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Review article

Propolis and bee health: the natural history and significance of resin use by honey bees*

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Abstract – Social immunity, which describes how individual behaviors of group members effectively reduce disease and parasite transmission at the colony level, is an emerging field in social insect biology. An understudied, but significant behavioral disease resistance mechanism in honey bees is their collection and use of plant resins. Honey bees harvest resins with antimicrobial properties from various plant species and bring them back to the colony where they are then mixed with varying amounts of wax and utilized as propolis. Propolis is an apicultural term for the resins when used by bees within a hive. While numerous studies have investigated the chemical components of propolis that could be used to treat human diseases, there is a lack of information on the importance of propolis in regards to bee health. This review serves to provide a compilation of recent research concerning the behavior of bees in relation to resins and propolis, focusing more on the bees themselves and the potential evolutionary benefits of resin collection. Future research goals are also established in order to create a new focus within the literature on the natural history of resin use among the social insects and role that propolis plays in disease resistance.

Apis mellifera **/ social immunity / antimicrobial defense / ecological immunity**

1. INTRODUCTION

Social immunity, which describes how individual behaviors of group members effectively reduce disease and parasite transmission at the colony level, is an emerging field in social insect biology (Cremer et al., 2007; Cremer and Sixt, 2009; Wilson-Rich et al., 2009). This phenomenon is widespread across the social bees, ants, wasps and termites. The behaviors range from more common acts like grooming of nestmates (i.e. in termites, Rosengaus et al., 1998) and removal of dead material from the main nest area (i.e. in ants, Currie and Stuart, 2001; Hart et al., 2002) to "social fever" in honey bees that is used to kill pathogens (Starks et al., 2000) and the detection and removal of pre-infectious diseased or parasitized brood (hygienic behavior in honey bees; Rothenbuhler, 1964; reviewed in Wilson-Rich et al., 2009). Since social insects generally live in large groups of constantly interacting, related individuals, there is an increased risk of disease outbreaks and evolution of specialized parasites (Schmid-Hempel, 1998). In light of this, the finding that honey bee immune pathways have a decreased number of family members or paralogs as compared to other non-social insects with complete genomes was surprising, as it indicates that honey bees may have reduced individual mechanisms of physiological defense (Evans et al., 2006). It is interesting to consider the suite of behavioral mechanisms or other traits that may have evolved at the individual and colony levels to compensate for this (Evans and Spivak, 2010).

One possible mechanism of social immunity in honey bees is the collection and in-hive use of resins, complex plant secretions with

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diverse antimicrobial properties. Honey bees harvest resins from various plant species and bring them back to the colony where they are then utilized as propolis (propolis is an apicultural term for the resins when used by bees within a hive). The harvesting of antimicrobial compounds (resins) from the environment and their incorporation into the social nest architecture as propolis is an exciting but relatively unexplored colony-level defense against pathogens. Much of the current literature concerning propolis has focused on the chemical constituents and biological activity of propolis and the botanical origins of the resins from which the propolis mixtures are derived (see Banskota et al., 2001; Bankova et al., 2008). Although this work is certainly interesting due to the pharmacological benefits to humans that may be available by better understanding these compounds, we still remain largely unaware of the benefits of resin collection to honey bees and the basic mechanisms that drive resin foraging at both the individual and colony levels. This review serves to provide a compilation of recent research concerning the behavior of bees in relation to resins and propolis, focusing more on the bees themselves and the potential evolutionary benefits of resin collection and not on chemical analyses of propolis and plant resins or implications for human health.

2. RESIN COLLECTION AND PROPOLIS USE BY HONEY BEES

Honey bees use propolis in varying degrees, some species and races rely very little on the substance, while others use resins and propolis extensively (Butler, 1949; Crane, 1990; Page et al., 1995). In fact propolis can be replaced by wax in honey bee colonies (Meyer, 1956; Crane, 1990). Colonies of *Apis dorsata*, the giant honey bee, may use resin occasionally to strengthen the site of comb attachment on a branch, while *A. cerana* colonies are not thought to use resins at all (Seeley and Morse, 1976; Crane, 1990). On the other hand, resins are thought to be essential to *A. florea* (the dwarf honey bee). To prevent ants from invading their exposed nests, *A. florea* places a ring of resin on the branches leading to a nest (Crane, 1990; Seeley et al., 1982). Very limited information exists on the use of resins by these Asian species of honey bees.

