

Aspects of the reproductive biology of the
European wool-carder bee, *Anthidium*
manicatum (Hymenoptera: Megachilidae),
with a focus on nest site selection

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There is a sort of wild bee frequenting the garden-campion for the sake of its tomentum, which probably it turns to some purpose in the business of nidification. It is very pleasant to see with what address it strips off the pubes, running from the top to the bottom of a branch, and shaving it bare with all the dexterity of a hoop-shaver.

- Rev. Gilbert White, *The Natural History of Selbourne* (1795)

When newly-felted and not yet made sticky with honey, the wadded purse [of the wool carder bee] is quite the most elegant specimen of entomological nest-building... No bird's-nest, however deserving of our admiration, can vie in fineness of flock, in gracefulness of form, in delicacy of felting with this wonderful bag...

- Jean Henri Fabre, *Bramble-bees and Others* (1915)

Abstract

The European wool-carder bee, *Anthidium manicatum* (L.) (Hymenoptera: Megachilidae), is a Palearctic megachilid bee species best known for its resource defense polygynous mating system, its remarkably aggressive males, and its recent range expansion into North America, South America, and New Zealand. This study, conducted during the summers of 2009 and 2010, examines further aspects of *A. manicatum*'s reproductive biology, with a particular focus on female nest site selection.

Using a captive population of bees collected at field sites in Brooklyn, New York, USA, and near Boston, Massachusetts, USA, I demonstrate: i.) that females overwhelmingly prefer to initiate nests in high areas; ii.) that significant size differences exist between geographically distinct non-native populations; iii.) that visual cues may be necessary and sufficient for males assessing territorial challenges; iv.) that females are capable of initiating multiple nests in parallel, and v.) that females can appropriate in-progress nests abandoned by conspecifics. This study also highlights advances in the methods used to house experimental populations of *A. manicatum*, and briefly explores the species' potential as a model system for behavioral ecological research.

Acknowledgements

Special thanks go to Dr. Philip Starks, who served as this project's chief academic advisor from start to finish. Thanks also to my other committee members, Dr. Francie Chew and Dr. Sara Lewis; to my colleagues in the Starks Lab (specifically Christopher Perrone, Dustin Schildroth, Shantal Richards, David Peck, Noah Wilson-Rich, Anne Madden, and Susan Weiner) for help with various and sundry projects; to Mona McKindley at the Longfellow National Historic Site, Dr. Gerry Moore at Brooklyn Botanic Garden, Jessica Arcate Schuler at the New York Botanical Garden, and David Fiske and Clark Bryan at the Massachusetts Horticultural Society for their patience with the bee collections; to Tegan Morton, for collection assistance; to Dr. Jerome Rozen at the American Museum of Natural History for help with the *Anthidium* literature; and to Adam South for advice on insect dissections. Financial support came from a National Science Foundation REU site award to Tufts University (DBI-0649190) and from a 2010 Tufts University Graduate Student Research Award. Special thanks are also due to the bees, who did most of the work, mostly without complaint.

The thanks I owe my wife, L. Mairin Odle, are of an entirely different order of magnitude: NM, NT, SN.

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Chapter I

Introduction

1.1. The magic spring, or Why study solitary bees?

With the possible exception of the laboratory fruit fly, no insect has been more intensely studied than the honeybee, *Apis mellifera* (Michener 2007). Beginning with Aristotle's *History of Animals* and extending well beyond the recent publication of the honeybee genome (The Honeybee Genome Sequencing Consortium et al. 2006), a comprehensive account of bee research would span over 2,000 years of publications (Wilson 1971). If for no other reason -- and there are many, including the species' use as a cultural metaphor (Kupperman 1995), its position in the public imagination, and its role in global agriculture (Aizen et al. 2009) -- this long history of research entitles *A. mellifera* to its position as "most famous of all arthropods" (Grimaldi & Engel 2005).

That said, we might do well to keep the honeybee in perspective, and to remember that it represents just one (or approximately 0.00006%) of the world's more than 17,000¹ bee species (Michener 2007). At least when compared to *A. mellifera*, the vast majority of these species remain poorly understood: In the worst cases, they are known by no more than a holotype and a brief, decades old description. For many of the rest, basic information such as geographic range,

¹ This figure does not appear to be widely known among the lay public. Most people interviewed by the author during his collection work expressed surprise at the numerical, morphological, and behavioral diversity of bees (A. Payne, pers. observ.).

diet, phenology, and behavior are completely unknown. Overall, what we *do not* know far outweighs what we *do* know when it comes to the biology of the bees.

The European wool-carder bee, *Anthidium manicatum*, exists somewhere between the extremes of obscure specimen and model species. Thanks to several 19th century naturalist sketches and 20th century research papers, we now understand the broad outlines of its phenology and natural history. We know, for instance, what its nests look like (Kurtak 1973), and how its males interact (Ward 1928). On the other hand, we have no idea how females utilize sperm, discriminate among nest sites (although see Payne et al. 2010), determine offspring size, or recognize food resources. We do not know how males recognize each other, conspecific females, or individuals of other species. Broad patterns of development are unknown, as are the genetic structures of native and invasive populations.

This thesis attempts to address some of those questions using experimental, enclosure-based methods. The author's hope is that it will inspire others to attempt more audacious and ambitious studies, and to prove that what von Frisch (1966) said of the honeybee is at least as true for all other bee species: namely that their lives are "like a magic spring. The more one lets it run, the more abundantly it flows."

1.2. The study system: Biogeography and range expansion

Anthidium manicatum (L.) (Hymenoptera: Megachilidae) is a robust, black-and-yellow, megachiliform bee (**Fig 1.1**) whose presumptive native range extends from northern Europe (southern Britain and northern Germany) to the Mediterranean coast of North Africa and the western edge of Central Asia (Müller 1987). A remarkably successful introduced species, *A. manicatum* has now expanded its range to include territories in the mid-Atlantic, midwestern, and northeastern regions of North America (Jaycox 1967; Smith 1991; Miller et al. 2002); isolated pockets of the American West and Pacific coast (Zavortnink & Shanks 2008); the Canary Islands (Müller 1987); coastal South America, including Brazil, Uruguay, and Argentina (Gibbs & Sheffield 2009); Transbaikalian Siberia (Proshchalykin 2007); and New Zealand (Gibbs & Sheffield 2009) (**Fig. 1.2**).

According to Jaycox (1967), the bee's presence in North America dates to at least 1963, when specimens were reared from a series of ¼ inch (~6.4 mm) diameter trap nests recovered from field sites located near Ithaca, New York. Since then, the bee has rapidly expanded its North American range to include much of the Northeast, as well as several regions further west; as of 2010, it was a common visitor to urban gardens in several northeastern American cities, including Boston, Massachusetts, and New York, New York (A. Payne, pers. obs.).



Fig. 1.1 Male *Anthidium manicatum* resting on a leaf of *Stachys byzantina*. Note the black-and-yellow markings on the head, legs, and abdomen. Paint marks on the thorax were added by the author.

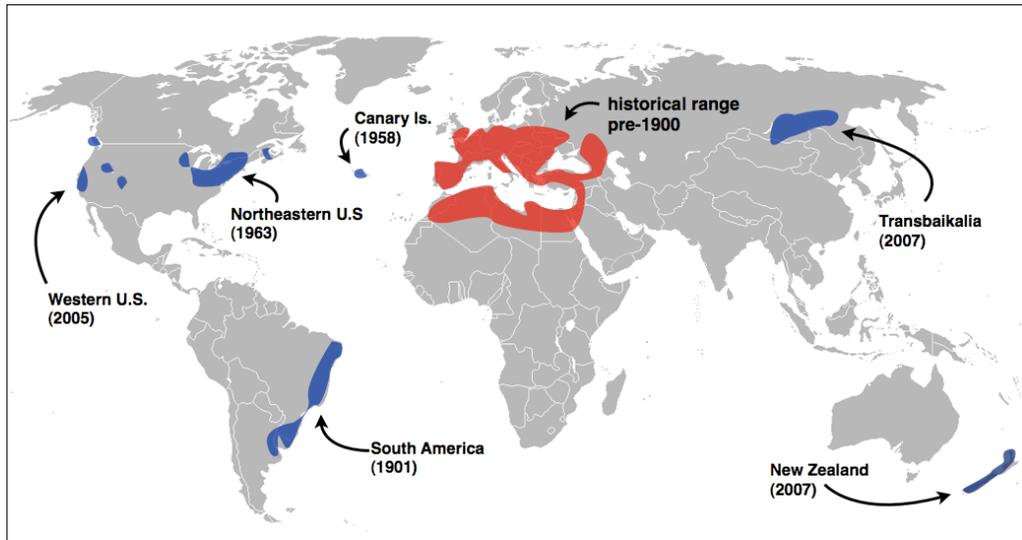


Fig. 1.2 The known 2009 distribution of *Anthidium manicatum*, based on a review by Gibbs and Sheffield (2009). Years in parentheses mark latest possible introductions. This map almost certainly underestimates the bee's true 2010 range.

This range expansion is perhaps unsurprising considering the species' habitat requirements, behavior, and phenology. As Jaycox (1967) pointed out, *A. manicatum*'s readiness to nest inside the pre-existing cavities found in various manmade objects (e.g., furniture, wooden packing crates; see section 1.3 below) has probably facilitated its accidental introduction to new regions. In addition, the species appears to be well adapted to garden conditions found throughout the world's Mediterranean and temperate climates: among its preferred floral hosts are several ornamental plants (e.g., *Lamium*, *Salvia*, *Stachys*, *Nepeta*, *Digitalis*) commonly cultivated throughout Europe and North America. Through its exploitation of human managed landscapes, *A. manicatum* may thus be pre-adapted to thrive in the expanding suburban developments that now define large portions of the North American landscape (Fettridge, et al. 2008).

The recent North American introduction and range expansion of a second non-native *Anthidium* species (*A. oblongatum* Illiger) may soon rival that of *A. manicatum*; since its initial sighting in Pennsylvania in 1995 (Hoebeke & Wheeler 1999), this Old World bee has already expanded its range to include parts of New York, New Jersey, Maryland, Ohio, Connecticut, Massachusetts, New Hampshire, Rhode Island, and Vermont (Miller, et al. 2002; Maier 2009). During the 2009 field season, I collected two *A. oblongatum* females (the first on 26 June, the second on 28 July; determined by J.S. Ascher, pers. comm.) from an eastern Massachusetts field site (Longfellow National Historic Site, 42°22.63'N, 71°07.56'W) where they foraged on the same plants visited by *A. manicatum*. (No

A. oblongatum individuals were seen at the site in the summer of 2010, despite similar collection efforts.) Since both species have similar habitat requirements and host plant preferences, the dynamics of these concurrent, overlapping, and congeneric range expansions may present an interesting opportunity for future research.

1.3. The study system: Floral preferences and nesting behavior

A. manicatum is a narrowly polylectic bee species whose floral hosts are restricted mostly to the Lamiaceae, Scrophulariaceae, Fabaceae, Compositae, and Boraginaceae (Kurtak 1973; Müller 1987; A. Payne, pers. obs.; **Fig. 1.3**).

Representatives of these plant families are common in residential and botanical gardens throughout the northeastern United States, where they provide both nectar and pollen sources for many bee species, including *A. manicatum* (**Fig.1.4**).

In addition to supplying nectar and pollen, some of these plants also provide raw materials essential for *A. manicatum* nest construction. The species' common name, the wool-carder bee, derives from its habit of harvesting plant trichomes -- plant pubescence, or "wool" -- for use in the construction of its brood cells. Using their sharply toothed mandibles, females pull these trichomes away from the plant's cuticular surface and roll them into a compact ball that they subsequently transport to some pre-existing cavity. Once inside, the bees manipulate the trichome mass into a brood cell in which they lay an egg atop a provisioning mass of nectar and pollen. A single cavity may host between one and



Fig. 1.3 Male *A. manicatum* feeding on the nectar of *Nepeta* (family Lamiaceae; species unknown). The bees are narrowly polylectic, with preferences for the plant families Lamiaceae, Scrophulariaceae, Fabaceae, Compositae, and Boraginaceae.



Fig. 1.4 Typical North American habitat of *A. manicatum*: intensely cultivated flower beds located at the Brooklyn Botanic Garden, Brooklyn, New York.

five such cells, each containing a single egg; once the cells are complete, the bee closes the entrance to each nest with a final terminal plug made of plant fibers and several centimeters of organic and inorganic debris (Kurtak 1973).

From a phylogenetic perspective, the nesting behavior of *A. manicatum* is a variation on a theme common within the subfamily Megachilinae (Hymenoptera: Megachilidae), namely the use of harvested plant materials in the construction of nest cells. Within the tribe Anthidiini, most species exhibit one of three distinct nesting phenotypes: the wool-carding (described above), resin-building, or cleptoparasitic habits (Michener 2007). Resin-builders most often construct cells on exposed surfaces, using plant resins as foundational building materials into which other organic or inorganic materials are occasionally embedded (Michener 2007). While it seems reasonable to assume, as Michener did (2007), that wool-carding is a synapomorphic trait arising from a paraphyletic assemblage of resin-builders, comprehensive and/or convincing phylogenetic analyses have yet to be performed (although see Müller 1996). Until they are, any postulates of homology or character polarity, as well as any proposed evolutionary relationships, remain untested.

1.4. The study system: Reproductive behavior and male aggression

The mating system of *A. manicatum* is marked by an extreme form of resource defense polygyny, wherein males obtain and defend floral territories that females visit for food resources and nesting materials (Kurtak 1973; Severinghaus

et al. 1981; Müller 1987). This territorial defense entails extreme, occasionally lethal, aggression directed at both conspecific males and heterospecific pollinators (Ward 1928; Severinghaus et al. 1981; Wirtz et al. 1988). The species also exhibits a male-biased sexual size dimorphism unusual among bees (Darwin 1871; Severinghaus et al. 1981; Shreeves & Field 2008; **Fig. 1.5**). Male size appears to correlate with mating success (Müller 1987; Starks & Reeve 1999), and small males routinely adopt alternative mating strategies if unable to defend a floral territory from larger usurpers (Starks & Reeve 1999). While females mate repeatedly with multiple males, details of sperm utilization and population genetic structure remain unclear.



Fig. 1.5 Typical male (left) and female *A. manicatum*; note the pronounced, reversed sexual size dimorphism characteristic of the species. Both specimens were captured on the same day in July 2010 at the same floral site on the Tufts University campus, Medford, Massachusetts. Accompanying scale in centimeters.

