenotype matching. Given the basal position of *Manuelia* in the phylogeny of the Apidae, kin recognition may represent an ancestral recognition mechanism in Apidae species phylogenetically more derived than *M. postica*.

Introduction

Throughout the animal kingdom, recognition of kin is a central issue to social behaviour. For example, kin recognition is a prerequisite for maximizing inclusive fitness (Hamilton 1964), and for choosing mating partners that represent the optimal balance between inbreeding and outbreeding (Bateson 1983). Kin recognition can occur by phenotype matching, whereby an individual learns some phenotypic trait of its kin (kin reference) or of itself (self reference), stores its representation in memory as a template, and later matches this template to the phenotype of an unfamiliar conspecific (Mateo 2004). In eusocial hymenopterans there is ample evidence to support kin recognition mainly by kin-referent phenotype matching (reviews: Page & Breed 1987; Michener & Smith 1987). In contrast, there is no evidence to support kin recognition in non-social or solitary hymenopterans.

Manuelia postica (Apidae, Xylocopinae) is a largely solitary bee species whose nests are found mainly in stems of *Chusquea quila* (Poaceae). A female excavates a tunnel, constructs a food mass, lays an egg on it, builds a partition with wood particles, and repeats these last three processes up to seven times before the nest is finished (Flores-Prado et al. 2008b). According to previous and detailed observations of nests ($n = 400$) during the breeding period from November 2005 to April 2006, neither omission of cell partitions nor evidence of their destruction were recorded (Flores-Prado et al. 2008b); these phenomena have been frequently reported in some species of *Ceratina*, a genus closely related to *Manuelia* (Sakagami & Laroca 1971; Sakagami & Maeta 1977; Maeta et al. 1997). In some such *Ceratina* species, the mother opens the cells to inspect and remove the fecal rests of her developing offspring. On the contrary, in *M. postica* fecal particles are always present inside cells occupied by developing

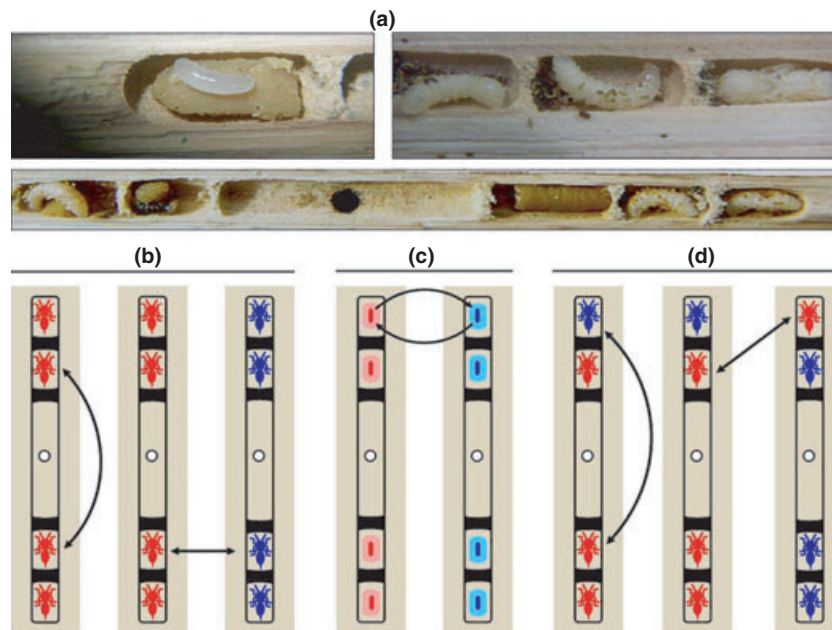


Fig. 1: (a) Sagittal cross-sections of nests showing individual cells occupied by *Manuelia postica* in different stages of development, growing in isolation. (b) Design of experiments described in Flores-Prado et al. (2008a) which showed the occurrence of nestmate recognition between *M. postica* females. Arrows indicate on-manipulated pairs of nestmate females and non-nestmate females as they were tested in behavioural assays. (c) The cross-fostering experiment reported herein; arrows indicate the manipulations performed. (d) Behavioural assays performed between two non-kin females (a foster and a non-manipulated female) developed in the same nest and between two kin females developed in different nests; arrows indicate the pairs of females used in the bioassays.