Use of resins by *A. mellifera* colonies is much more widespread. While there is considerable variation among colonies in resin collection and propolis use, all colonies do appear to use at least some (Seeley and Morse, 1976; Page et al., 1995; Manrique and Soares, 2002; M. Simone-Finstrom, pers. obs.). A feral colony nesting in a tree cavity coats the entire inner walls with a thin (0.3 to 0.5 mm) layer of propolis forming what has been termed a "propolis envelope" around the nest interior (Seeley and Morse, 1976; Fig. 1). Propolis is continually added to the nest walls during colony development, and is first placed at areas prior to comb attachment, which not only creates a clean, smooth surface, but may also reinforce new comb (Seeley and Morse, 1976; Visscher, 1980). Both feral colonies in tree cavities and domesticated colonies in commercial hive boxes, generally use propolis for covering holes and crevices in the nest, and narrowing the hive entrance (Huber, 1814; Haydak, 1953; Ghisalberti, 1979), which is evident from the origin of the word propolis ("pro": in front of; "polis": the city). Utilizing propolis in this manner is thought to function as a way for colonies to better maintain homeostasis of the nest environment. This could be a result of reducing microbial growth on hive walls, preventing uncontrolled airflow into the nest, and waterproofing walls against sap (if tree-cavity nesting) and external moisture, in addition to creating some protection against invaders (Seeley and Morse, 1976; Ghisalberti, 1979; reviewed in Visscher, 1980).

Because of the range of uses for propolis, it has been noted that propolis is essential to honey bees, particularly those in the wild (Haydak, 1953; Hoyt, 1965). However, domesticating bees has resulted in a reduction of propolis collection across races (Fearnley, 2001), likely because its use by bees often makes opening hives more difficult for beekeepers. Hoyt (1965) said that propolis "is the bane of a beekeeper's existence", so it is no surprise that apiculturists have selected lines that happened to produce less propolis.

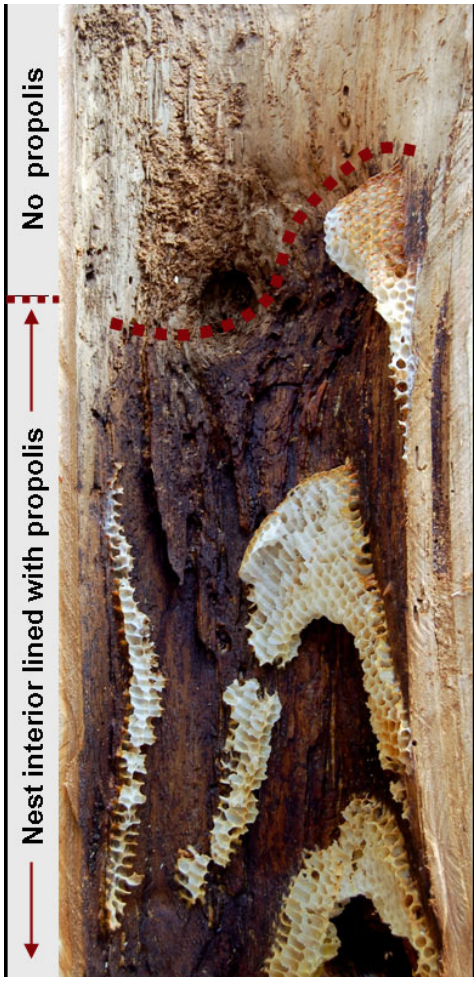


Figure 1. A cross-section of a feral honey bee hive within a tree cavity found September 2009 in the residential area of Bloomington, Minnesota. The nest interior, where comb is present, is coated in a thin layer of propolis (plant resins mixed with wax) creating a "propolis envelope" around the colony. The upper portion of the cavity had not been lined with propolis, as the colony had not begun to use that space.

3. SIGNIFICANCE TO BEE HEALTH

Propolis is highly regarded for its medicinal properties for humans, especially in Eastern Europe, South America, and Asia. The antimicrobial properties of propolis against human pathogens have been known since antiquity (see Ghisalberti, 1979). A number of studies have presented evidence that propolis has strong hepatoprotective, antitumor, antioxidative, antimicrobial and antiinflammatory properties (for recent reviews see Banskota et al., 2001; Sforcin, 2007; Viuda-Martos et al., 2008). Curiously, few studies have examined the antimicrobial properties of propolis against *bee* pathogens or on honey bee immune responses. Since much of the background on biological activity of propolis involves using propolis or components of propolis as treatments of disease, there has been a logical transition into studying propolis as a treatment to use in honey bee colonies (i.e. Samšiňáková et al., 1977; Garedew et al., 2004; Antúnez et al., 2008). However, there should also be a combined focus on the natural function of propolis, specifically determining if its presence in a honey bee hive either directly or indirectly affects pathogen and parasite loads. There is some evidence that it may both serve as a natural mechanism of disease resistance and have the potential to be further applied as an in-hive treatment. Here we describe completed research on the potential significance of propolis for bee health, and then discuss the future direction of this work.

3.1. American foulbrood

The majority of studies relating to the effectiveness of propolis against hive diseases have investigated propolis versus the bee pathogen *Paenibacillus larvae*, the causative agent of American foulbrood (Lindenfelser, 1967, 1968; Mlagan and Sulimanovic, 1982; Bastos, et al., 2008; Antúnez et al., 2008). This is largely because American foulbrood is a highly pathenogenic disease and in recent years has become resistant to conventional antibiotics (Evans, 2003). The main focus of this research has involved in vitro laboratory studies concerning the activity of a few propolis extracts against one or several strains of *P. larvae* grown in the laboratory. One recent study compared the antibacterial activity of a variety of propolis extracts from Minnesota and southeastern Brazil and found that green

propolis from Brazil (derived from *B. drancuncilifolia*) had significantly greater activity against *P. larvae* in vitro compared to propolis from north temperate Minnesota (derived largely from *Populus* spp), but that both inhibited growth of *P. larvae* (Bastos et al., 2008).