1.5. The study system: Historical perspective

A. manicatum, first described by Linnaeus as *Apis manicata* in 1758, has been the subject of casual ethological interest since at least the late 18th century, when the naturalist Gilbert White (1795) described a “sort of wild bee” harvesting trichomes from the cuticles of his garden plants. By the middle of the 19th century, the species had attracted Darwin’s (1871) attention as an unusual example of reversed sexual size dimorphism in Hymenoptera. While brief descriptions of *A. manicatum*’s life history followed throughout the 19th and 20th centuries (Westrich 1989 and references therein; K. Hartfelder unpubl. data), the first complete account of female nesting behavior did not arrive until Kurtak’s 1973 masters thesis. In it, she described the contents of several trap nests recovered from field sites in Ithaca, New York, as well as the results of nesting observations conducted inside a small screen enclosures. Some of these reports, as well as additional contributions on male behavior, appear in Severinghaus and colleagues (1981).

From 1985 to 1986, Müller conducted a series of morphometric and behavioral studies on male *A. manicatum* in select European and North American populations. In his unpublished masters thesis (1987), he described an intriguing, bimodal distribution of male head sizes within the North American population, a pattern that remained consistent from generation to generation (such a distribution could not be established for the European populations). Head size distributions were “invariably unimodal” among females from both the European and North

American populations. Müller also demonstrated a correlation between male size and reproductive success. Much of the rest of his thesis is concerned with explaining the persistence of the small male phenotype in the face of dramatic reproductive skew.

Later work by Starks and Reeve (1999) addressed male size in relation to reproductive strategy. They demonstrated that alternative male reproductive tactics -- in this case explicit territorial defense versus “wandering” among territories in the hopes of surreptitious copulation -- represent plastic, conditionally-dependent behavioral phenotypes. When larger, territorial males were removed from the study sites, formerly wandering males adopted the territorial tactic. Such behavioral plasticity seems at odds with the dimorphic male size distribution described by Müller (1987).

1.6. The outline of this work

This thesis is the product of two field seasons spent researching various aspects of the reproductive biology of *A. manicatum*. The topics covered include male intrasexual recognition, female nest site selection, parallel nesting, and nest site usurpation.

In chapter 2, I report the results of a 2009 study of the bee’s potential for nest site selection, a critical component of female reproductive fitness (published as Payne et al. 2010). In chapter 3, I expand upon those results with a series of follow up studies conducted during the summer of 2010. These investigations

addressed questions of nest site selection, parallel nest site utilization, and male intrasexual aggression. Since these results are best understood within a recognition systems framework (Starks 2004), Appendix A includes a more general outline of recognition systems theory (published as Payne & Starks 2010) illustrated by specific examples from the social insect literature.

Chapter II

Nest site selection in the European wool-carder bee, *Anthidium manicatum*, with methods for an emerging model species

Payne A, Schildroth DA, Starks PT. 2010.

Apidologie. doi:10.1051/apido/2010050

2.1. Abstract

For many organisms, choosing an appropriate nest site is a critical component of reproductive fitness. Here we examine nest site selection in the solitary, resource defense polygynous bee, *Anthidium manicatum*. Using a wood-framed screen enclosure outfitted with food sources, nesting materials, and bamboo trap nests, we show that female bees prefer to initiate nests in sites located high above the ground. We also show that nest sites located at higher levels are less likely to contain spiderwebs, suggesting an adaptive explanation for nest site height preferences. We report size differences between this study's source populations in Boston, Massachusetts and Brooklyn, New York; male bees collected in Boston have smaller mean head widths than males collected in Brooklyn. Finally, we argue that methods for studying captive populations of *A. manicatum* hold great promise for research into sexual selection, alternative phenotypes, recognition systems, and the evolution of nesting behavior.

Keywords: megachilidae / introduced species / solitary bee / enclosure methods

2.2. Introduction

The European wool-carder bee, *Anthidium manicatum* (Hymenoptera: Megachilidae), is a Palearctic solitary bee species best known for its hyper-aggressive males and resource defense polygynous mating system (Ward 1928; Pechuman 1967; Severinghaus et al. 1981; Starks and Reeve 1999). Males obtain and defend floral territories that females visit for food resources and nesting materials (Kurtak 1973, Severinghaus et al. 1981, Müller 1987); in the process, they routinely attack both conspecific males and heterospecific pollinators, sometimes lethally injuring the latter (Ward 1928; Severinghaus et al. 1981; Wirtz et al. 1988). *A. manicatum* also exhibits a male-biased sexual size dimorphism unusual among bees (Darwin 1871; Severinghaus et al. 1981; Shreeves and Field 2008). Previous research has shown that male size correlates with mating success (Müller 1987; Starks and Reeve 1999), and that small males routinely adopt alternative mating tactics if unable to wrest control of territories from larger rivals (Starks and Reeve 1999). All of these reasons, along with the bee's status as an introduced species in North America, South America, and New Zealand (Gibbs and Sheffield 2009), make *A. manicatum* a promising system for the study of sexual selection, alternative phenotypes, and invasion ecology.

Less appreciated is the species' potential as a model organism in studies of aculeate nesting behavior and of the recognition systems that make it possible. *A. manicatum* is a member of the Anthidiini, a tribe of megachilid bees that engage in elaborate and highly derived nesting behaviors (Melander 1902; Michener

2007). Some of these genera, e.g. *Dianthidium*, construct exposed nest cells made of tiny pebbles embedded in conifer resin, while others, such as *Hoplostelis*, are obligate cleptoparasites of other bee species. Still other genera, including *Anthidium*, build their nests in pre-existing cavities that they line with the harvested trichomes of wooly plants (Michener 2007).

A. manicatum is perhaps the best studied of these “wool-carder” bees. Several European authors have contributed notes on the species' nesting biology (Westrich 1989 and references therein; K. Hartfelder, personal communication) and brief descriptions of the nests themselves date back to at least the middle of the 19th century, when Darwin's correspondent Frederick Smith (1855) outlined their basic structure. Shortly after, Kirby and Spence (1857) reported seeing females at least twice build their nests inside “the lock of a garden-gate,” but suggested that nests were more likely to be found in cavities in old trees. In spite of this long history, detailed descriptions of female nesting behavior did not emerge until Kurtak explored the topic in an unpublished 1973 masters thesis. In it, she described a small number of nests recovered from traps placed in the greater Ithaca, New York, region shortly after the bee's putative introduction to North America. Utilizing a small screen enclosure, she also reported the first known observations of females in the act of nest construction.

Using their sharply toothed mandibles, female wool-carder bees strip the trichomes, or pubescence, from the leaves and stems of various plants, roll this pubescence into a ball, and carry it to some pre-existing cavity. Once inside, the

bees shape the pubescence into cells where they deposit an egg and a provisioning mass made of nectar and pollen. A female constructs at least one, but usually several, cells within a single cavity, then seals up the entrance with various bits of organic and inorganic material that it carries to the nest; taken together, this material forms the nest's "terminal plug." New bees either emerge later that summer as the second generation of a bivoltine life cycle, or overwinter as prepupae and emerge the following spring (Kurtak 1973).

Ultimately, Kurtak (1973) based her observations on only a limited number of incomplete nests ($N = 2$) built inside her screen enclosure. To date, few nests have been recovered in nature (Pechuman 1967; Kurtak 1973; Severinghaus et al. 1981) and, despite Sugiura's (1994) success trap nesting captive *A. septemspinosum*, a similar effort using *A. manicatum* was largely unsuccessful (Kurtak 1973). To our knowledge, there have been no previous experimental studies of either nesting behavior or nest site selection in this species.

Here we describe an experimental study of nest site selection undertaken with a captive population of *A. manicatum* during the summer of 2009. We present findings on size differences between two populations within the northeastern United States, and we test the hypothesis, based on suggestive findings by Severinghaus and colleagues (1981), that females prefer to initiate nests in locations high above the ground. We also present a possible adaptive explanation for this behavior. Finally, we argue that this study's methods allow for much greater experimental control than can be achieved through traditional field

methods. We suggest that our methods therefore hold great promise for future studies of sexual selection, alternative phenotypes, recognition systems, the evolution of nesting behavior, and many other topics in evolutionary biology.

2.3. Materials and Methods

Collection methods.

Between 26 June and 11 August 2009, we captured live *Anthidium manicatum* from two urban field sites near Boston, Massachusetts, USA (Tufts University campus: 42°24.4'N, 71°07.1'W, $N = 11$; Longfellow National Historic Site: 42°22.63'N, 71°07.56'W, $N = 11$) and from one in Brooklyn, New York, USA (Brooklyn Botanic Garden: 40°40.1'N, 73°57.8'W, $N = 35$). Each of these sites contained cultivated flower beds of perennial plants visited by *A. manicatum* (at Tufts: *Nepeta* sp. L. (Lamiaceae); at Longfellow National Historic Site: *Salvia x superba* (Lamiaceae), *Antirrhinum majus* L. (Scrophulariaceae), *Digitalis purpurea* L. (Scrophulariaceae), *Artemisia* sp. L. (Asteraceae); at Brooklyn Botanic Garden: *Teucrium chamaedrys* L. (Lamiaceae), *Calamintha nepeta* (L.) Savi (Lamiaceae), *Lamium maculatum* L. (Lamiaceae), *Salvia sclerea* L. (Lamiaceae), *Antirrhinum majus* L., *Nepeta* sp. L.). All collections were made between 9:30 and 15:30 hr using aerial insect nets.

Using individual glass vials, we transported captured bees to the International Social Insect Research Facility (I.S.I.R.F.) at Tufts University, where we chilled them on ice for 3 minutes, temporarily immobilizing them. While the

bees were inactive, we measured the widths of their heads to the nearest 0.05 mm using Dialmax SPi2000 dial calipers, repeating each measurement 3 times per individual and averaging the values as in Müller (1987). We then marked the dorsal sides of the bees' thoraces with individual-specific color combinations of Testors enamel paint. After marking, we allowed the bees to recover within the wood-framed screen enclosure described below.

In addition to these 57 bees, we collected 14 *A. manicatum* at the Brooklyn site on 19 August 2009. We killed these bees on-site by submersion in 100% ethanol, then transported them to I.S.I.R.F. to obtain head width measurements using the protocol described above. These bees, as well as dead individuals recovered from the enclosure throughout the season ($N = 37$) and those bees remaining alive at the end of the study on 24 August 2009 ($N = 2$), were placed in individual tubes in 100% ethanol and stored at -20°C . Several bees ($N = 18$) were not recovered from inside the enclosure, and their bodies were presumed lost inside crevices in the floor or in cracks in the wooden walls; given the precautionary double doors (see below) and periodic checks on the integrity of the screen, it is unlikely that any of these bees escaped.

Animal husbandry and the field enclosure.

To simulate natural conditions, we outfitted a previously existing, wood-framed screen enclosure (4.8 m long X 2.7 m wide X 2.0 m tall) with large pots containing plants used by *A. manicatum* for nectar and pollen (*Salvia* sp L.,

Nepeta sp. L.) and for nesting materials (*Stachys byzantina* Kock (Lamiaceae)) (Fig. 2.1). If a plant appeared unhealthy, we moved it outside the enclosure for a short time to maximize its exposure to full sunlight; thus, the total number of pots within the enclosure varied throughout the season ($N =$ between 3 and 12 for each species). We also planted *Rosmarinus officinalis* L. (Lamiaceae), *Origanum vulgare* L. (Lamiaceae), and *Ocimum basilicum* L. (Lamiaceae) (itself a source of nectar and pollen), in two small herb plots located within the enclosure. Double doors at the front of the structure prevented bees from escaping, while a plexiglass roof and screened openings on the northwest and southeast walls allowed for direct sunlight for at least six hours on sunny days. In addition, we provided bees with shallow dishes of water *ad libitum*. To prevent the loss of nests to foraging ants, on 16 July we distributed TAT ® Ant Traps ($N = 6$) and Terro ® Ant Killer Liquid Baits ($N = 6$) throughout the enclosure and at various heights along the walls.

Trap nesting.

On each wall of the enclosure, we mounted horizontal trap nests ($N = 295$) constructed from lengths of dried bamboo (55-245 mm; mean = 145.30 mm \pm 41.76) opened at one end and closed at the other by a node. The entrance diameters ranged from 4.2-19.2 mm (mean = 13.27 mm \pm 2.10). We bundled these trap nests with bailing twine into groups of between 4 and 8 with entrances pointing in both directions parallel to the walls. We then placed the traps at one of

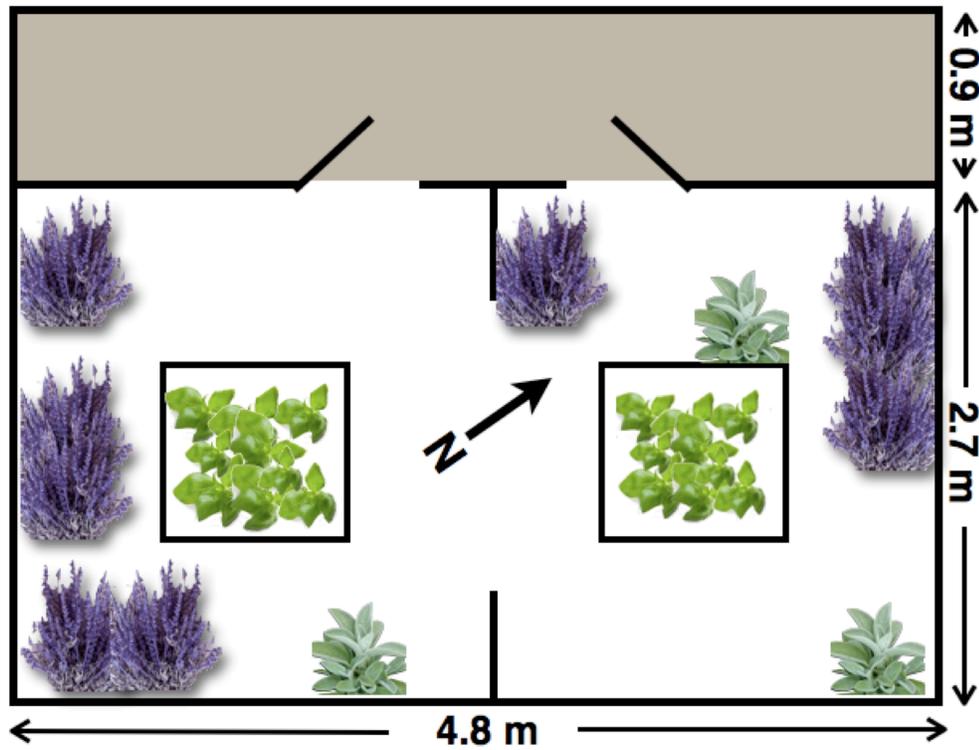


Fig. 2.1 Stylized diagram of the I.S.I.R.F. *Anthidium* enclosure, a wood-framed screen structure open to sunlight through the northwest and southeast walls and through the plexiglass roof. The enclosure contained two small herb plots planted with *Rosmarinus officinalis* L., *Origanum vulgare* L., and *Ocimum basilicum* L., and pots containing *Salvia* sp. L., *Nepeta* sp. L., and *Stachys byzantina* Koch. We attached bamboo trap nests ($N = 295$) to each wall of the enclosure in bundles of between 4 and 8.

three height levels within the enclosure (low: < 35 cm above ground, $N = 70$; middle: 80-95 cm, $N = 98$; high: > 165 cm, $N = 127$) and, due to the original placement of the structure, oriented each trap nest's entrance toward one of four intermediate cardinal directions (northeast, $N = 70$; southwest, $N = 82$; southeast, $N = 71$; northwest, $N = 72$).