offspring. Additionally, we have never observed more than one individual developing inside a cell (Fig. 1a), i.e. physical contact between individuals and movement of larvae between the cells has never been observed (Flores-Prado et al. 2008b). Although evidence points to the absence of physical contact between developing individuals, chemical interactions cannot be excluded because low molecular weight volatile compounds could permeate through cell partitions. However, nestmate recognition in *M. postica* has been demonstrated to occur mostly through non-volatile epicuticular compounds (Flores-Prado et al. 2008a). Notwithstanding this lack of physical contact, newly emerged adult females prior to the destruction of cell partitions showed nestmate recognition evidenced by bioassays carried out between pairs of such nestmate females and between pairs of such non-nestmate females (Fig. 1b): nestmate females were more tolerant (and less intolerant) to each other than non-nestmate females were to each other (Flores-Prado et al. 2008a). According to such previous study, nestmate discrimination in newly emerged females of *M. postica* prior to destruction of cell partitions can be explained mainly on the basis of: (i) cues acquired

from food masses or nest materials, or (ii) cues acquired by females from themselves.

To test these two possibilities, we designed a cross-fostering experiment manipulating the rearing environment of females based on the design described by Mateo & Holmes (2004): two non-kin females (a foster and a non-manipulated female) developed inside isolated cells within the same nest and two kin females developed inside isolated cells in different nests.

Materials and Methods

Cross-fostering experiments were set up at Altos de Lircay National Park, Chile (35°29'S; 70°58'W). One translucent or pre-defaecating larva (Flores-Prado et al. 2008b) was withdrawn from a cell *a* of nest *A*, and another from a cell *b* of a different nest *B*. The larva withdrawn from cell *a* was placed in cell *b*, and vice versa (Fig. 1c). The cells with transferred larvae were marked. In this way, each manipulated nest contained one foster larva and one or more non-manipulated larvae. Larval exchanges were started during the last week of November 2005 and continued until the first week of January 2006.

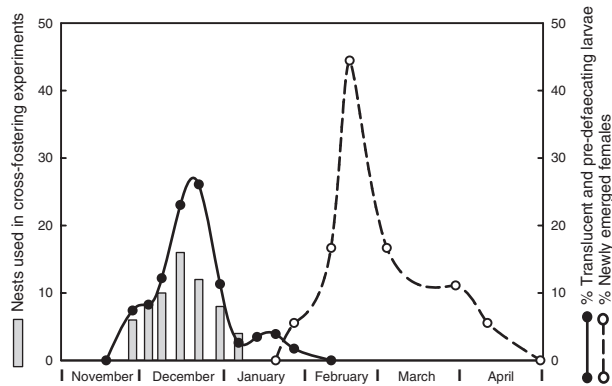


Fig. 2: Abundance of translucent and pre-defaecating larvae and newly emerged females prior to destruction of cell partitions of *Manuelia postica* between November 2005 and April 2006, and instances when cross-fostering experiments were set up.

According to our previous observations (Flores-Prado et al. 2008b) this is the period when nests are most often found with translucent and pre-defaecating larvae inside (Fig. 2). Nests were sagittally opened, and exchanges of larvae were performed. Thereafter, nests were closed and left in the field for approx. 80 d, a period sufficient for the larvae to develop into adults prior to destruction of cell partitions (Fig. 2) (Flores-Prado et al. 2008b). Nests were then brought to the laboratory and kept at 9°C until they were re-opened in order to withdraw females to be used in recognition experiments.

Ninety-four nests were opened in the field, but 30 of them could not be used because they contained less than two recently enclosed larvae inside. Exchanges of larvae were performed in 64 nests. Ten nests suffered damage during field exposure. Of the 54 nests brought to the laboratory, only 40 nests could finally be used in the behavioural bioassays, since in the remaining nests either: (i) the partitions between cells had been destroyed by the growing individuals, i.e. hibernating assemblages had formed ($n = 3$), (ii) only dead females were found ($n = 3$), or (iii) foster larvae turned out to be males ($n = 8$).