Limited field studies have been conducted on the effects of treating colonies with propolis against this bacterial pathogen in colonies. Mlagan and Sulimanovic (1982) fed small, 1-frame honey bee colonies propolis extract in either an aqueous or alcohol solution. They determined that while both treatments reduced the total number of diseased larvae compared to a control colony, the propolis treatments in this manner would not be sufficient to eliminate the disease from the hive. Lindenfelser (1968) found similar results indicating that while propolis treatments may temporarily reduce the spread of disease it is not enough to cure colonies. A more recent study determined that feeding colonies ethanol extracts of propolis mixed into sugar syrup can reduce the amount of *P. larvae* spores found in colony honey stores (Antúnez et al., 2008). However, none of these colonies exhibited clinical symptoms of the disease during the course of the experiment; future experiments could determine if the reduced spore load in honey reduces pathogen transmission in colonies (Antúnez et al., 2008). Furthermore, honey bees do not appear to actively ingest propolis and it is unclear if an oral method of treatment would be at all effective against more severe infections.

3.2. *Varroa destructor*

V. destructor is currently the most damaging parasite affecting honey bee colonies, even with the current colony collapse issues (Cox-Foster et al., 2007; Johnson et al., 2009). Information on the effect of propolis against this parasitic mite has the possibility to shed light on the use of propolis as an in-hive treatment, but also on a possible natural benefit of propolis use by honey bees. A series of laboratory assays have shown that directly exposing mites held in petri dishes to relatively low concentrations of ethanolic propolis extracts caused high mortality (100% due to contact with 10% extract; Garedew et al., 2002). Furthermore exposure to extracts at concentrations as low as 0.5% caused narcotic effects leading to reduced heat production and metabolic rates (Garedew et al., 2002, 2003). These effects could influence the ability of mites to cope with other stressors (i.e. temperature changes, Garedew et al., 2003) or to successfully infest larval cells and maintain the normal course of the parasite's population growth. Taking these findings into a field setting, we have been conducting an ongoing study on the effects of propolis on the reproductive success of *V. destructor* in field colonies. Colonies with experimentally controlled mite levels have been treated by painting the inside walls with propolis extracts (collected from colonies in Minnesota and Brazil) or by spraying empty combs with the propolis extract. Frames containing developing pupae infested with the parasitic mites were removed and percent infestations and reproductive success of mites within the cells were calculated. Preliminary results (M. Simone-Finstrom and M. Spivak, unpubl. data) suggest that the propolis treatments may reduce the number of mature females produced within a single cell. Altering this level of reproductive output would effectively reduce population growth of the parasites within a colony and hopefully reduce the need for the more caustic chemicals currently utilized as treatments against *V. destructor*. Furthermore, as a single propolis sample can contain up to 300 chemical components (i.e. Salatino et al., 2005), it may be more difficult for the mites to develop resistance against a suite of combined compounds.

3.3. Other large parasites and pests

Honey bee colonies also must defend themselves against a number of larger parasites and pests. Two studies have examined the effectiveness of propolis extracts against the greater wax moth, an opportunistic parasite that mainly affects weakened hives (Johnson et al., 1994; Garedew et al., 2004). In laboratory experiments similar to those conducted



Figure 2. A mouse skull that was encased in propolis found within a honey bee colony in an apiary of the University of Minnesota. If a colony intruder has been killed within the hive and is too large for the bees to remove, they will embalm it using propolis to prevent the corpse from decaying.

with *Varroa*, propolis extracts caused larval mortality and reduced metabolic rates of wax moth larvae and adults (Garedew et al., 2004). The implication here is that contact or possibly volatile emissions from propolis may reduce the ability of the moths to effectively reproduce and develop within a hive.

With respect to other large invaders, Cape honeybees, *A. m. capensis*, have been observed encapsulating the parasitic small hive beetle, *Aethina tumida*, in "propolis prisons" which serves to prevent the beetles from successfully reproducing (Neumann et al., 2001). The European honey bee, *A. mellifera*, will also embalm other intruders that are presumably too large to remove from the nest after being killed; Hoyt (1965) observed a mouse encased in propolis and suggested that the bees covered it in propolis to prevent odor and decay from affecting the rest of the hive (Fig. 2). Colonies of *A. dorsata* have also been noted to coat foreign objects in propolis (Seeley and Morse, 1976), as have the stingless bee *Trigona carbonaria* that "mummify" beetle parasites alive using a mixture of wax, plant resins and mud (also known as batumen; Greco et al., 2009). It may be that this behavior of embalming predators or parasites may be a relatively widespread phenomenon among the social bees. Particularly with respect to this entombment behavior, the use of propolis by bees can be described analogously to individual immune function. If we consider a honey bee colony as one entity or "superorganism", then this behavior would be equivalent to cellular encapsulation of foreign microbes or parasites seen at the individual level (see Cremer and Sixt, 2009). The propolis envelope itself, also fits into this analogy as it is a type of mechanical barrier to both reduce parasites from entering the nest (or superorganism) and potentially prevents parasites and microbes from developing once inside (i.e. Simone et al., 2009).