We also mounted a small number of vertical trap nests ($N = 17$) at a height of approximately 140 cm along the southeastern wall of the enclosure; approximately half ($N = 9$) of these traps were arranged with their entrances pointing downward, while the others opened upward. These trap nests were ignored by bees and thus are not included in subsequent analyses of height or orientation preferences or of spiderweb presence.

In mid-October, we opened a small number of trap nests containing wool ($N = 9$) and photographed the contents. Three of these nests are depicted in **Fig. 2.2** and **2.3**. We left the remainder of the nests inside the enclosure to await spring eclosion.

Data collection and analysis.

Between 23 July and 23 August 2009, we surveyed each trap nest twice weekly with a small flashlight to record the presence or absence of plant pubescence. As this pubescence (hereafter “wool”) could only have been transported to the traps by *A. manicatum* females, we used its presence as a proxy for nest initiation. The wool was easy to spot, as its distinctive glistening

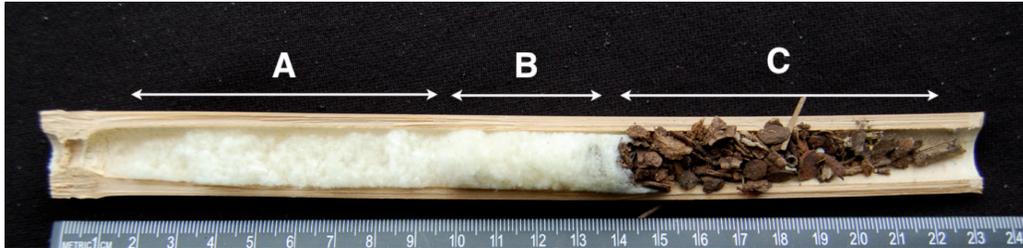


Fig. 2.2 A completed nest opened by the authors on 9 October 2009. This nest contained loose flocculence at the distal end of the tube (A), followed by a single cell (B), and a terminal plug composed of detritus from the herb plots (C). *Stachys byzantina* plants located inside the enclosure provided the source of pubescence. The reference scale is in centimeters.

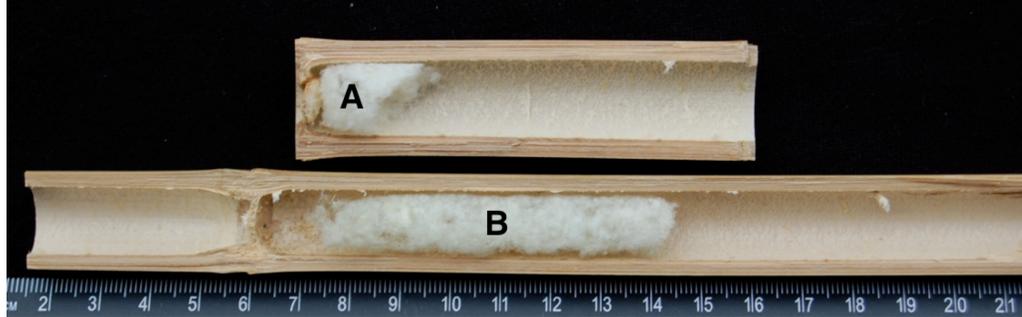


Fig. 2.3 Two incomplete nests opened by the authors on 10 October 2009. The top nest contained an unused and incomplete cell (A), while the second nest contained loose flocculence without cells (B). These structures were typical of those nests that lacked completed cells. The reference scale is in centimeters.

appearance set it apart from the light background of the trap nest interior. During the late August surveys, we also recorded any nests that contained a quantity of organic or inorganic matter indicative of a terminal nest plug. The presence of a plug implied that the female had finished her nest, and we scored these nests as “completed.”

During this period, we also monitored trap nests for the presence of spiderwebs built on or inside the trap nest entrances. Once detected, spiderwebs were removed and their presence recorded. In the final analysis, we scored each trap nest according to a.) whether or not it had ever contained wool, b.) whether or not it was completed with a terminal plug, and c.) whether or not it had ever contained a spiderweb. These scores were applied regardless of the trap nest’s status on the last day of the surveys.

Before analyzing head width data, we first established the normality of each distribution using a Shapiro-Wilk test (for each sex by location, $W > 0.92$, $p > 0.35$) and the homogeneity of variances using Levene’s test ($W = 2.692$, $p > 0.05$). Differences in mean head width among sex/locations categories were analyzed using a one-way ANOVA, followed by a post-hoc Tukey-Kramer test in SPSS GradPack® software (SPSS 2008). Nest site preferences and spiderweb data were analyzed using chi-square tests; for small sample sizes, we used Fisher’s exact test carried out in StatXact 6® software (Cytel 2003).

2.4 Results

Head width as a function of sex and location.

Males were significantly larger than their female counterparts in both the Boston ($p < 0.05$) and Brooklyn populations ($p < 0.001$) (**Fig. 2.4**). There was also a significant size difference between Brooklyn males and Boston males ($p < 0.05$), but Brooklyn females were not significantly larger than Boston females ($p > 0.20$). There was no significant size difference between Boston males and Brooklyn females ($p > 0.20$).

Survival rate and behavior within the enclosure.

Mortality within the enclosure was high; 35 (61.4%) of the introduced bees were not seen alive again after the day of introduction (**Fig. 2.5**). Of these, 23 were among the cohort of 35 bees transported from Brooklyn to the Tufts University campus on 5 August 2009. Nevertheless, bees that survived the first day quickly adapted to life within the enclosures, and among this group the average life spans of females (23.0 days \pm 8.67) and males (13.6 days \pm 13.31) were long enough to exhibit the full range of behaviors recorded under natural conditions. These behaviors included feeding, territorial patrols by large males, aggression between males, successful and unsuccessful copulation attempts, collection of pubescence by females, and nest construction (Kurtak 1973). As the enclosure was closed to heterospecifics, we did not observe the interspecies

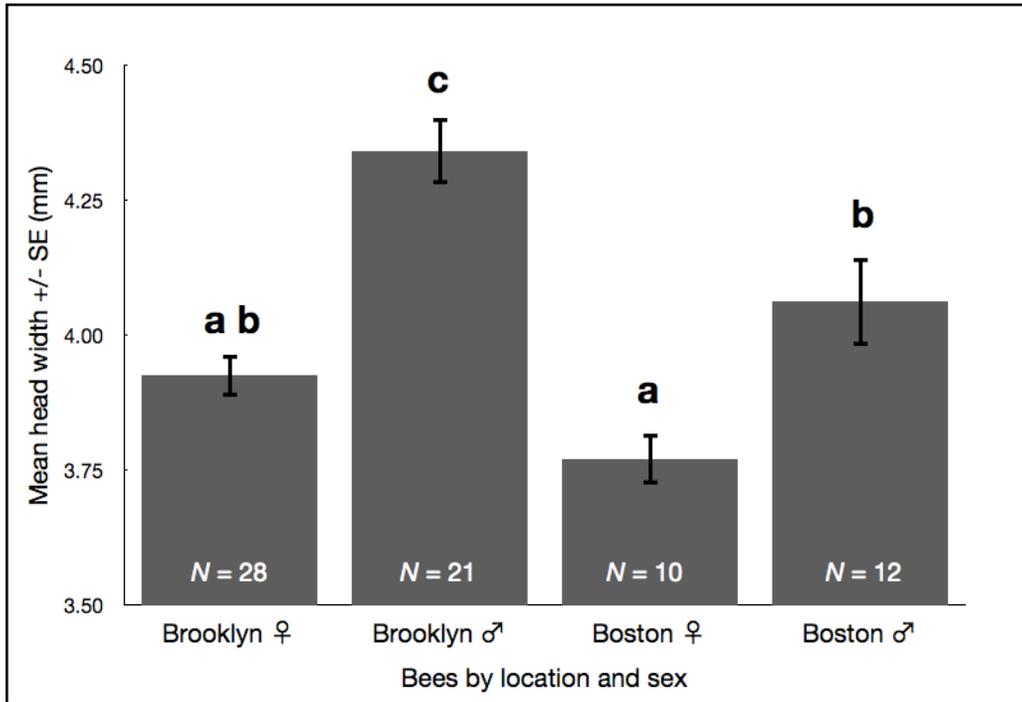


Fig. 2.4. Mean head widths (+/- 1 SE), arranged by location and sex. Different letters represent significant differences.

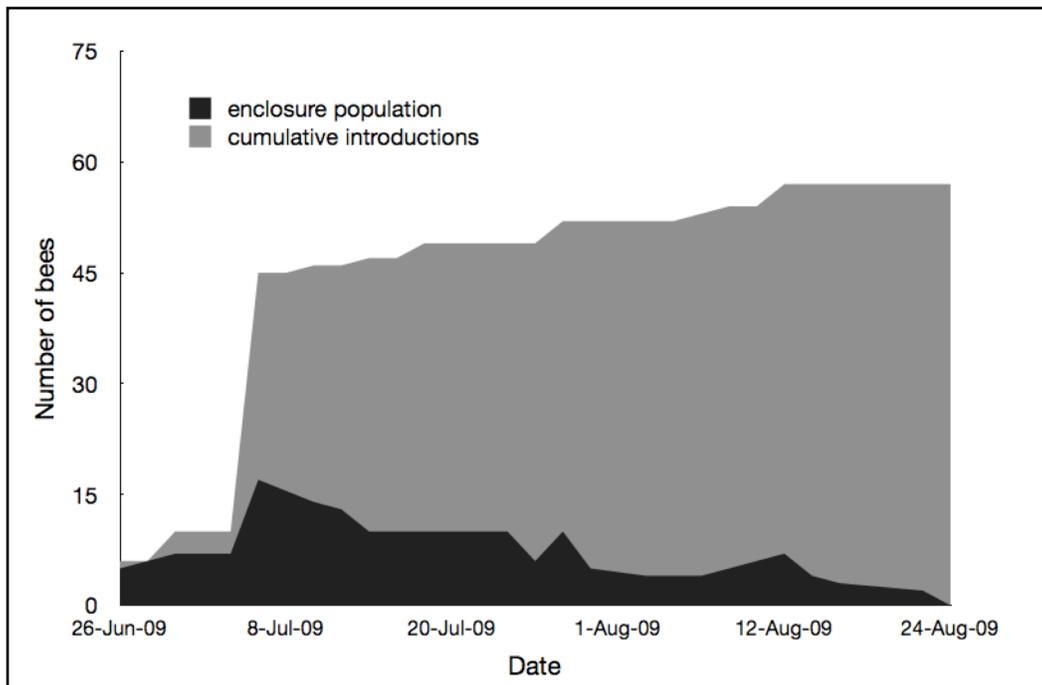


Fig. 2.5 Cumulative introductions of bees and surviving bee populations by date. We surveyed bees at irregular intervals throughout the field season to record which individuals were present inside the enclosure.

aggression described by several authors (Ward 1928; Severinghaus et al. 1980; Wirtz et al. 1988).

Trap nesting results.

On days when the bees were active, we observed individual females delivering wool to, and presumably provisioning, multiple nests concurrently. At the end of the field season, 50 of the enclosure's 295 trap nests contained wool, and 5 of these contained terminal plugs indicative of completed nests. The bees used trap nests with diameters ranging from 9.5-15.6 mm, and lengths ranging from 79-222 mm. Trap nest orientation had no significant effect on female nest site selection (northeast = 17, southwest = 16, southeast = 9, northwest = 8; $\chi^2_{df 3} = 4.773$, $p = 0.189$), although there was a slight trend in favor of trap nests oriented toward the northeast and southwest. Height, meanwhile, had an extremely significant effect on nest site selection: females overwhelmingly chose to initiate nests in traps positioned at the highest level within the enclosure ($\chi^2_{df 2} = 34.89$, $p < 0.0001$), though there was no significant difference between traps located at the low and middle levels (Fisher's exact test, $\chi^2_{df 1} = 2.806$, $p = 0.15$) (**Fig. 2.6**). Females also completed significantly more nests in the high traps than in either middle or low traps (Fisher's exact test, $\chi^2_{df 2} = 6.601$, $p = 0.0317$) (**Fig. 2.7**).

We found no evidence of wool in any of the vertical trap nests, though the sample size was too small to achieve statistical significance. In fact, with the

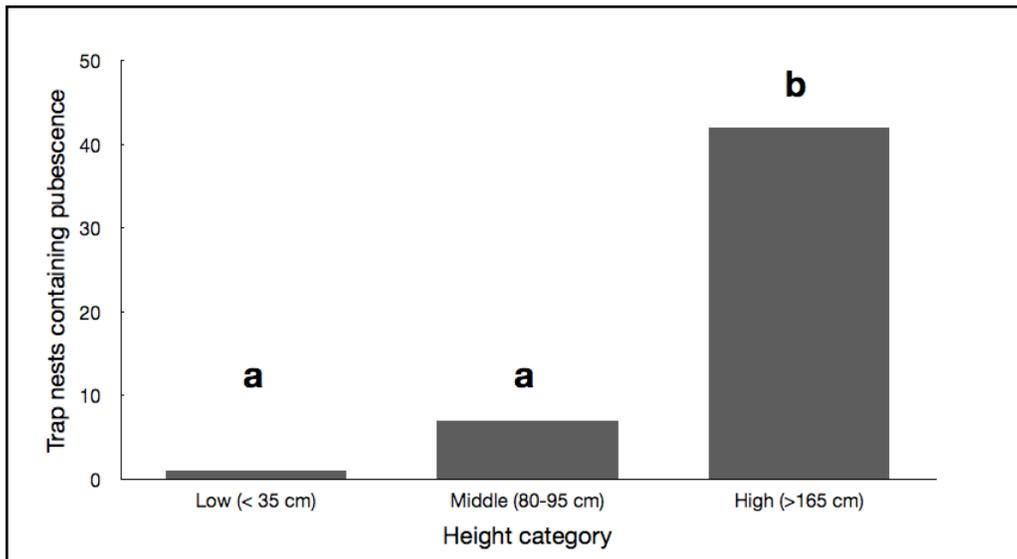


Fig. 2.6 Number of pubescence containing trap nests arranged by height level. Bees showed a significant preference for transporting pubescence to high trap nests over low and middle traps; there was no significant difference between the number of pubescence containing trap nests at the low and middle levels.