Kin recognition is implied if after some period of development the fostered individual distinguishes between unfamiliar kin and non-kin (Mateo & Holmes 2004). In this design, unfamiliar individuals refer to individuals reared apart from each other (in different litters) (Mateo & Holmes 2004). As individuals of *M. postica* always develop apart from each other, i.e. even if two (or more) females develop (under natural or manipulative conditions) in the same nest they do so in isolation from each other, all individuals developing in a nest are unfamiliar to

each other. Given the occurrence of nestmate recognition in this system (Flores-Prado et al. 2008a), based on either cues from food masses and nest materials or on cues acquired by females from themselves, we have modified the experimental design described above (Mateo & Holmes 2004) as follows. Behavioural experiments were performed between two non-kin females (a foster and a non-manipulated female) developed in the same nest ($n = 14$), and between two kin females developed in different nests ($n = 13$). The pairs of non-kin females were extracted from one nest, and the pairs of kin females were extracted from two nests which were not used as source of other test females (Fig. 1d). Each female was used only once in the behavioural experiments. A female was placed at one end of a 7 cm long glass tube whose 5 mm internal diameter was similar to that of the galleries where the bees live in nature. A second female was placed at the opposite end of the tube, whose ends were then sealed with Teflon stoppers. The glass tubes were held horizontally in the indoor experimental arena and temperature was maintained between 23 and 25°C during the experiments. The activity of females was video recorded for 15 min. Videotapes were scored by a person without knowledge of the nature of the pair being observed. This allowed the determination of occurrence of behavioural events and duration of behavioural states, which were classified as tolerant or intolerant on the basis of behaviours described by several authors for species of Apoidea (e.g. Breed & Julian 1992; Wcislo 1997; Pabalan et al. 2000; Flores-Prado et al. 2008a). Thus, a behavioural event was scored as tolerant if one female passed by the other venter to venter, and as intolerant if a female exhibited a C-posture, was observed pushing, biting, stinging, or touching with the legs the other female, or if one female facing the other moved backwards. A behavioural state was scored as tolerant if females remained near each other or if they were in contact with each other with no signs of mutual aggression, and as intolerant if they were in contact and exhibited aggressive behaviours, remained far away from each other, or one of them moved away from the other. Behavioural events and states were analysed using the software THE OBSERVER v. 3.0 (Noldus).

Results

The number of intolerant behavioural events and the duration of intolerant behavioural states was higher between non-kin females from the same nest ($n = 14$) than between kin females from different

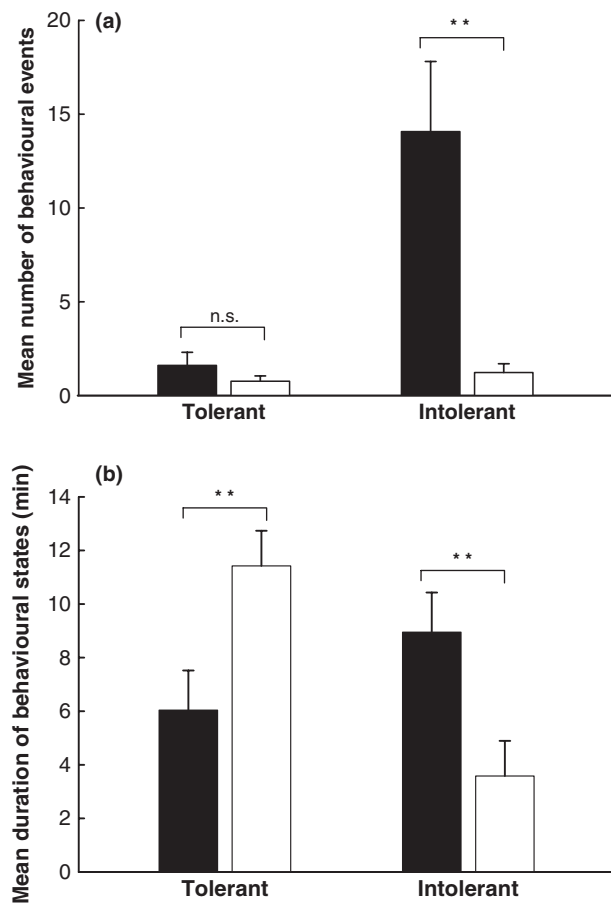


Fig. 3: Mean number of tolerant and intolerant behavioural events (a) and behavioural states (b) of *Manuelia postica* females in the presence of non-kin females reared in the same nest and of kin females reared in different nests. The observation period was 15 min. ns = non-significant; **: $p < 0.01$. □ = kin females; ■ = non-kin females.

nests ($n = 13$) ($F_{1,25} = 10.3$, $p < 0.01$; and $F_{1,25} = 7.82$; $p < 0.01$, respectively) (Fig. 3a). Furthermore, the duration of tolerant behavioural states was higher between kin females from different nests than between non-kin females from the same nest ($F_{1,25} = 7.82$; $p < 0.01$) (Fig. 3b).