3.4. Social immunity

Recent evidence indicates that propolis in honey bee colonies may play a more subtle role in colony level immunity than direct defense against parasites and pathogens. In 2007 we conducted a field study to determine how a resin-rich environment affects immune-gene expression in honey bees (Simone et al., 2009). Colonies were experimentally enriched with propolis by painting the interior walls with extracts of either Brazilian green propolis or propolis collected from Minnesota. Age-marked bees were collected from these colonies and analyzed for immune-gene expression using real-time PCR. We found that 7-day old bees from propolis treated colonies had significantly lower expression of two immune-related genes as compared to bees collected from control colonies (hymenoptaecin, an antibacterial peptide, and AmEater, a gene involved in cellular immunity). Furthermore, this reduction in immune expression appeared to be due to a reduction in the overall bacterial loads of these colonies, as determined by 16S eubacterial gene transcript levels. This finding was significant because an elevated immune response

has an associated fitness cost, as demonstrated by reduced colony productivity in honey bees (Evans and Pettis, 2005) and decreased individual survival in bumble bees (Moret and Schmid-Hempel, 2000). This was also the first study to document effects of a component of the nest environment on individual immunity in honey bees. The results of this study support those of previous laboratory studies on another social insect, the wood ant *Formica paralugubris*, which collects and distributes resin from spruce trees throughout its nest material. Soil collected from ant nests experimentally enriched with this resin had significantly fewer pathogenic bacteria and fungi (Christe et al., 2003), and adult ants in colonies experimentally made resin-rich did not invest as much in individual immunity (indicated by decreased antibacterial ability of the hemolymph) compared to ants in resin-deprived colonies (Castella et al., 2008a), which resulted in increased survival during a pathogen challenge (Chapuisat et al., 2007). Therefore, as with *F. paralugubris* ants, it is likely the presence of propolis in a honey bee colony may reduce the investment in the innate immune response by acting as an external immune defense mechanism.

3.5. Self medication

In light of all of this information, one obvious question concerns the idea of "self-medication". Resin collection may be constitutive (i.e., collected regardless of physiological demand or pathogen level) or inducible (i.e., a conditional response to infection; Schmid-Hempel and Ebert, 2003). If it is inducible, it might be considered a form of self-medication, defined as the "defense against [pathogens and] parasites by one species using substances produced by another species" (Clayton and Wolfe, 1993). There are number of vertebrates that self-medicate by ingesting, absorbing, topically applying or living in proximity to plants with medicinal compounds (reviewed in Clayton and Wolfe, 1993; Lozano, 1998). Examples of self-medication in the insect literature, particularly with respect to social insects are less common. When *F. paralugubris* ant colonies were challenged with the fungal pathogen *Metarhizium anisopliae*, they did not respond by increasing the rate or quantity of resin collection, and the authors concluded that the use of resin by this species was a constitutive rather than inducible response, and therefore not an example of self-medication (Castella et al., 2008b). Honey bee colonies infected with diseases or parasitic mites do not appear to respond by collecting more resin (M. Simone-Finstrom, M. Spivak, pers. obs.) but studies to quantify resin collection after pathogen challenge are ongoing. The trade-off between the energetic costs to individual bees of collecting resin may have been offset by the antimicrobial properties of the resins which benefited the individuals' immune systems and increased colony fitness, leading to continued selection for resin collection regardless of pathogen or parasite levels.

3.6. Future studies on bee health

There have been a number of studies on the effectiveness of propolis against bacterial pathogens. Further studies should be conducted with respect to propolis against hive diseases both alone and in combination with other disease resistance mechanisms (i.e. hygienic behavior) to better determine how valuable propolis could be as a direct treatment. In Europe, there are currently plans to study how propolis may be used against bee pathogens and parasites as a form of treatment (see Moritz et al., 2010). Research at the University of Minnesota currently underway has a similar, but more specific focus. We are completing a series of studies to identify specific fractions and components of propolis extracts that are active against the bacterial agent of American foulbrood and common honey bee viruses (M. Wilson, J. Cohen, G. Gardner, J. Burtness, M. Spivak, unpubl. data). Propolis extracts in general have been shown to be active against some human viruses in vitro (i.e., HIV-1, Gekker et al., 2005), and the results of this work on honey bee viruses could have implications for human health by identifying possible compounds for further study.



Figure 3. Particularly in feral colonies nesting in tree cavities, honey bees secure the site of comb attachment on the hive wall with propolis. In some cases, as can be seen here (same feral colony as in Fig. 1), the rim of comb cells will also have a thin coating of propolis. The function and significance of this behavior is currently unknown.