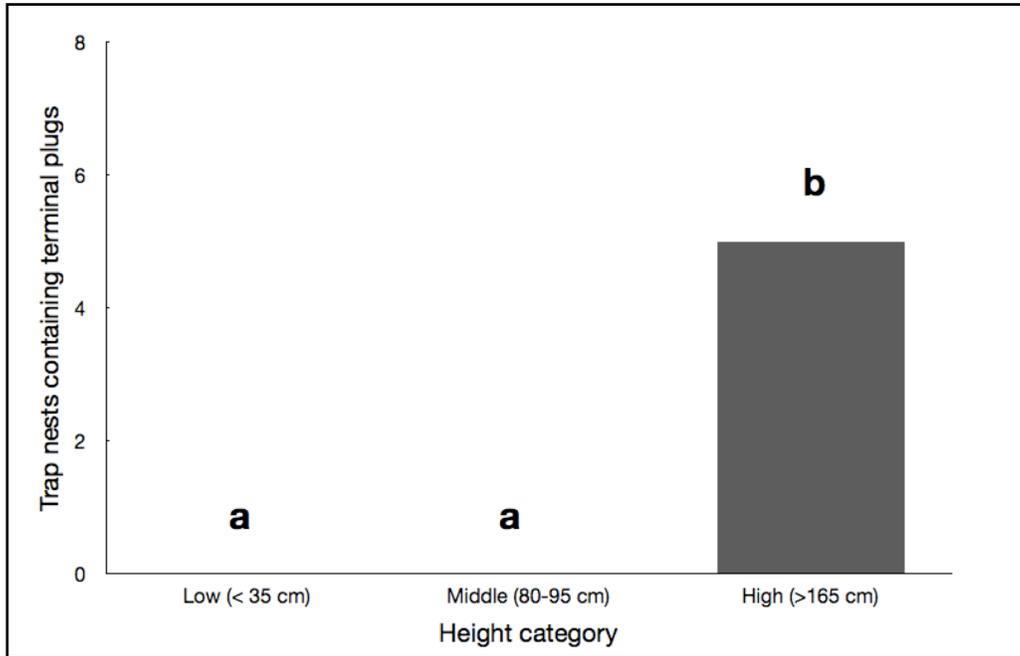


Fig. 2.7 Number of trap nests containing a terminal plug (i.e., “completed” trap nests) arranged by height level. Bees showed a significant preference for initiating terminal plug construction in high trap nests; we found no evidence of terminal plugs at either the low or middle levels.

exception of a single male that climbed into a downward facing trap nest during a storm, we never saw bees enter vertical traps.

By the end of the field season, 62 of the 295 horizontal trap nests had contained at least one spiderweb either on or within their entrances; two of these traps, one at the high level and another at the middle level, also contained wool.

Height had a significant effect on spiderweb construction, though the pattern was the reverse of that seen in wool deposition ($\chi^2_{df2} = 20.26$, $p << 0.0001$) (**Fig. 2.8**).

Ant presence within nests

On 15 July, we observed a number of large bodied ants (*Formica* sp.) inside a high level, wool containing trap nest. The ants were concentrated in the area immediately in front of the wool, and several of them appeared to be feeding on this material. Bits of loose flocculence hung from the edge of the nest entrance, and there was a small amount of terminal plug-like debris (bits of sawdust, wood splinters, and at least one very small [< 2 mm] snail shell) located in the trap nest's proximal end. When we returned to the nest the next day, the ants were still inside, but the wool was almost completely gone and the pseudo-plug doubled in size. We removed the ants from the trap nest and placed them in 100% ethanol at -20°C .

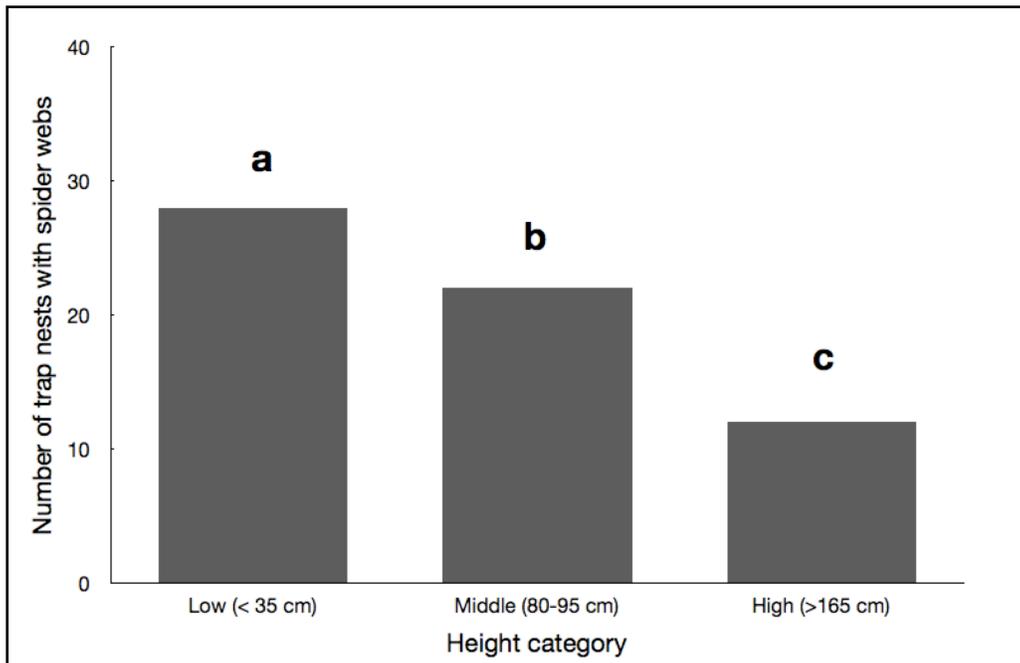


Fig. 2.8 Number of trap nests with spiderwebs constructed either on or in the trap entrance, arranged by height category. High traps were significantly less likely than middle traps ($\chi^2_{df 1} = 6.159$, $p = 0.013$), and middle traps significantly less likely than low traps ($\chi^2_{df 1} = 4.206$, $p = 0.0403$), to contain spiderwebs. Two of these trap nests (one at the middle level and another at the high level) contained pubescence prior to the construction of the spiderwebs.

Also on 15 July, we discovered a number of individuals of a smaller ant species (*Crematogaster lineolata*) inside four more high level, wool containing trap nests. We removed these ants from the nests and stored a small number of individuals using the same protocol as above. All four of these trap nests still contained wool at the end of the field season.

Anti-ant measures taken on 16 July (see methods) seemed to prevent further attacks. We included all five ant-parasitized nests in our analysis of nest initiation preferences; we did not, however, include the pseudo-plugged nest in our analysis of “completed” nests.

Female aggression at nest entrances.

On 17 July, we observed a female attempt to enter a nest as another female was exiting. The exiting female chased the first away from the nest entrance and into the center of the enclosure, where the two bees circled each other in a manner reminiscent of the aggressive spiral flights of males (Severinghaus 1981). This was the only time we observed such behavior either within the enclosures or under natural conditions.

2.5 Discussion

Relative size is an important determinant of male behavior in *A. manicatum*, a species in which large males consistently outcompete smaller males in the fight for territories and their associated fitness benefits (Müller 1987; Starks and Reeve 1999). Size differences between populations are intriguing, then, as

they may shed light on the factors determining body size. Male bees taken from the Brooklyn population of *A. manicatum* were significantly larger than their male counterparts captured near Boston; this effect was so marked that the mean head size of Boston males converged upon that of Brooklyn females, erasing any sexual dimorphism between these groups. The reason for this difference may lie in the field sites themselves. Brooklyn Botanic Garden comprises 52 acres of cultivated plantings that include several species visited by *A. manicatum*; by contrast, the Longfellow National Historic Site contains a single formal flower garden, approximately 20 m X 30 m, and a more limited number of plant species used by the bees. The floral territories from which we captured bees on the Tufts University campus were even smaller, were spaced at a considerable distance from one another, and contained only a single species used by *A. manicatum*. Indeed, Müller (1987) found similar size differences between a group of bees captured in a European botanical garden and another captured from smaller gardens in Ithaca, New York. Taken together, these results may support previous assertions (Sugiura 1994) that nutrition plays a key role in the determination of body size in megachilid bee species, though we cannot yet rule out genetic differences between populations.

Earlier reports of trap nesting in wild populations of *A. manicatum* suggested that females might preferentially build their nests in locations high above the ground; in fact, all four such nests reported by Kurtak (1973) and by Severinghaus and colleagues (1981) were collected from the upper stories of man-

made structures. Our results support this hypothesis, and show that under semi-natural conditions females overwhelmingly choose the highest available cavities in which to build their nests. This behavior may serve an adaptive purpose by limiting the exposure of nest sites to interference by predators or parasites; indeed, the presence of spiderwebs on or within the trap nests in our enclosures was significantly skewed toward lower elevation traps. While we did find ants in high level traps within our enclosure, this may not reflect what happens in nature, where nests may be considerably higher than the options available to captive bees.

To our knowledge, ours is the first report of possible ant predation on nests of *A. manicatum*. Nevertheless, there have been reports of nest parasitism by unidentified arthropods (Kurtak 1973) and by cleptoparasitic Anthidiini (reviewed in Müller 1987), and Sugiura (1994) reported high levels of infestation (21.8% of all nests) of *A. septemspinosum* nests by a meloid beetle, *Zonitis japonica*. Whether ants feed on *A. manicatum* larvae, the pollen mass, or on the unidentified secretions that bind the nest material together, remains an open question.

Females may also prefer to build nests high above the ground and far from floral territories (Severinghaus et al. 1981) as a means of escaping nest usurpation by other *A. manicatum* females. Vinson and Frankie (2000) reported on conspecific nest usurpation in the trap nesting bee *Centris bicornuta* and suggested that the oily entrance plugs with which these females close their nests may be an adaptation against such usurpation. It is at least plausible that the heavy fortifications of the *Anthidium* terminal plug serve a similar purpose. While our

study did not rigorously track individual female nest visitations, the seemingly aggressive interaction between two females at a nest entrance warrants further investigation.

A. manicatum holds great promise as a model organism in studies of sexual selection, alternative phenotypes, recognition systems, and aculeate nesting behavior. Unfortunately, this promise remains largely unfulfilled due to the limitations of field based research protocols. Indeed, studies of nesting behavior face particular difficulties, as natural nests have proven difficult to locate, and attempts to keep *A. manicatum* in captivity have been relatively unfruitful (Kurtak 1973). Here we describe methods for the successful maintenance of both male and female *A. manicatum* in a field enclosure under semi-natural conditions. Our results echo Sugiura's (1994) success using similar methods to study the congeneric *A. semitemspinus* in Japan. His study of the effects of parental investment on offspring sex ratio, as well as ours of nest site selection preferences, demonstrate the power of these methods to resolve previously unanswerable questions about the biology of anthidiine bees.

2.6 Acknowledgments

We owe our thanks to several individuals without whose assistance this work could not have been completed: Dr. Gerry Moore at Brooklyn Botanic Garden and Mona McKindley and Jim Shea at the National Park Service were exceedingly patient with periodic visits from the bee collectors. At Tufts, Dr.

Frances Chew and Dr. Sara Lewis loaned equipment and provided feedback, Tegan Morton assisted with collections at the Brooklyn field site, and Susan Weiner and Dr. George Ellmore helped with the identification of ants and plants, respectively. Noah Wilson-Rich provided assistance with data analysis. L. Mairin Odle, Burton Payne, as well as Dr. Klaus Hartfelder and two anonymous referees, reviewed previous drafts of this paper and contributed valuable suggestions for improvement. In addition, this research was supported by a National Science Foundation REU site award to Tufts University (DBI-0649190).

Chapter III

Further studies of recognition systems and nest site selection in the European wool-carder bee, *Anthidium manicatum*

3.1 Abstract

Due to its unusual behavior, reversed sexual size dimorphism, and recent cosmopolitan range expansion, the European wool-carder bee, *Anthidium manicatum*, is an intriguing subject for behavioral research. In this chapter, I build upon a previous study of the bee's capacity for nest site selection with a new series of field- and enclosure-based investigations. These experiments a.) address further questions regarding the templates females use to identify nest sites, b.) track individual females to reveal patterns in nest site utilization, and c.) attempt to uncover the criteria by which males recognize one another during aggressive interactions. Results indicate that females prefer to nest in longer (i.e., deeper) cavities. They also demonstrate that some females are capable of maintaining multiple nest sites in parallel, and of appropriating abandoned, unfinished nests for their own use. Meanwhile, preliminary results suggest that visual stimuli may be both necessary and sufficient for the initiation of male aggressive behavior. Despite modest success with enclosure-based experimental methods, high mortality remains a serious concern.

3.2 Introduction

Due to its unusual behavior, reversed sexual size dimorphism, and recent cosmopolitan range expansion (Payne et al. 2010), the European wool-carder bee, *Anthidium manicatum* (L.) (Hymenoptera: Megachilidae), is an intriguing subject for behavioral research. While several studies have investigated aspects of the organism's behavior under natural field conditions (Ward 1928; Pechuman 1963; Kurtak 1973; Severinghaus et al. 1981; Müller 1987; Wirtz et al. 1988; Starks & Reeve 1999), only one (Payne et al. 2010) describes experimental research using a captive, enclosure-bound population (although see Kurtak 1973, as well as Sugiura's 1994 study of the congeneric *A. septemspinosum*). Here I expand upon that study with further investigations into the reproductive biology of *A. manicatum*. Using both wild and captive populations, I attempt to answer new questions regarding female nest site selection, patterns of nest site utilization, and the recognition systems behind male intrasexual aggression; I also address the utility of enclosure-based methods in the study of anthidiine biology.

Nesting studies

For animals that build nests in pre-existing cavities, choosing an appropriate nest site can be a critical component of reproductive fitness. It follows that when these sites vary greatly in quality, i.e. in their ability to foster and protect offspring, we should expect natural selection to favor those females best able to locate and recognize high quality nest sites (Payne & Starks 2010). In

the absence of evolutionary constraints, the cumulative effect of this selection should be the evolution of highly precise nest site recognition systems (Payne & Starks 2010).

Many of these systems are amenable to empirical analysis through carefully designed experiments (examples in Seeley & Morse 1976; Seeley & Morse 1978; Vinson & Frankie 2000). In a recent example, Payne and colleagues (2010) investigated *A. manicatum*'s capacity for nest site selection using a captive population of wild-caught bees. When presented with potential nest sites arranged at three altitudinal levels within an experimental enclosure, females overwhelmingly preferred to build in the highest available sites. These results are similar to those obtained by Seeley and Morse (1978) in their field studies of honeybee nest site selection (although while Seeley and Morse found a significant preference based on nest entrance direction, Payne and colleagues found only a non-significant trend).

Unfortunately, the limitations of their experimental design prevented those authors from drawing further conclusions regarding *A. manicatum* nest site preferences. Here I expand upon their work by exploring the degree to which female nest site selection is sensitive to another spatial variable, cavity depth. Following up on preliminary investigations of nest site utilization patterns (Payne et al. 2010), I also track individual females to determine whether or not they a.) maintain multiple nests in parallel, or b.) appropriate abandoned, unfinished nests for their own use.