Discussion

In previous experiments carried out with non-fostered females of *M. postica* (Fig. 1b) we demonstrated nestmate recognition capacity evidenced through behavioural discrimination: pairs of nestmate females (developed inside a single nest whose larvae had not been manipulated) were more tolerant and less intolerant than non-nestmate females (developed inside different nests whose larvae had not been manipulated) (Flores-Prado et al. 2008a). As each pair of such nestmate females developed

from egg to adult stage in physical isolation from any conspecific within the same breeding environment, the results suggest the occurrence of kin recognition. However, as females could have learned or acquired similar environmental cues (i.e. chemicals from the nest material or from the food mass), the nestmate discrimination pattern observed may also be explained on the basis of nestmate recognition mediated by cues acquired from the intra nest environment.

In order to distinguish between these two interpretations, we manipulated the breeding environment of test females so that non-kin females developed in the same breeding environment, and kin females developed in different breeding environments. We observed that non-kin females developed in the same nest were more intolerant and less tolerant than kin females developed in different nests, thus showing the occurrence of kin recognition.

In *Apis mellifera*, neurobiological studies suggest the existence of a critical period from 3 to 8 d after adult emergence during which the olfactory system of adult bees is most sensitive to environmental odours (Masson et al. 1993). Although maturation of synaptic circuitry is likely to occur after adult emergence, synaptic connections established during metamorphosis provide the structural basis for transmission of sensory information to the mushroom bodies (Ganeshina et al. 2006). These structures are associative centres in the brain that mediate olfactory learning and memory (Menzel et al. 1996) and are probably involved in the kin recognition phenomenon (Wyatt 2003). Some evidence also suggests that larvae of the solitary bee species, *Colletes fulgidus longiplumosus* (Colletidae) has olfactory learning capacity (Dobson 1987). Learning has also been shown to occur in larvae of the social ant species, *Cataglyphis cursor* (Isigrini et al. 1985). According to these antecedents and based on the results herein reported, we propose that the template in *M. postica* females is formed in the pre-adult stages and/or during the first days after adult emergence while they are in physical isolation from nestmates, before the destruction of inter-cell partitions by emerged adults (Flores-Prado et al. 2008b). When partitions are destroyed by newly emerged adults, these become familiar individuals (physical and probably chemical contact occurring between them) forming a pre-hibernating assemblage (Flores-Prado et al. 2008b), a stage in which reinforcement of the template with cues from relatives inside the nest cannot be excluded.

In our experiment, individuals of *M. postica* develop inside individual cells and in physical

isolation from each other up to the adult stage prior to destruction of cell partitions, and there is no contact between them and their mother (Fig. 1a) (Flores-Prado et al. 2008a). Considering that non-volatile compounds have been demonstrated to mediate nestmate recognition in *M. postica* (Flores-Prado et al. 2008a), it is likely that cues used in the recognition phenomenon described above do not correspond to kin cues, consequently suggesting that kin recognition has occurred through self-referent phenotype matching. In eusocial hymenopterans there is evidence to support kin-referent phenotype matching; however, evidence for self-referent phenotype matching in the honeybee (Getz & Smith 1983, 1986) has been criticised due to the difficulty of entirely eliminating pre-imaginal (Alexander 1991) and social (Hauber & Sherman 2000) learning in such social species. Finally, we can not discard kin recognition by kin-referent phenotype matching if volatile low molecular weight compounds from siblings permeate through cell partitions and if their role in kin recognition is demonstrated.

The present report provides arguments for the occurrence of kin recognition in a mainly solitary hymenopteran. Given the basal position of *Manuelia* within the phylogeny of Apidae (Michener 2000), a family that contains species ranging from solitary to eusocial, this capacity may represent the retention of an ancestral mechanism of recognition in phylogenetically more derived eusocial species.