Future research should also be directed toward the natural use of propolis by honey bees as a disease resistance mechanism. Propolis will occasionally be used for tasks other than smoothing hive walls and reducing entrances. Huber (1814) observed honey bees embedding strands of propolis in cleaned and polished cells. Ribbands (1953) believed that bees used propolis in this manner to prevent disease transmission when reusing cells. It is unclear how common this behavior is, but at least feral colonies can be found with propolis on the rims of cells (Fig. 3). Recent evidence also indicates that honey bees may "entomb" chemically contaminated pollen in cells with propolis, but the frequency of this behavior and subsequent effect on colony health is currently unclear (vanEngelsdorp et al., 2009).

It is possible that the antimicrobial properties of materials used and stored in combs (e.g. royal jelly, honey) are enhanced by the addition of propolis (Visscher, 1980; Tautz, 2008). In particular, the modes of action of propolis against microbes and parasites are currently unknown and could be due to contact (e.g. Garedew et al., 2002) and/or volatile emissions (e.g. Messer, 1985). The two modes are not necessarily mutually exclusive and could have varying effects depending on the organism, and must be considered when investigating the use of propolis both as a colony treatment and its natural effectiveness in the hive. Additionally the persistence of the activity of propolis in the hive is something that needs to be known, particularly if the goal is to utilize propolis for colony treatments.

While knowledge concerning the role that propolis plays in disease resistance in honey bee colonies is growing, studies are needed on the behavioral ecology of resin collection to fully understand how it can impact bee health. We need more information concerning the mechanisms of resin collection and use as propolis within the colony, and the regulation of resin foraging, both at the individual and colony levels. The remainder of this review will focus on these issues, discussing what is currently known and identifying major areas for future study.

4. SOURCES OF RESIN AND THE PROCESS OF RESIN COLLECTION

In tropical climates honey bees mostly collect resins from *Clusia minor* and *Clusia rosea* flowers and from alecrim plants (e.g. *Baccharis dracuncufolia*), which is similar to other tropical bee species (Pereira et al., 2003; Salatino et al. 2005). Recently a leguminous species (*Dalbergia* sp.) has also been identified as a common source in tropical regions (i.e. Silva et al., 2008). In temperate climates poplar trees (*Populus* sp.) appear to be the primary source for resins (Popravko and Sokolov, 1980; Nagy et al., 1986; Greenaway et al., 1987; Bankova et al., 1992; 2006; Markham et al., 1996; Salatino et al., 2005). However,

it is clear that other trees, like pine, birch, elm, alder, beech and horse-chestnut species, are adequate resin sources for temperate honey bees, particularly when poplar species are unavailable (Alfonsus, 1933; Ghisalberti, 1979; Crane, 1990). Additionally, honey bees in Uganda appear to forage for resins selectively on *Alnus* sp. and can actually defoliate these trees; whether there are other possible sources in the region remains unclear (Nyeko et al., 2002).

Honey bees will forage for resins from droplets appearing on the bark of the trunks or limbs of trees (Alfonsus, 1933), from the surfaces of some fruits (i.e. *Macaranga tanarius*; Kumazawa et al. 2008), or more typically on the vegetative apices (buds, leaf primordia and young leaves). The bees must extract the resins from the trichomes and ducts by fragmenting these early leaves using their mandibles (Meyer, 1956; Nyeko et al., 2002; Teixeira et al., 2005). Resin-foragers have shown a preference for young leaves and vegetative buds over more expanded leaves (Park et al., 2004).

The cues that honey bees rely on to find resinous plant sources are currently unknown. Huber (1814) placed a bunch of poplar branches "that had very large buds coated both on the outside and inside with a viscous, reddish and odoriferous sap" in front of his honey bee colonies and observed bees collecting resins within 15 minutes. It is clear that foragers select specific sources, and rely on currently unknown cues. Honey bees have been observed probing the apex of one plant with their antennae then moving to another one, probing it and subsequently collecting resin from it (Teixeira et al., 2005). The same study also provided evidence that the resin-foragers preferred female versus male *Baccharis dracuncufolia* as resin sources. The young leaves and buds have a similar chemical composition that changes as the leaves become more expanded (Park et al., 2004), which implies that there may be a chemical cue released by the resin source that the foragers are able to detect.

Once the bees find the resin source, they then have to collect it. Huber (1814), Haydak (1953), and Meyer (1956) have described this process in great detail. There are four basic steps (taken from Meyer, 1956) that a resin forager follows to pack her corbicula: (1) Break off a particle of propolis with the mandibles; (2) work it with the mandibles and take it with the forelegs; (3) transfer it from the forelegs to the middle leg; (4) transfer it from the middle leg to the corbicula on the same side. This sequence is repeated until there is a full resin load on both corbicula (see Fig. 4). No corbiculate bees can collect resin and pollen during a single foraging trip because of this behavior (Armbruster, 1984; Roubik, 1989). After completing the four steps, bees have been observed flying around for a few seconds above the resin source, then landing again to add more to each corbicula (Alfonsus, 1933; Haydak, 1953). The purpose of these flights is unknown but may be used to assess the weight of the current corbicular load. The process of obtaining a full corbicular load of resin has been noted to take about seven minutes (Teixeira et al., 2005; Kumazawa et al., 2008), but can take from 15 min to and hour depending on the weather (Haydak, 1953).