Male aggression study

While the extreme inter- and intraspecific aggression of *A. manicatum* males is well known (Ward 1928; Kurtak 1973; Severinghaus et al. 1981; Wirtz et al. 1988; Starks & Reeve 1999), the recognition systems that facilitate that aggression remain obscure. In the classical ethological tradition (e.g., the discussion of sign stimuli and fixed action patterns in Tinbergen 1951), both Ward (1928) and Kurtak (1973) used decoy models to try to induce aggressive behavior in *A. manicatum* males under natural conditions. In Ward's case, the decoy was a recently-killed leafcutter bee (*Megachile centuncularis*) discretely pinned to the flower stalk of a *Lamium* plant. In Kurtak's, the decoys were various models including "freshly killed Hymenoptera...a black balsa wood model the size of a large male *A. manicatum*, a black and yellow striped fishing fly, and colored paper discs," some of which were affixed to flowers, while others were moved through floral territories on thin wires.

None of these experiments appear to have elicited aggressive behavior: in Ward's words, "[The male wool-carder bee] acted as if the dead *Megachile* did not represent an insect to it." Kurtak, meanwhile, failed to report the results of her experiments (despite describing them in her methods section), an omission that I interpret, perhaps incorrectly, as a failure to obtain positive results.

Throughout the 2009 and 2010 field seasons, I repeatedly observed that resting, perching males appeared to escape the notice of other males so long as they remained flightless; this, combined with Kurtak's and Ward's results, seems

to suggest that males initiate aggressive behavior only in the presence of visual stimuli, specifically those formed by the flight patterns of other males or of heterospecific pollinators.

To test the related hypothesis that visual stimuli are *sufficient* to initiate aggressive behavior, I designed a mirror-based experiment that produced realistic visual stimuli (the sight of a live male exhibiting fully characteristic flight patterns) in the absence of non-visual, i.e. auditory or olfactory, cues.

3.3 Materials and methods

Modifications to the experimental enclosure

Prior to the 2010 field season, I made extensive modifications to the wood-framed screen enclosure described by Payne and colleagues (2010). Much of the stone flooring surrounding the small herb plots (Chapter II, **Fig. 2.1**) was removed to create two large areas for the planting and cultivation of *A. manicatum*'s preferred host plants (**Fig. 3.1**); these areas were then outfitted with a remotely-operated watering system consisting of drip hoses buried beneath a loose layer of potting soil. In order to address concerns regarding adequate light exposure, I also installed a SunSystem® III 1,000 watt, commercial greenhouse fixture with high-pressure sodium bulb in the space above the enclosure's screen ceiling; this light was turned on sporadically throughout the field season to supplement daily sun exposure and to counteract the effects of extended cloud cover.



Fig. 3.1 Modifications to the I.S.I.R.F. Anthidium enclosure. Much of the stone floor present in 2009 (left) was removed in 2010 to accommodate extensive plantings of *A. manicatum*'s preferred host plants (right).

Following these modifications, 8 plants of *Salvia nemorosa* L. “Osterfriesland” (Lamiaceae), 8 of *Nepeta* sp. L. “Walker’s Low” (Lamiaceae), 4 of *Lamium maculatum* L. (Lamiaceae), and 8 of *Stachys byzantina* Koch (Lamiaceae) were transplanted directly into the enclosure’s dirt floor. Each of these four species provides a source of nectar and pollen for *A. manicatum*, while *S. byzantina* also provides a source of nesting materials in the form of abundant leaf pubescence (Kurtak 1973; Payne et al. 2010). Plants were watered as needed, and spent flower heads were removed (“deadheaded”) periodically to encourage flowering. Later in the field season (on 9 July 2010) I added two mobile pots containing *Stachys monnieri* (Gouan) Ball (Lamiaceae) for use as a supplemental food source. Throughout the season, I also implemented measures (described in detail in Payne et al. 2010) aimed at preventing ant predation within the enclosure.

Trap nesting

As in Payne et al. (2010), all trap nests were constructed from lengths of dried bamboo, opened at one end and closed at the other by a node. Trap nests ($N = 192$) were cut to one of three standardized lengths (“short”: 8 cm, “medium”: 13 cm, “long”: 18 cm.), and bundled together into 64 “choice blocks,” each with one trap nest of each length (**Fig. 3.2**). The three trap nests in any given choice block were matched by entrance diameter (range = 11.3-14.9 mm; mean = 12.98 mm \pm 0.96); all entrance diameters and trap nest lengths were within the ranges used by



Fig. 3.2 Three of the 64 trap nest “choice blocks” distributed along the upper walls of the *Anthidium* enclosure.

female *A. manicatum* during the 2009 field season (diameter: 9.5-15.6 mm; length: 7.9-15.6 cm) (Payne et al. 2010).

Choice blocks were distributed along three of the four enclosure walls (northeastern wall: $N = 15$; southwestern wall: $N = 15$; southeastern wall: $N = 34$), at more or less even intervals, with their entrances pointing in toward the center of the enclosure at a standard height of approximately 1.9 m above the floor.

Collection locations

Between 15 June 2010 and 9 July 2010, I collected a total of 40 adult *A. manicatum* individuals ($\text{♀} = 26$, $\text{♂} = 14$) for introduction into the experimental enclosure. **Table 3.1** presents the results of the collection trips by date, location, and sex.

As in Payne et al. (2010), live *A. manicatum* were collected at several sites within the greater Boston area, specifically: a.) on the suburban campus of Tufts University, in Medford, Massachusetts ($42^{\circ}24.4'N$, $71^{\circ}07.1'W$); b.) in the Alice Longfellow Garden at the Longfellow National Historic Site, Cambridge, Massachusetts ($42^{\circ}22.63'N$, $71^{\circ}07.56'W$); and c.) at the Massachusetts Horticultural Society's Elm Bank Reservation in Wellesley, Massachusetts ($42^{\circ}16.51'N$, $71^{\circ}18.36'W$).

The first of these sites ("Tufts campus") occupies approximately 150-acres of suburban landscape with infrequent, small-scale plantings of an ornamental *Nepeta* species and of the spotted dead-nettle, *Lamium maculatum* (**Fig.3.3**). The

Table 3.1 The results of *A. manicatum* collection trips by date, location, and sex.

Collection date	Collection location	Bees by sex
15 June 2010	Tufts campus	♀ = 3 , ♂ = 3
16 June 2010	Longfellow historic site	♀ = 11 , ♂ = 3
23 June 2010	Longfellow historic site	♀ = 4 , ♂ = 0
6 July 2010	Tufts campus	♀ = 1 , ♂ = 2
7 July 2010	Tufts campus	♀ = 1 , ♂ = 1
9 July 2010	Elm Bank	♀ = 6 , ♂ = 5



Fig. 3.3 A *Nepeta* bush located near the President's House on the Tufts University campus, Medford, Massachusetts. Frequently visited by both male and female *A. manicatum*, this site provided several of the individuals used in this study.

second (“Longfellow historic site”) is an approximately 0.1-acre formal garden featuring numerous ornamentals, including the *A. manicatum* host plants *Salvia x superba* (Lamiaceae), *Antirrhinum majus* L. (Scrophulariaceae), *Digitalis purpurea* L. (Scrophulariaceae), and *Artemisia* sp. L. (Asteraceae) (**Fig.3.4**) The Elm Bank Reservation (“Elm Bank”) consists of 182 acres of carefully tended gardens that include both agricultural and ornamental plants.

While the Elm Bank site contained a large number of plants known to host *A. manicatum*, I was only able to locate the bees on a small patch of *Stachys monnieri*, and on a single *Nepeta* bush located nearby. This extreme localization of *A. manicatum* despite the presence of widespread host diversity is unusual, and did not occur at the other two sites.

Collection methods and color marking

All bees were captured using aerial insect nets then transferred directly into individually labelled 15mL Falcon tubes with lightly punctured caps. These tubes were then transferred to a cool, dark box for transportation to the International Social Insect Research Facility (I.S.I.R.F.) at Tufts University.

At I.S.I.R.F., the caps of the tubes were removed and replaced with nylon screens in order to allow for maximum gas exchange. With the bees inside, the tubes were placed one at a time into a small plastic chamber and exposed to a direct flow of CO₂ for between 1 and 2 minutes. Immobile bees were then removed from their tubes and marked on their dorsal mesosomas using



Fig. 3.4 The Alice Longfellow Garden at the Longfellow National Historic Site, Cambridge, Massachusetts. The small, formal beds contain several plants visited by male and female *A. manicatum*.

individual-specific color combinations of Testor's enamel paint (**Fig. 3.5**). After marking, the bees were released together into a temporary, nylon mesh cage (approximately 35 X 35 X 61 cm) for transportation to the *Anthidium* enclosure. All bees were introduced to the enclosures on the same days they were captured.

Mirror experiments and male aggression

To test the hypothesis that visual stimuli are sufficient to initiate aggressive behavior, I mounted a large (76 X 100 cm) household mirror approximately 25 cm from a *Nepeta* bush located on the Tufts University campus (**Fig. 3.6**). For two hours, I monitored both male and female reactions to the mirror, recording them in a non-quantitative manner. These experimental observations were limited to a single sunny day, 30 July 2010.

Data collection and analysis

Between 21 June and 27 July 2010, I surveyed each trap nest at least twice weekly with a small flashlight to record the presence or absence of plant pubescence, an appropriate proxy for nest site initiation. Trap nests that contained pubescence at any point during the season were scored as "utilized" in the final trap nesting database. These data were then analyzed using chi-squared tests (or, in cases in which "expected" values were less than 5, Fisher's exact test for goodness-of-fit). All analyses were carried out using StatXact 9® software (Cytel 2010).



Fig. 3.5 Color marking: Each individual was labeled with three dots of Testor's enamel paint applied directly to the dorsal surface of the mesosoma; these unique color codes (in this case, *yellow-green-red*) allowed for the tracking of specific individuals throughout the field season.



Fig. 3.6 An experimental mirror, positioned approximately 25 cm from a *Nepeta* bush located on the Tufts University campus in Medford, Massachusetts.

For the nest site utilization studies, each female present in the enclosure was tracked for half-hour intervals at least four times each week between 16 June 2010 and 27 July 2010. Females were associated with specific nests only when they were observed carrying plant pubescence and/or scopal pollen loads for deposition inside those nests.

3.4 Results

Survival rate

As was the case in 2009 (Payne et al. 2010), mortality rates were high within the enclosure (**Fig. 3.7**). Nineteen (47.5%) of the 40 bees introduced were not seen alive again after their first day; many were later found dead, including one caught in a spiderweb built by an unknown species. Meanwhile, at least one of the 21 bees that survived the first day managed to escape from the enclosure; this male was later spotted patrolling a *Nepeta* bush on the Tufts University campus (approximately 300 m to the southwest of the *Anthidium* enclosure and 150 m northeast of the original capture location), still sporting the paint marks applied earlier in the season. This indicates that earlier confidence in the enclosure's security (Payne et al. 2010) may have been unwarranted.

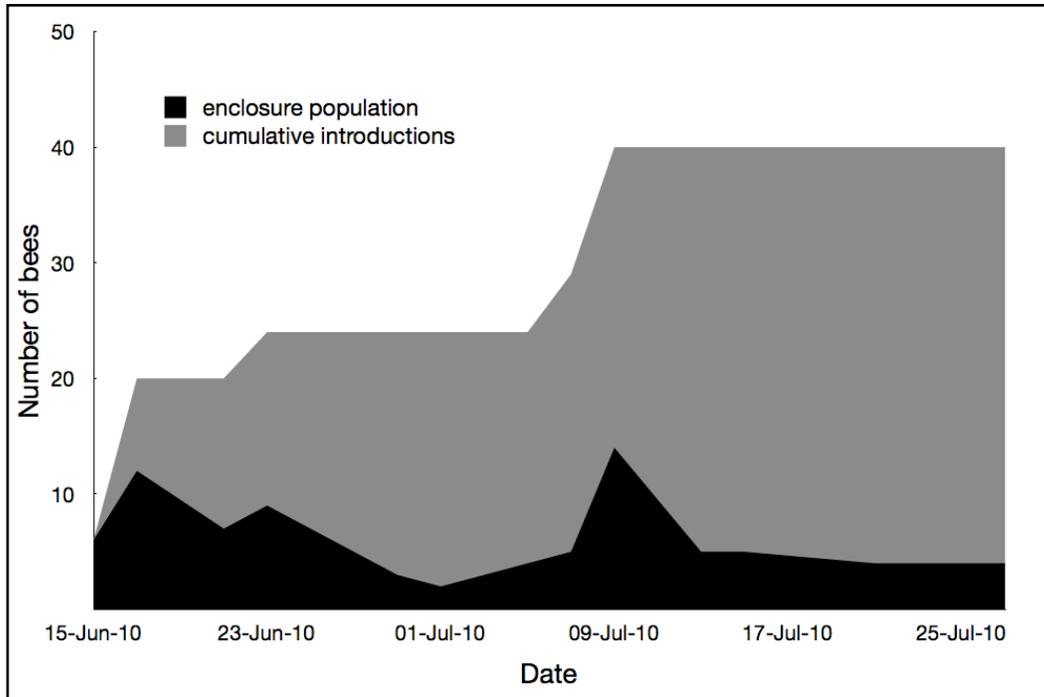


Fig. 3.7 Cumulative introductions of bees and surviving bee populations by date. We surveyed bees at irregular intervals throughout the field season to record which individuals were present inside the enclosure.

Trap nesting results

By the end of the surveys on 27 July 2010, 12 trap nests had been provisioned with plant pubescence (i.e., “utilized”) by *A. manicatum* females. On 30 July, I opened each of those nests and evaluated their contents. Most contained only a small amount of pubescence, either in the form of loose flocculence or incomplete cells; two nests contained a single complete cell each. None had more than one complete cell. No nests had been completed, and no terminal plugs begun. The contents of each nest are listed in **Table 3.2** (see also **Fig. 3.8 & 3.9**).

Trap nest utilization was non-random with respect to cavity length (Fisher’s exact test for goodness-of-fit, $\chi^2_{df2} = 4.5$, $p < 0.05$; **Fig 3.10**), with bees preferentially utilizing medium and long over short nests (combined “medium/long” versus “short,” chi-square test, $\chi^2_{df1} = 8.333$, $p < 0.005$). No other pairwise or subset comparisons (e.g., “short” versus “long,” “medium” versus “long,” “medium/short” versus “long”) revealed significant differences.

Several females delivered plant pubescence to multiple nests concurrently. At least one also delivered pubescence to a nest begun by a female that had died a week earlier (**Table 3.2; Fig. 3.9**).