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Literature Cited

- Alexander, R. D. 1991: Social learning and kin recognition. *Ethol. Sociobiol.* **12**, 387–399.
- Bateson, P. 1983: Optimal outbreeding. In: *Mate Choice* (Bateson, P., ed.). Cambridge Univ. Press, Cambridge, pp. 257–275.
- Breed, M. D. & Julian, G. E. 1992: Do simple rules apply in honey-bee nestmate recognition? *Nature* **357**, 685–686.
- Dobson, H. E. M. 1987: Role of flower and pollen aromas in host-plant recognition by solitary bees. *Oecologia* **72**, 618–623.
- Flores-Prado, L., Aguilera-Olivares, D. & Niemeyer, H. M. 2008a: Nest-mate recognition in *Manuelia postica* (Apidae: Xylocopinae): an eusocial trait is present in a solitary bee. *Proc. Biol. Sci.* **275**, 285–291.
- Flores-Prado, L., Chiappa, E. & Niemeyer, H. M. 2008b: Nesting biology, life cycle, and interactions between females of *Manuelia postica*, a solitary species of the Xylocopinae (Hymenoptera: Apidae). *N.Z. J. Zool.* **35**, 93–102.
- Ganeshina, O., Vorobyev, M. & Menzel, R. 2006: Synaptogenesis in the mushroom body calyx during metamorphosis in the honeybee *Apis mellifera*: an electron microscopic study. *J. Comp. Neurol.* **497**, 876–897.
- Getz, W. M. & Smith, K. B. 1983: Genetic kin recognition: honey bees discriminate between full and half sisters. *Nature* **302**, 147–148.
- Getz, W. M. & Smith, K. B. 1986: Honey bee kin recognition: learning self and nestmate phenotypes. *Anim. Behav.* **34**, 1617–1626.
- Hamilton, W. D. 1964: The genetical evolution of social behaviour, I, II. *J. Theor. Biol.* **7**, 1–52.
- Hauber, M. E. & Sherman, P. W. 2000: The armpit effect in hamster kin recognition. *Trends Ecol. Evol.* **15**, 349–350.
- Isigrini, M., Lenoir, A. & Jaisson, P. 1985: Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proc. Natl Acad. Sci. USA* **82**, 8545–8547.
- Maeta, Y., de la Sierra, E. A. & Sakagami, S. F. 1997: Comparative studies on the in-nest behaviors of small carpenter bees, the genus *Ceratina* (Hymenoptera, Anthophoridae, Xylocopinae) I. *Ceratina* (*Ceratina*) *cucurbitina*, Part 2. *Jpn. J. Entomol.* **65**, 471–481.
- Masson, C., Pham-Delègue, M. H., Fonta, C., Gascuel, J., Arnold, G., Nicolas, G. & Kerszberg, M. 1993: Recent advances in the concepts of adaptation to natural odour signals in the honeybee, *Apis mellifera* L. *Apidologie* **24**, 169–194.
- Mateo, J. M. 2004: Recognition systems and biological organisation: the perception component of social recognition. *Ann. Zool. Fenn.* **41**, 729–745.
- Mateo, J. M. & Holmes, W. G. 2004: Cross-fostering as a means to study kin recognition. *Anim. Behav.* **68**, 1451–1459.
- Menzel, R., Hammer, M., Müller, U. & Rosenboom, H. 1996: Behavioral, neural and cellular components underlying olfactory learning in the honeybee. *J. Physiol.* **90**, 395–398.
- Michener, C. D. 2000: *The Bees of the World*. John Hopkins Univ. Press, Baltimore.
- Michener, C. D. & Smith, B. H. 1987: Kin recognition in primitively social insects. In: *Kin Recognition in Animals* (Fletcher, D. J. C. & Michener, C. D., eds). John Wiley and Sons Press, Chichester, NY, pp. 209–242.

- Pabalan, N., Davey, K. G. & Packer, L. 2000: Escalation of aggressive interactions during staged encounters in *Halictus ligatus* Say (Hymenoptera: Halictidae), with a comparison of circle tube behaviors with others Halictine species. *J. Insect. Behav.* **13**, 627—650.
- Page, R. E. & Breed, M. E. 1987: Kin recognition in social bees. *Trends Ecol. Evol.* **2**, 272—275.
- Sakagami, S. F. & Laroca, S. 1971: Observations on the bionomics of some neotropical Xylocopine bees, with comparative and biofaunistic notes (Hymenoptera, Anthophoridae). *J. Fac. Sci. Hokkaido Univ. Ser. VI Zool.* **18**, 57—127.
- Sakagami, S. F. & Maeta, Y. 1977: Some presumably presocial habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. *Insectes Soc.* **2**, 319—343.
- Wcislo, W. T. 1997: Social interaction and behavioral context in a largely solitary bee, *Lasioglossum (Dialictus) figueresi* (Hymenoptera, Halictidae). *Insectes Soc.* **44**, 199—208.
- Wyatt, T. D. 2003: *Pheromones and Animal Behaviour: Communication by Smell and Taste*. Cambridge Univ. Press, Cambridge.