Figure 4. As a resin forager returns to the nest with a load of resin on her corbicula, they go deep into the hive at a "cementing" site, where the resin will be used. Pictured in a colony located at the University of Minnesota, a resin forager within a hive must wait for other bees to remove the resin from her hind legs. Propolis is used in this area and can be seen here on the tops of the two frames.

Once the bee has a full load, she returns to her colony to unload the resin from her corbiculae. The unloading process typically takes approximately 15 minutes, but can extend from one to seven hours or even overnight (Alfonsus, 1933; Haydak, 1953; Ratnieks and Anderson, 1999; Nakamura and Seeley, 2006). A resin-forager cannot unload her corbiculae herself, but rather must rely on her nestmates to take the resins off of her. Once the resin-forager returns with a full load, she will go to a site within the hive where propolis is needed, where she waits until other bees, known as cementing bees, bite off chunks of resin from her corbiculae (Betts, 1921; Alfonsus, 1933; Haydak, 1953; Meyer, 1956; von Frisch, 1993; Nakamura and Seeley, 2006). Cementing bees immediately attach the resin to a site along the hive wall. The cementing bee then smoothes the resin, now officially propolis, with her mandibles in a manner that is similar to that of wax construction (Alfonsus, 1933; Nakamura and Seeley, 2006). The resins may also be placed in a storage area where bees can grab chunks of propolis to later place in comb cells or other areas (Huber, 1814; Haydak, 1953; Fearnley, 2001; Tautz, 2008). Many of the few resin-foragers in a colony will perform cementing behavior, but not all cementing bees will forage for resins (Huber, 1814; Meyer, 1956; Nakamura and Seeley, 2006). Meyer (1956) found that forager-aged bees with atrophied wax glands do most of the cementing work. Recent evidence from Nakamura and Seeley (2006), however, indicated that the bees they observed using resin in the nest performed these behaviors prior to foraging. This suggests that cementing and other in-hive resin activities are performed by the middle-aged bees that typically perform nest construction tasks in addition to those bees foraging for resins.

During the cementing process, the resins do not appear to be chemically modified. While there is some evidence that the general chemical profiles of resins collected directly from a forager and in-hive collected propolis can vary slightly from the leaf buds of the plant source (i.e. Ghisalberti, 1979; Peev et al., 2009), it is likely that some variation could occur to due volatilization of some chemicals during the course of the return foraging trip. In addition, propolis sampled from a single colony likely contains an amalgam of various sources at least to some degree in addition to wax and is essentially a concentration of some of the compounds collected directly from the plants. Thus, some compounds would expect to be more or less represented in propolis samples, but the general chemistry would remain similar as has been found (i.e. Greenaway et al., 1990; Park et al., 2004; Teixeira et al., 2005; Vardar-Ünlü et al., 2008). For other bee species, however, there is some suggestive evidence that bees add secretions to the resins. Workers of the stingless bee *Plebeia emerina* reach maximum development of the head and intramandibular glands during the age of most frequent resin handling, which may be utilized to maintain the viscosity of resins during use (dos Santos et al., 2009). How this may change the chemical properties of the resins has yet to be investigated.

Honey bee resin foragers follow a fairly strict diurnal pattern in foraging and cementing behaviors. Foraging for resins is typically observed between 10 am and 3:30 pm on sunny days, likely due to the increased pliability of resins at higher temperatures (Alfonsus, 1933; Meyer, 1956; Hoyt, 1965; Nyeko et al., 2002). Cementing behavior occurs most often in late afternoon with the foragers participating in the behavior once their loads have been removed (Meyer, 1956; Ratnieks and Anderson, 1999). Additionally while resin foragers can almost always be found from May through November in temperate regions (Crane, 1990), there appears to be some seasonality in resin collection and propolis use. Resin is said to be collected most frequently in late summer (end of June) through autumn when the honey flow is greatly reduced (Alfonsus, 1933; Meyer, 1956; Crane, 1990). Meyer (1956) hypothesized that more regular propolis collection in late summer and early fall is the result of a seasonal change in foraging behavior and not the result of climatic changes or the need to prepare the hive for winter, as has been suggested (Ghisalberti, 1979). This idea is supported by the fact that honey bees can be induced to collect resin during any part of

the season (Butler, 1949; Meyer, 1956; M. Simone-Finstrom, pers. obs.). However, it is also likely that higher levels of resin collection late in the season are due to the reduced nectar flows, as resin foragers are not necessarily committed to resin collection for their foraging lives. During periods of greater nectar and pollen availability a resin forager may be more motivated to forage for pollen and nectar depending on colony need. Nakamura and Seeley (2006) found that while resin foragers did not switch to other resources through the course of a single day in September, 33% switched to either pollen or nectar on subsequent days.