Mirror experiments and male aggression

Throughout the observation period, both males and females collided repeatedly with the surface of the mirror; only males, however, displayed aggressive behavior toward their reflected images. Female collisions were



Fig. 3.8 Trap nest (A.13.2), opened on 30 July 2010, with a single unfinished cell at the extreme distal end. A dead female was found inside the trap nest, still clutching a ball of plant pubescence between her legs and mandibles.

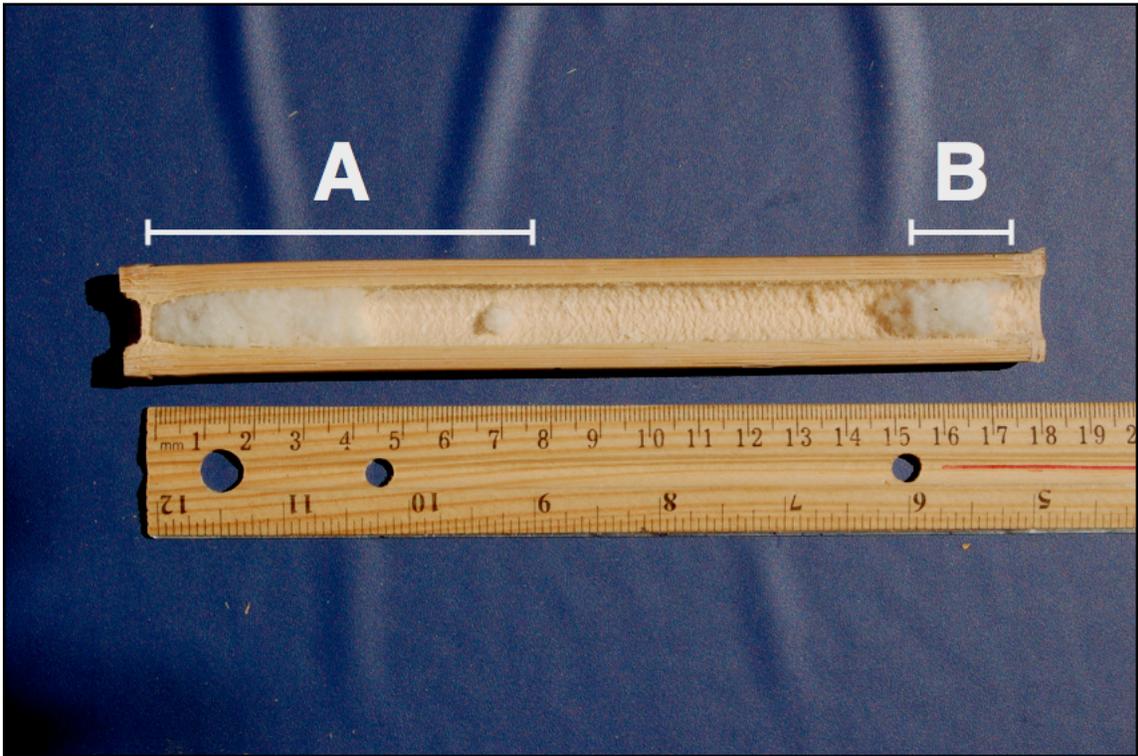


Fig. 3.9 Opened trap nest (C.13.3) demonstrating nest appropriation by *A. manicatum* females. The female that initiated the nest (single cell in **A**) on 9 July 2010 was dead by 13 July. Seven days later, another female was provisioning the cavity with pubescence (**B**).

Table 3.2 Contents of “utilized” trap nests from the 2010 enclosure study. *Distal end* refers to the closed end of the trap nest, the point most distal from the entrance. *Presumed start date* is the first time wool was observed inside the nest.

Nest	Length	Presumed start date	Minimum # of females utilizing	Trap nest contents on 30 July 2010
B.6.3	18 cm	21-Jun-10	1	Loose flocculence beginning 11.0 cm from distal end of trap nest, extending for 3.6 cm.
B.3.1	8 cm	21-Jun-10	1	Completed cell, 3.4 cm long, beginning at extreme distal end of trap nest. Contains visible pollen ball.
A13.2	13 cm	23-Jun-10	1	Single incomplete cell, 2.9 cm long, beginning at distal end of trap nest. Body of dead female bee inside, still clutching pubescence.
A12.2	13 cm	23-Jun-10	1	Very little wool at distal end of trap nest.
A.14.2	13 cm	23-Jun-10	1	Tightly packed flocculence, 4.0 cm long, but without cells. Begins 0.3 cm from distal end.
A.16.3	18 cm	28-Jun-10	1	Single incomplete cell, 3.6 cm long, starting 0.4 cm from distal end of trap nest.
C.8.3	18 cm	30-Jun-10	1	Very limited amount of wool at extreme distal end of trap nest.
C.5.3	18 cm	30-Jun-10	1	Single unfinished cell, 2.6 cm long, beginning 2.4 cm from distal end of trap nest.
A6.3	18 cm	06-Jul-10	1	Very small amount of wool, 1.9 cm long, beginning at distal end of trap nest.
C.13.3	18 cm	09-Jul-10	2	Completed cell with loose flocculence, together extending 4.1 cm, beginning at distal end of trap nest. Followed by a large gap, then flocculence starting 14.0 cm from distal end and extending 2.0 cm. Each section is the work of a different female.
C.14.3	18 cm	26-Jul-10	1	10.8 cm of loose flocculence beginning at extreme distal end of trap nest.
C.13.2	13 cm	26-Jul-10	1	Single unfinished cell, 3.2 cm long, beginning 0.4 cm from distal end of trap nest. 1.1 cm of loose flocculence begins 5.3 cm from distal end of trap nest.

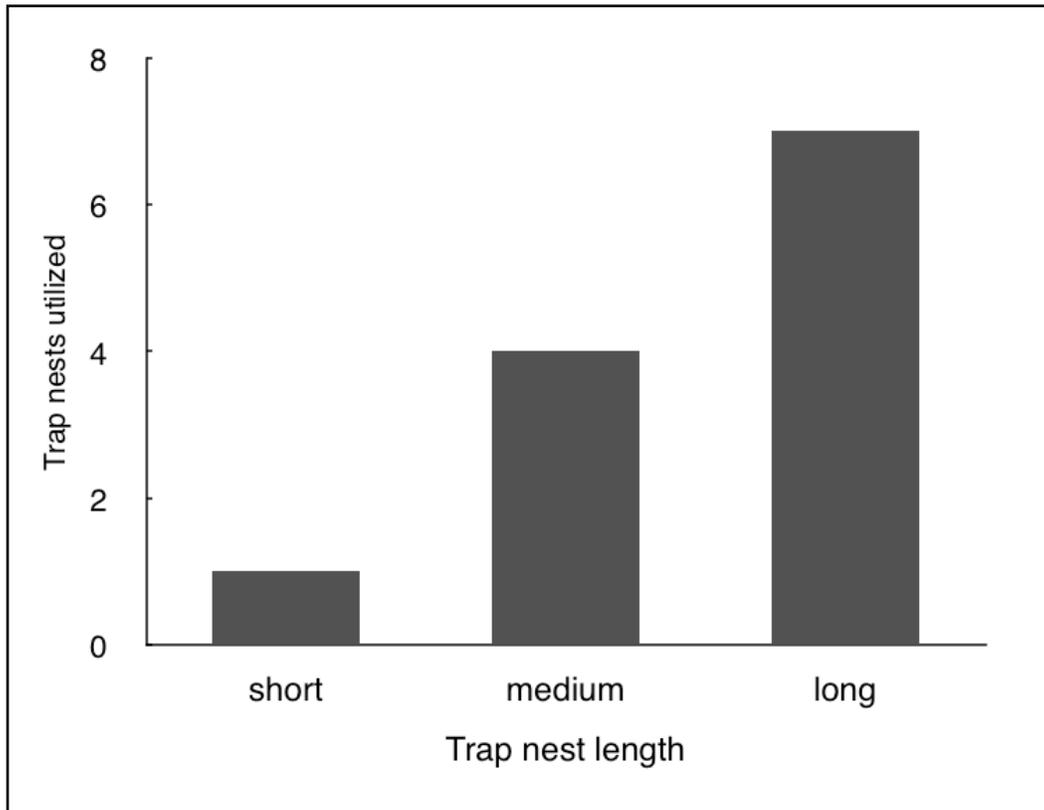


Fig. 3.10 Trap nest utilization by length (short: 8 cm; medium: 13 cm; long: 18 cm). Trap nest utilization was non-random with respect to cavity length (Fisher's exact test, $\chi^2_{df 2} = 4.5$, $p < 0.05$). This appears to be due to a preference for medium and long over short nests (combined "medium/long" versus "short," chi-square test, $\chi^2_{df 1} = 8.333$, $p < 0.005$)

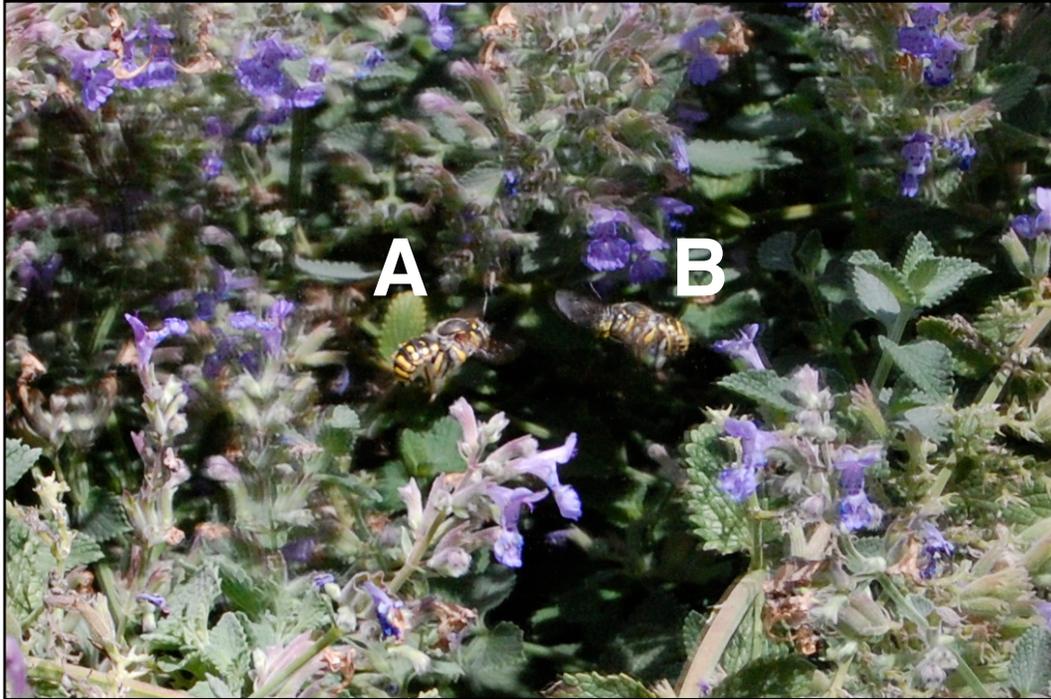


Fig. 3.11 Male *A. manicatum* (A) confronts its own reflection (B) in a large household mirror.

presumably the result of attempts to reach flowers reflected in the mirror's surface.

Males hovered in front of their reflected images and made repeated attempts to grapple with them (**Fig. 3.11**). Each of the males observed ($N = 2$) eventually gave up the fight, temporarily leaving the floral territory and presumably ceding it to the pseudo-rival.

3.5 Discussion

Despite extensive modifications to the I.S.I.R.F. *Anthidium* enclosure, mortality remained a serious concern throughout the 2010 field season. Behavioral observations suggest that poor plant performance may be mostly or entirely to blame; despite strong vegetative growth, the plants never produced the number of flowers necessary to maintain a sizable captive population. This is particularly frustrating, of course, given the efforts made here to enhance growing conditions through updated watering and lighting systems. Future researchers are thus encouraged to first ensure adequate floral production before beginning any enclosure-based study of *A. manicatum* behavior.

Regardless of the limitations imposed by high mortality, females in this study did manage to initiate several nests within the enclosure. While none of those nests were completed, it seems clear that females “intended” to finish them; harvesting plant materials and depositing them inside trap nests is a costly activity, and one that implies a high investment in the future of the site. Given that

females had a large number of potential sites to choose from, non-random patterns of nest site utilization should indicate discrimination among the available options. Statistical analyses (chi-square and Fisher's exact tests) reveal that non-random patterns do exist with regard to nest site length (i.e., cavity depth), at least under these conditions.

Among females initiating nest sites within the enclosure, most deposited plant pubescence in more than one trap nest concurrently. In some cases, bees made provisioning visits to widely separated trap nests on the same day. This would seem to imply that females can maintain multiple nests at the same time, and that they perhaps engage in similar behavior under natural conditions. Caution is necessary, however, when extrapolating enclosure-based results to wild populations. It is unclear, for example, whether wild females are presented with anywhere near the number of potential quality nest sites available inside the I.S.I.R.F. enclosure. Likewise with female appropriation of in-progress, abandoned nest sites; while I observed a single female depositing pubescence inside a previously-used trap nets, this may not represent a common behavior in nature. After all, wild female *A. manicatum* may encounter in-progress nest sites so infrequently that appropriation rarely, if ever, becomes a possibility.

Finally, preliminary results suggest that visual stimuli, specifically the form and visual pattern of a pollinating insect in flight, are both necessary and sufficient for the initiation of aggressive behavior by *A. manicatum* males. Again, however, caution is necessary when interpreting these results. Interpretations of

behavioral patterns can be highly subjective, and researcher bias can lead to differing interpretations of the same observations (Pfungst 1911). More experiments are thus necessary before we can say for certain that males demonstrate the same behavior toward their reflected images as they do toward each other.

Chapter IV

Conclusions and future directions

The results of these studies demonstrate: i.) that female *Anthidium manicatum* overwhelmingly prefer to initiate nests in high areas; ii.) that significant size differences exist between geographically distinct non-native populations; iii.) that visual cues may be necessary and sufficient for males assessing territorial challenges; iv.) that females are capable of initiating and maintaining multiple nests in parallel, and v.) that females can appropriate in-progress nests abandoned by conspecifics. They also make a strong case for the use of enclosure methods in the study of solitary bees, while at the same time addressing potential shortcomings and drawbacks (e.g., high mortality).

Of course, much work remains to be done. At the moment, nothing is known about the genetic structure of *A. manicatum* populations, including the distribution of paternity among males, the degree of genetic differentiation among local populations, and the allelic composition of widely disjunct populations within the species' non-native range. The development of microsatellite primers, either through ground up library development or via appropriation from related megachilid species, would go far toward meeting those challenges.

Meanwhile, refinements to the enclosure methods outlined above could result in researchers maintaining multiple successful generations reared entirely under experimental conditions. Once that system is in place, researchers would be

able to manipulate several environmental variables (temperature, food abundance, host plant availability, breeding male size) to determine their effects on offspring size, female fecundity, sex ratio, and many other evolutionary metrics.