5. STIMULI INVOLVED IN RESIN FORAGING

In general social insect foraging has been studied extensively both in relation to the individual mechanisms involved as well as issues related to division of labor and task allocation within a colony. Although there is abundant research on the regulation of foraging behaviors at both the individual and colony levels with respect to pollen and nectar (reviewed in Page and Fondrk, 2004; Hunt et al., 2007), the behavior of foragers collecting nest-building supplies like resins is understudied. It appears that there are bees within a colony that are specialized to forage for resin (Meyer, 1956; Ranger and O'Donnell, 1999; Nakamura and Seeley, 2006) and that this likely has a genetic component similar to that of pollen and nectar specialized foragers. However the cues that resin foragers use to both initiate foraging and find a resin source are virtually unknown. One leading hypothesis is that volatile compounds released from the resin play a large role in locating resins (Armbruster, 1984; Roubik, 1989; Bankova et al., 2000; Patricio et al., 2002; Teixeira et al., 2005), though it has yet to be investigated.

5.1. Regulation at the individual level

Individual bees may detect the need for resin and then use communication signals (e.g., waggle dances, trembling) inside the nest to recruit nest mates to forage for it, as they do to recruit nest mates to food resources. The cues bees use to detect the need for resin may be the presence of gaps, crevices or irregularities in the nest architecture that may allow the entry of microbes, intruders, air currents and sunlight (Butler, 1949; Ribbands, 1953; Seeley and Morse, 1976; Crane, 1990). Since the nest interior is completely dark, bees must rely on non-visual senses to detect stimuli within the nest environment. The bees' antennae are an integral tool for this type of information assessment (Erber and Pribbenow, 2001; Johnson, 2008). Bees, and specifically some resin handlers and foragers, have been noted to detect crevices by inserting the antenna into gaps in nest architecture (Nakamura and Seeley, 2006).

We have started to investigate whether resin foragers are more sensitive to certain stimuli as compared to other foragers to begin to understand what stimuli resin foragers may detect in order to initiate foraging behaviors. We have conducted a series of experiments using proboscis extension conditioning response to determine if resin foragers are able to learn tactile stimuli more effectively than pollen foragers (for general methods see Erber et al., 1997). Using this technique, we found that resin foragers are better able to learn a tactile stimulus (a gap between two metal plates) and may be better able to distinguish between two other tacitle stimuli (rough sandpaper or smooth paper) as compared to pollen foragers (M. Simone-Finstrom, J. Gardner, M. Spivak, unpubl. data). These differences were not due to a greater ability of resin foragers to learn all stimuli, as resin and pollen foragers equally learned the odor geraniol. While this data is merely suggestive of the possible stimuli that may be involved in initiating resin foraging, it provides a general starting point for future research to examine how resin foragers assess information related to initiating their task. Use of tactile information for initiating nest construction tasks holds true for other social insects, like some species of paper wasps (*Polistes fuscatus*) and termites (*Nasutitermes costalis*, *Coptotermes formosanus*) that have been noted to detect nest damage and determine building sites using antennation (Jones,

1980; Downing and Jeanne, 1990; Lee et al., 2008).

5.2. Colony-level organization of resin foraging

A recent study by Nakamura and Seeley (2006) documented detailed observations of both resin foragers and cementers (bees that manipulate resins in the hive) in order to understand how resin foraging is regulated at the colony-level. They proposed two hypotheses, and neither proved to be mutually exclusive: (1) the "unloading difficulty hypothesis", which proposes that individuals determine resin need based on how long it takes another bee to help them remove the resin from their legs; and (2) the "caulking activity hypothesis", which states that resin foragers manipulate resins within the hive and thus sense the need to forage based on available caulking sites. Of 77 resin foragers monitored closely, 26% performed tremble dances, which appeared to function as a signal to stimulate other bees to handle resin within the nest (Nakamura and Seeley, 2006). Trembling by resin foragers appears to be an unloading signal similar to how it stimulates nest bees to receive and store nectar from nectar foragers (Seeley et al., 1996). In addition, 8% of the 77 resin foragers and cementers performed "crevice-detecting" behavior, which was defined as "walking along crevices and inserting the antenna into them" (Nakamura and Seeley, 2006, p. 340). Based on their findings, they surmised that resin foraging is a "demand-driven" process in response to sensing the need for it.

After resin foraging has been initiated by one or several bees, it is possible bees use waggle dances as a colony-level recruitment signal, in a similar way to how pollen and nectar-foragers use dances as communication signals to recruit other foragers to their food sources. Nakamura and Seeley (2006) found that 26% of the 77 observed resin foragers performed dances near cementing sites deep within the hive (unlike pollen and nectar dances, which are done near the hive entrance). Waggle dances by resin foragers have also been observed near cementing sites by Milum (1955), Meyer (1956) and von Frisch (1993). However the purpose and subsequent effect of these dances is unknown, and could simply be a vestige of more general foraging behaviors.

One way to better address questions concerning the mechanisms of resin foraging would be to maintain a line of bees selectively bred that consistently collects large quantities of resin and a corresponding line that consistently collects little resin. Research on lines of bees bred for hygienic and non-hygienic behavior (i.e. Spivak, 1996; reviewed in Wilson-Rich et al., 2009 and Evans and Spivak, 2010), and for high- and low-pollen hoarding (e.g. Page and Fondrk, 1995) has been instrumental in uncovering a host of information on the genetic mechanisms regulating honey bee social behaviors.