The author hopes that the research described here, in conjunction with the preceding discussion of future directions, will serve to encourage more work on *A. manicatum* in particular, and solitary bees in general.

Appendix A

Recognition systems in the social insects

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Synopsis: Constrained by definitions and limited in scope, the study of recognition remains an undeveloped science. A growing number of researchers are now abandoning these constraints in favor of a holistic, “wide-angle” approach to recognition. Here we illustrate this approach with examples from the lives of the social insects and demonstrate how ants, honeybees, wasps, and termites rely on recognition systems to survive and reproduce. Specifically, we show how the wide-angle view frames our study of four seemingly unrelated behaviors: selecting nest sites, choosing mates, recognizing relations, and detecting parasites.

Keywords: chemical mimicry, cuticular hydrocarbons, dulosis, inclusive fitness, kin recognition, mate choice, nest site selection, recognition systems, social insects

A.1: Introduction: A wide-angle view of recognition systems

Readers of scientific journals are no strangers to recognition: a recent keyword search by the authors turned up over 100,000 papers related to that subject. Of these, only a fraction were from the animal behavior literature; the balance came from journals of molecular biology, immunology, cellular medicine, or cognitive psychology. There were papers on human facial recognition and on the immune detection of viral proteins, papers on nepotism in ground squirrels and on the specificity of restriction enzymes -- papers, in other words, from almost every branch of biology. The only question was what, if anything, they had in common.

During the last fifteen years, some researchers have begun to converge upon an answer. They argue that these studies are united both by a common theme and by the need for a common framework, and that the time has come for a unified approach to the study of recognition (Blaustein & Porter 1996; Sherman et al. 1997; Starks 2004; Liebert & Starks 2004). They point out that all recognition research, no matter what the model system, is about explaining how evaluators identify and discriminate among entities. To these scientists, it makes little difference whether that evaluator is a red-tailed hawk or a human lymphocyte; both bird and blood cell have evolved to recognize significant entities accurately and efficiently. In this expanded view -- what we call the "wide-angle" approach to recognition -- all biological recognition systems are variations on a theme, a

theme best understood through a shared vocabulary and a common theoretical framework.

And yet, despite the promise of new insights and new collaborations, this wide-angle perspective remains rare. Even after well-reasoned arguments by Blaustein and Porter (1996) and Sherman and colleagues (1997), many still think of kin recognition not as a limited case, but rather as the *only* case, of animal recognition. While this may be due to poor coverage in the textbooks -- recognition is often given little more than a short mention in a chapter on kin selection -- it also owes something to long-standing inconsistencies in the technical language, inconsistencies that persist despite efforts at standardization.

In this article, we explore how students of behavioral ecology might benefit by adopting the wide-angle approach. We offer a quick review of current terms and make the case for recognition as a ubiquitous biological process. We then illustrate the approach by bringing it to bear upon the study of social insects, and by showing how four seemingly unrelated behaviors -- selecting nest sites, choosing mates, recognizing relations, and detecting parasites -- all depend on the ability to recognize and discriminate.

A.2: Describing recognition: the need for a common vocabulary.

Despite its widespread use in the literature, “recognition” remains a vague term. Almost of all of us have some sense of what it means to “recognize an opportunity” or to “recognize a face in a crowd,” but few, if pressed, could

provide a rigorous definition. What do we really mean when we say we recognize an old friend on the train? For that matter, what do we mean when we say that a honeybee recognizes olfactory cues, or that a restriction enzyme recognizes a nucleotide sequence? If we expect to get a handle on recognition systems, we must first get a handle on the terms we use to describe them.

To that end, we propose the following definition: recognition occurs whenever an evaluating entity, regardless of its level of biological organization, identifies another entity with reference to a previously-existing template. In short, the ability to recognize is the ability to identify encountered entities. While no one would argue that an enzyme identifies its substrate through the same mechanisms that we use to identify a car in a parking lot, the overall structures and the ultimate outcomes of those processes are the same. A recognition system does not require cognitive processing to achieve these goals: Neither gated channels nor restriction enzymes nor tRNA molecules require ghosts in the machines -- or in this case, in the alpha helices -- to meet the basic requirements for recognition.

Any attempt at synthesis must also designate those features that all recognition systems share. Perhaps the most obvious of these is the condition of having two or more participants, one that does the recognizing and at least one other that gets recognized. Following Liebert and Starks (2004), we call these the “evaluator” and the “cue-bearer,” respectively. These terms are particularly helpful as they replace a confusing set of words (“signaler” vs. “receiver”;

“recipient” vs. “actor”) that grew out of the exigencies of communication and kin recognition work.

The act of recognizing also means that evaluators must possess criteria against which to judge cue-bearers. Taken together, these criteria form the “template,” a term we use regardless of whether those criteria are learned or fixed. Learned templates may crystalize early on or they may require constant updates throughout the evaluator’s lifetime, but either way they only form after exposure to example stimuli, so-called “referents.” For example, one of the first things that newly emerged *Polistes* paper wasps are exposed to is the odor of the natal nest. This unique hydrocarbon signature becomes the referent by which the wasps form nestmate recognition templates, and future interactions between adults depend on how well newly encountered wasps and those templates match (see “Recognizing kin,” below).

Though we usually think of recognition as a single behavior, it is often helpful to break it down into three essential components: expression, perception, and action. The expression component refers to the production or acquisition of identity cues by the cue-bearer, the perception component to the detection and interpretation of those cues, and the action component to all those behaviors elicited by recognition on the part of the evaluator. Expression thus falls to the cue-bearer (which need not be a biological entity), while the other two are exclusive to the evaluator. Each of these components is explored in detail elsewhere by one of the authors (Starks 2004).

There is one more subtle distinction in the terms used to describe recognition systems, namely the difference between “discrimination” and “recognition.” While authors have sometimes used them interchangeably, the former properly refers to observable behavioral changes, while the latter refers only to an invisible process occurring inside the evaluator. In other words, only one, discrimination, is amenable to traditional behavioral analysis. Some organisms almost certainly recognize objects or other individuals without outwardly changing their behavior, but short of brain imaging assays or EEGs, we have no way of knowing for sure. (These techniques are, however, becoming more common. See the discussion of pheromone detection in “Recognizing mates” below.)

In the next sections, we show how these terms apply to some behaviors of the social insects and how the wide-angle view helps us make sense of those behaviors.

A.3: Evaluating nest sites

This is a common enough scene in the summertime forests of North America: a swarm of honeybees, *Apis mellifera*, is dangling from the high branches of a tree, a compact mass made up of thousands of homeless workers. Hours before, this swarm split from its parent hive, leaving the old nest in the hands of a young queen and striking out for a new place to call home. Now it

hangs in a moment of indecision, exploring its surroundings, weighing its options, and figuring out where to begin the task of building a new colony.

This decision is not to be taken lightly. Many hives do not survive their first year, and much of their success or failure depends on finding a quality nest site. But how do the bees even begin to decide? The forest and surrounding fields are full of potential homes: every tree cavity, every old stump, every farmer's empty bee box is a possibility. To complicate matters, most workers are inexperienced at choosing real-estate; hardly any of them are more than a few weeks old, and for many the swarm marks the first real journey into the outside world. What's more, this corporation of workers must arrive at a decision without the help of central management because the queen, buried deep within a writhing mass of bees, is in no position to affect the process.

So how to begin? First, the scout bees, the workers charged with finding the new nest site, must know what to look for and how to rank the cue-bearing sites they encounter. In other words, it must possess a nest template. Seeley and Morse (1976) first investigated this template in the late 1970s by evaluating the nest sites of feral bees in the forests around Ithaca, New York. They found that bees tended to prefer tree cavities that fell within a circumscribed range of volumes, usually between thirty and sixty liters, and that they tended to choose cavities with small exterior openings over those with larger entrances. The data did not imply any preference for one tree species over another, and suggested only a small preference for living versus dead trees.

Following their forest observations, Seeley and Morse (1978) designed a series of experiments to see what other cues might influence nest site preference. The researchers built a series of side-by-side nest boxes, each modified according to a single variable, and presented them as choice experiments to bee swarms. Scout bees would encounter both cue-bearing boxes at the same time, evaluate the cues with reference to the nest site template, and then convince the swarm to settle in one of the two boxes. When one type of box was chosen significantly more often than another, the researchers interpreted this as evidence of a preference.

The results showed that scout bees possess far more complicated templates than one might expect given their small brains. As it turns out, the scout bees were looking not only at cavity volume and entrance diameter, but also at nest height (higher boxes were preferable to lower ones), distance from the previous nest (somewhere between 400 and 1000 meters was ideal) and nest entrance direction (south-facing was better than north-facing). Data for nest entrances supported the previous observation that bees preferred small over large holes, but the bees showed no preference when it came to the shape of the entrance. Meanwhile, the position of that entrance, either near the bottom or the top of the nest box, did seem to matter, while the bees showed no preference when it came to cavity shape, dryness, or draftiness. Through careful observation and well-designed experiments, Seeley and Morse were beginning to shed light on the nature of the honeybee nest template.

Of course, there is more to the perception component of a recognition system than just the template; on an even more fundamental level, evaluators must first be able to perceive identity cues. In another project, Seeley (1977) investigated the proximate mechanisms behind the scout bees' ability to perceive differences in nest site cavity volume. Clearly, the bees were able to accurately and consistently discriminate between cavity sizes, but the means by which they did so were far from clear. Seeley had previously observed that the bees spent a great deal of time walking and flying around the interior of the nest cavity; through a series of elegant experiments, he was able to demonstrate that this movement was actually a method for measuring and calculating interior volumes. When the bees were deprived of visual information by means of a light baffle over the cavity entrance, they were still able to choose those nests closest to the ideal volume. However, when Seeley placed rotating cylinders inside the nest boxes and created a sort of treadmill for incoming bees, he was able to confuse their spatial assessments. Regardless of whether the bees were forced to do more or less walking than the cavity size required, their perceptions of volume correlated to the distances they walked, and not to the real size of the cavity. Thus Seeley showed that the bees measured not with their eyes, as one might expect, but rather through a complex calculus of distances walked and angles turned.

Recent studies by Seeley and colleagues (Seeley & Burhman 2001; Seeley & Visscher 2003; Seeley & Visscher 2004) have begun to shed light on the action component of nest site recognition, the decision making process that leads a

swarm to move into a site. Removing colonies to a mostly treeless island off the coast of Maine, the researchers offered scout bees their choice of several nestboxes, only one of which was a high quality site. Previous studies had demonstrated that individual scouts visit only a single site, evaluate it thoroughly, and then return to the hive to share their assessment with the other workers. The scout bees then advertise their site through a series of waggle-dances containing information about both the location and the quality of the cue-bearer. Stronger dances lead more bees to investigate the sites, until eventually the swarm becomes a miniature political convention, each faction dancing in support of its nest site choice. When a quorum is reached, the scouts begin to make faint piping noises, and the swarm takes off in the direction of their new home.

The problem of finding a nest site is not, of course, limited to bees. Starks (2003) investigated some of the same questions using European paper wasps, *Polistes dominulus*, and found that they too recognize quality nest sites. Given a choice between long, medium, and short nest boxes, the wasps preferred to initiate nests in the medium ones. While several foundresses started nests in the small boxes, almost none chose the large ones, perhaps because more exposed sites raise the risk of predation by birds or brood parasites. Unlike honeybees, however, these wasps had no preference for higher versus lower nest sites, and settled equally often into boxes at all elevations in the enclosure. Thus, as expected, recognition templates vary between species and almost certainly reflect the unique selection pressures faced by each.

As an interesting side note, Starks found that female wasps emerging from hibernation preferentially stopped to perch on fragments of the nests they were raised on, even when those fragments had been moved from their original sites. Since these nest materials were in new locations and had been cut up into smaller pieces, the wasps must have used chemical cues to discriminate between them. It is well known that wasps acquire hydrocarbon signatures from their nests and use those signatures as referents when creating nestmate recognition templates; Starks suggests that returning to the nest may be a way of reconnecting with the previous season's sisters or of updating a learned template by new exposure to the original referent. Either way, returning to the natal nest may be a proximate mechanism by which wasps facilitate cooperation between kin, a behavior that is facilitated in turn by the ability to recognize.

Despite advances such as these, we still have much to learn about the expression, perception, and action components of nest site recognition systems. While we now know that honeybees possess a nest cavity template that includes ideal measurements of volume, entrance size, and height, we know practically nothing about the development of that template. Somehow, by the time scout bees are searching the landscape for appropriate nest cavities, they have developed a Platonic vision of their ideal home; how they and other insects are able to form such templates, sometimes in the absence of clear referents, is an exciting area for future research.

A.4 Identifying mates

Among the social insects, sex tends to be a limited affair. For the workers who make up the bulk of any given colony, life is a chaste, and often brief, exercise in altruism. That much is to be expected. But even among reproductives -- the winged queens and frenetic males that fill the skies in early spring and autumn -- the mating season is often little more than a brief interlude in a longer life cycle.

Of course, we must not mistake brief for boring; more often than not, these mating periods are every bit as extravagant as they are short. Reproductive female paper wasps fly out to meet some of their male counterparts on high, well-lit structures where the latter congregate en masse. Honeybees meet each other high above the ground where males hone in on and mate with polyandrous queens mid-flight. Winged reproductive ants rendezvous in swirling, carousing clouds, several hundred strong, and naturalists lucky enough -- or unlucky enough -- to wander into them do not soon forget the experience. In the high stakes game of finding a mate under such chaotic conditions, recognition is a critical component of fitness.

This is especially true for males. With the exception of one genus of ant (*Cardiocondyla*, see below), all social Hymenoptera species seem to have sperm-limited males with life expectancies that are significantly shorter than their queens'. Once the males' sperm supplies are exhausted, so too is their biological relevance, and selection seems to favor individuals who bow out gracefully (though "gracefully" may be too charitable a word for a species like the

honeybee: its drones are famous for explosively rupturing immediately after they mate.) Honeybee males not only mate just once before they die; they also have just one *opportunity* to mate, a single high altitude flight in which they must compete with hundreds of other males to find and inseminate a new queen. It makes sense for them to maximize that opportunity and to locate receptive females as quickly as they can.

To this end, selection has favored honeybee males with large eyes and large antennae to serve as well-honed, queen detection devices. Recent studies by Wanner and colleagues (2007) investigated the perception component of queen recognition and showed that drones possess sex-specific odorant receptors that respond to a chemical (9-oxo-2-decenoic acid, or 9-ODA) found in the queen retinue pheromone. Meanwhile, using calcium-imaging techniques, Sandoz (2006) demonstrated that brain regions found only in the males respond specifically to this and to two other components of the queen pheromone. These long distance chemical signals, along with a highly developed detection apparatus, help drones perceive flying cue-bearers long before they can detect them visually. Their sterile sisters, meanwhile, recognize and respond to some of the same chemicals, but with an entirely different behavior: in their case, recognition of 9-ODA leads them to gather around the queen and to suppress the development of their ovaries. The same expression component, the production and emission of 9-ODA, leads to two very different action component outcomes in the honeybee.

While males have a lot invested in finding females, they do sometimes misinterpret cues. An interesting example comes from *Cardiocondyla obscurior*, an ant species that exhibits strong dimorphism within the male population of each nest. Some males are wingless, or ergatoid, individuals who stay within their natal nests, mate with their relatives, and produce sperm throughout their lives. Others are winged, sperm-limited dispersers who mate with the same females as the ergatoids, but who also leave the nest to mate. Interestingly, while ergatoid males show extreme aggression toward each other and often kill new males before they emerge, they are highly accepting of the winged males (Anderson et al. 2003). In fact, they not only tolerate them, but also frequently mount them in attempts at copulation. Cremer and colleagues (2002) have recently shown that the wingless males escape the lethal aggression of their nestmates by mimicking the chemical signatures of virgin queens. By covering themselves in mimicked cues, these ants are able to fool the ergatoid recognition system, thus escaping aggression while still competing for mates.

While hymenopteran males must do everything they can to maximize their reproduction, most of them do not, at least, have to live with the consequences. Not so their newly-inseminated queens, who can live years or even decades longer than their mates. While some species, notably the honeybee and some bumblebees, are able to mate multiply with different males, many hymenoptera appear to be monandrous and to store their sperm for life. This leads to the curious instance, rare among animals, of sperm outliving the males that created

them. It should also lead to a great deal of pickiness on the part of the females, and to the development of highly precise mechanism for recognizing quality males. Unfortunately, the high-altitude nature of the nuptial flights means that we know far less than we would like about female mate choice in these species. Baer (2003) provided tantalizing details in a recent review of bumblebee male sexual selection -- apparently females in laboratory settings show a great deal of choosiness among potential mates and occasionally sting undesirable males to death -- but much of the necessary research remains to be done.

For termites, lifetime mating and the continuous production of sperm create a different set of selection pressures, but the need to recognize and evaluate potential mates remains the same. In a recent study of the dampwood termite, *Zootermopsis nevadensis*, Shellman-Reeve (2001) demonstrated that they were able to recognize and avoid close relatives during laboratory mating trials. Although termites often replace dead mates with offspring or other close relatives, they appear to avoid such inbreeding during initial colony formation.

As a result of their haplodiploid reproductive system, some hymenopteran species suffer disastrous consequences after inbreeding. When these females mate with close relatives, they increase their chances of producing diploid offspring that are homozygous at the complementary sex-determination locus; since heterozygosity at the *CSD* is required to develop as a female, these individuals grow up to become genetically abnormal males. If they do not die early or get identified and removed from the nest (as in honeybees), they may mate with

females, produce sterile triploid offspring, and thus increase the colony's genetic load. We know that several of these species demonstrate some form of kin recognition and so we expect some of them to recognize and avoid close relatives when choosing mates. Intriguingly, this kin discrimination may relax under extreme circumstances, such as those found in the genetic bottlenecks associated with invasions. A study by Keller and Fournier (2002) tested whether or not non-native French populations of Argentine ant *Linepithema humile* avoided inbreeding with siblings. They concluded that these individuals did not recognize their siblings in mate choice situations, but suggested that this might not be true of the populations in the native range.

Of course, inbreeding avoidance is just one way that animals benefit from the ability to recognize their kin. In the next section, we look at some more ways that the social insects rely on these abilities to survive, reproduce, and maintain their fitness advantages.

A.5 Recognizing kin.

Few evolutionary quandaries have caused as much puzzlement, or as much grief, as the question of how altruism persists in a Darwinian world. Consider, for instance, how long it took for an elegant and, more importantly, a mathematically rigorous solution to emerge. In the early 1960s, over a hundred years after Darwin hinted at it in the *Origin*, William Hamilton (1964a; 1964b) published his vision of inclusive fitness, a gene centered view of natural selection that explains why

some animals sacrifice so much for their relatives. The concept is simple: Hamilton argued that an altruism promoting gene could spread through a population so long as it caused organisms to preferentially direct care toward their relatives. If an organism sacrifices its own fitness, and if that sacrifice means that more copies of the sacrifice-inducing gene wind up in the next generation via the reproduction of close relatives, then the altruistic gene has an evolutionarily successful strategy. Inclusive fitness is the lens that clarifies, and allows us to see for the first time, the true nature of altruism.

Hamilton also suggested that if an animal could recognize its relatives and then discriminate between them and other individuals, then that ability to recognize would play a central role in the evolution of altruism. In the decades since the debut of inclusive fitness, behavioral ecologists have confirmed that kin recognition exists in multiple species and that it seems to be adaptive in ways predicted by theory. In fact, the recognition system framework described in this article largely grew out of that work and has blossomed most fully within it; while the examples that follow are covered by the authors elsewhere in this volume, it is worthwhile to briefly revisit them here in the context of social insect recognition.

Many insects are, it turns out, surprisingly smelly creatures -- at least to other individuals of the same species. Most of the scent cues studied so far belong to a class of molecules found embedded in the waxy outer surfaces of their exoskeletons and known to biochemists as cuticular hydrocarbons, or CHCs. These molecules have proven to be quite diverse, and the normal variability found

between species and even between individuals is sufficient to provide high resolution signals of identity. As such, CHCs are well suited to act as the cues by which insects recognize differences between kin and non-kin.

As predicted, researchers have indeed uncovered a central role for CHCs in the recognition systems of social insects. A classic example comes from the work of Gamboa and his colleagues on recognition in the primitively eusocial *Polistes* paper wasps (reviewed in Gamboa 2004). Females of the temperate species emerge in the spring and found nests dominated by a single reproductive queen; often this queen also receives a great deal of help from non-reproductive assistants who do everything from collect food to defend and enlarge the nest. By Hamiltonian logic, this kind of altruistic behavior makes little sense unless it is directed toward close relatives and leads to an increase in the helper's inclusive fitness, even as she reduces her personal fitness. Paper wasps, like most other animal altruists, would do well to tell relatives apart from other reproductive females.

This discrimination between kin and non-kin, or more accurately between nestmates and non-nestmates, is based on recognizing CHC cues specific to wasps from the same natal nest group. While there is some evidence to suggest that genetics may play a role in these chemical signatures, by far the most important influence appears to come from the papery nest itself. *Polistes* wasps are much more likely to accept individuals that eclosed on their own nest than they are to associate with foreign wasps, even if those foreign individuals are more

genetically related to themselves. Experimental manipulation of larval origin supports these findings and shows that wasps preferentially associate with nestmates even when overall relatedness is low. The nest carries the cues which, when acquired by a female, provide a passport to interacting with nestmates found off the original nest. While this kin recognition system is clearly susceptible to error, nestmate status is probably linked to kinship status often enough in nature that nest origin serves as a decent proxy.

There are at least two proximate mechanisms at work in *Polistes* nestmate recognition: first, the acquisition of cues by newly eclosed wasps (an expression component mechanism), and second, the learning of cues and the formation of a nestmate template by evaluator wasps (a perception component mechanism). For the expression component, each wasp appears to acquire and then bear the specific odor cues of the nest material on which it was raised; this means that while odor cues are homogenized across nestmates, no meaningful intra-colonial distinctions can be made on the basis of CHC dissimilarity. Experiments bear this out, and demonstrate that wasps appear to follow an “all or none” rule when encountering previously unmet individuals: when other wasps smell like nestmates, they are accepted as kin, but when they do not, they are treated as intruders and often met with aggression.

When it comes to the perception component, wasps seem to develop their nestmate templates in much the same way that they develop their own chemical cue profiles, namely through exposure to the natal nest. The nest odor itself

appears to be a mixture of environmental and wasp-based odors, and taken together these cues form the referent on which the nestmate recognition template is based. In this way, an inanimate referent (the nest) ends up having a profound effect on the way a wasp evaluates and discriminates between animate cue-bearers (other wasps).

Of course, this particular model of cue expression and template formation is not applicable across all social insects: that polyphyletic taxon is simply too diverse to allow for generalizations. Ants, for instance, seem to have evolved an array of different expression mechanisms that result in nest- or colony-specific CHC profiles. In some groups, for example in species of the genus *Camponatus*, the queens appear to be the sole source of colony-specific odors; in others, e.g. *Cataglyphis iberica* and *C. niger*, the queen has little influence on the cues, and the colony's odor is derived instead from a "gestalt" mixture of individual worker produced chemicals. For the vast majority of species, the mechanisms remain unknown; while research continues, the only conclusion we can draw so far is that there are no easily generalizable conclusions.

It appears that insects need not rely solely on chemical cues to recognize differences between conspecific individuals. To return to *Polistes* wasps, Tibbetts (2002) studied the mechanisms by which females identify individuals and maintain dominance hierarchies. After observing that individual brown paper wasps, *P. fuscatus*, varied greatly in individual facial markings, she decided to modify these marking with paint and then observe how other wasps responded.

All focal individuals received paint treatments on their faces, though those treatments did not effect the previously existing patterns on control wasps. As it turned out, individuals with altered facial patterns received more aggression from their nestmates than the controls did. None received aggression of the sort that would be used against non-nestmates -- presumably because they still bore chemical cues that tied them to that specific nest -- but they did lose their places in the dominance hierarchy and had to fight to regain positions of power. After a short span characterized by aggressive interactions, the wasps sorted themselves back into the previous dominance order, apparently familiarizing themselves with the new facial patterns. It seems that the wasps have flexible and updatable visual templates for who is in charge in the colony; when the disconnect between facial cues and other indicators of status, such as aggression and fighting ability, becomes too great, the wasps learn to look for a different set of cues.

While the existence of visual templates raises intriguing questions for future research, chemical recognition still seems to be the sine qua non of insect kin recognition. It also plays a vital role in another set of recognition systems, systems that have evolved to protect those resources that social insects work so hard to accumulate.

A.6 Avoiding parasites.

Social insect colonies are centers of wealth in the insect world; with hidden food stores, nutritious larvae, and armies of committed workers, they are cities

ripe for pillaging. The committed workforce is particularly appealing, and often essential, to a certain type of insect opportunist, the so-called social parasite. These organisms steal workers and occasionally the entire nests of other species, and use those resources to benefit their own offspring. But such coups require a certain delicacy. Most social insects have highly developed mechanisms by which to recognize and discriminate against other species, and the successful parasite must somehow find a way to subvert these systems. The means with by they do so are the focus of this section.

While social parasites exist in many different groups of insects, nowhere have they evolved to such an extraordinary degree as in the ants. In some of these species, the so-called dulotic or slave-making ants, workers routinely leave their own nests to make raids on the pupae of neighboring species. The captured pupae are returned to the slave-makers' nests, where they emerge as adults and, using their new masters as referents for template formation, quickly accept their faux nestmates as kin. Thus, an action component behavior suitable under normal conditions becomes terrifically maladaptive after a recognition error.

In one genus of dulotic ants (*Polyergus*), the slave-makers are so highly-specialized to their raiding tasks that they have lost most of the abilities required to take care of themselves, and have thus become obligatory social parasites. *Polyergus* species often raid the nests of closely related *Formica* species, returning the pupae to eclose inside their own colonies; occasionally, however, they have been observed to enter queen-right host colonies, kill the host queen,

and usurp her position among the workers. Recent work by Tsuneoka (2008) investigated the nature of these usurpations by the Japanese pirate ant, *Polyergus samurai*, against its host, *Formica japonica*.

Tsuneoka introduced *P. samurai* queens into *F. japonica* colonies raised in the laboratory and observed the parasite queens' behaviors under three experimental conditions: in queen-right, queenless, and workerless host colonies. Under none of these circumstances did the slave-making queen physically attack her host workers; instead, in both the queen-right and queenless colonies, she was able to stave off *F. japonica* attacks by raising her gaster and presumably emitting behavior modifying pheromones (which immobilized and sometimes killed host workers). Within a few hours, the host workers gradually ceased to attack the parasite and instead accepted her presence.

The relatively low level of aggression directed toward host workers was not seen in interactions between the queens in worker-filled colonies. When *P. samurai* females entered queen-right nests, they attacked the *F. japonica* queen immediately upon contact, grasping and biting the host with their mandibles. During these attacks, the parasite queens also directed their gasters toward the host queens, and spent some time after the attack licking their victims and grooming their own bodies; shortly after, the workers gradually accepted the new queen. In queenless colonies, meanwhile, *P. samurai* queens appeared to have more difficulty usurping colonies. Finally, in workerless colonies, most

introduced queens simply ignored the host queens, presumably avoiding conflicts in which there were no workers to be gained.

All of this adds up to a picture of *P. samurai* as a highly-skilled chemical cue mimic, a not uncommon type among the social parasites. Indeed, some social insects seem to use similar methods against their own species: the Cape honeybee, *Apis mellifera capensis*, has become a major pest for South African beekeepers who rely on colonies of its host, *A. m. scutella*. The workers of *A. m. capensis* are unusual among honeybee subspecies in their ability to lay diploid female eggs via asexual reproduction; most honeybee workers are only able to lay haploid male eggs and even then only successfully in colonies without queens. In queen-right colonies, workers usually police each other's egg-laying and remove eggs that they have not laid themselves. Thus, not only have Cape honeybees managed to evolve a novel method of asexual reproduction, but they have also developed a way to avoid detection by African honeybee workers intent on policing. Presumably they do so by means of chemical mimicry of queen-laid eggs.

Finally, the mixing and acceptance of heterospecifics are not limited to parasitic relationships. Mixed colonies of ants often live together in fungus gardens, symbiotic associations between ants and certain species of epiphytes; Orivel and colleagues (1997) investigated one such relationship between two species of ant, *Crematogaster limata parabiatica* and *Odontomachus mayi*. These two species coexist peacefully inside their ant gardens, sharing food resources and odor trails, but keeping their broods separate. Remarkably, the relationship

between these species goes beyond tolerance of each other to the exclusion of any individual not associated with the ant garden. Indeed, both species will readily attack members of their own species if these individuals come from outside the shared nests. Equally remarkable is that each of these species manages to maintain distinct hydrocarbon profiles throughout their association. This implies that these ants are able to build recognition templates using the individuals around them as non-exclusive referents and, in this respect, they share some aspects of their behavior with those ants that fall prey to dulotic parasites.

A.7 Conclusions.

This chapter provides only a limited tour of the many recognition behaviors performed by social insects; it does not address the identification of foreign debris within the nest, the recognition of specific floral odors, or the discovery and removal of diploid male larvae, to name but a few. Nevertheless, each of these -- and scores of other behaviors -- are best understood as acts of recognition.

The recognition systems framework first developed to study kin recognition is still the best way to approach these topics. Future research will no doubt reveal even more applications, and will hopefully explore the mechanisms behind template formation, cue expression, and behavioral modification in even more detail.

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