6. TOPICS TO CONSIDER

Resins are produced by a large variety of plants across taxa worldwide. Bees around the globe collect and utilize resins as propolis for a number of purposes, including sealing cracks in the nest, creating smooth surface for comb attachment, entombing parasites and predators, and reducing in-hive microbes. We are currently at the fringe of understanding all the facets involved in this process.

While investigations on the chemical components of propolis are currently growing at an almost exponential pace, there is still a host of information lacking from our knowledge base. In particular, one area of special interest to the beekeeping community is the presence of contaminants in-hive products, like propolis (i.e. Bogdanov, 2006). While commercial hives are often given a variety of chemical treatments to control various hive diseases and parasites, investigations into the residues that these may leave behind are relatively new. There is limited evidence that acaricides can occasionally be found in propolis collected from a hive (Bogdanov et al., 1998; Wallner, 1999), as well the antibiotic (tylosin) used to treat the bacterial diseases American foulbrood and European foulbrood (2 of 30

samples from China had detectable amounts; Zhou et al., 2009). Similarly low levels of pesticide residues likely from treatments on the plant sources have been detected in some propolis samples (Chen et al., 2009), but not in others (Santana dos Santos et al., 2008). Further study on the frequency and abundance of these chemicals in propolis samples needs to be conducted as well as the possible antagonistic effect that these compounds could have on the chemical constituents of propolis or possibly the synergistic effects that the residues have with those chemicals found in wax, honey and pollen stores (i.e. Frazier et al., 2008; Johnson et al., 2010).

The vast majority of current studies related to propolis, however, tend to focus on chemically identifying propolis components, while incorporating descriptions of the biological activity of samples. These studies are currently being conducted on samples collected globally and will not only provide some comparative information on the activity of propolis from varying regions and ecosystems, but will also help to narrow focus on identifying specific components and mixtures of components required for activity against various microbes and parasites. While we know that biological activity can often be correlated with phenolic content (i.e. da Silva et al., 2006; Popova et al., 2007; reviewed in Bankova et al., 2008; Viuda-Martos et al., 2008), it is currently unclear if these compounds work in synergy with other compounds or if some are antagonistic. Both scenarios are likely. Additionally, in some cases, biological activity has been shown to be equivalent regardless of the race of the honey bee, geographical region or season, even though the chemical profiles may be dissimilar (i.e. Kujumgiev et al., 1999; Sforcin et al., 2000; Silici and Kutluca, 2005; Silici et al., 2007). However, many other studies have shown that, while propolis is generally active against most gram-positive bacteria and some fungi, the level of activity depends on location, likely due to differences in plant sources (i.e. Popova et al., 2007; Seidel et al., 2008; Chaillou and Nazareno, 2009). In particular, it has been hypothesized that wet-tropical and lower latitude ecosystems may have plants with generally higher levels of antimicrobial compounds or at least access to a larger variety of plants that may differ in antimicrobial properties, and would thus lead to increased biological activity of propolis samples from those climates (Popova et al., 2007; Seidel et al., 2008).

The widespread use of resins by the various tropical bee species also should be investigated in greater detail. It is well known that many tropical euglossine, meliponine and megachilid bees use resins for nest construction (Armbruster, 1984; Roubik, 2006). These bees often mix resins with clay, soil and wax to form the nest itself and its supporting structures (Roubik, 1989). When resins are amalgamated with soil or clay material the resulting mixture is often termed geopropolis or batumen, whereas when it is only mixed with wax it is simply called propolis or cerumen with respect to non-honey bee species (Barth, 2004; Roubik, 2006). It is also apparent that some bees utilize these resin mixtures as protection against predators, like ants (Seeley et al., 1982; Roubik, 1989; Patricio et al., 2002; Lehmberg et al., 2008). One interesting case of resin collection among tropical bees involves *Chalicodoma pluto*, which uses resins and wood to construct nest cells and tunnels. A *C. pluto* female harvests resin from vertical trunk fissures by loosening it with her large mandibles then scraping it off with her specialized, elongate labrum (Messer, 1983). In this case it appears that the bee has a specialized morphological feature to collect and use this necessary resource; however in other cases it may be the resin source that has specialized to attract bees. Flowers of *Dalechampia* sp. and *Clusia* sp., which are visited by a host of tropical bees, are thought to produce resins as a pollinator reward instead of nectar (Armbruster, 1984; Gonçalves-Alvim, 2002; Salatino et al., 2005). *Trigona pallens* specifically has been noted to be attracted to the resin-producing flowers of *Clusia odorata*, and other species may also be utilizing this resin source (Armbruster, 1984). Since resin is essential nesting material for a number of tropical bees it is likely that some plants evolved mechanisms to produce resins that attract pollinator species. In terms of the evolution of the behavior it would be important to understand if these bees are simply exploiting this commonly available resource in

tropical climates for nest construction or if there are some other, possibly health-related benefits for nesting with resins. Limited studies have been done on the biological activity of tropical bee resins, and all have shown that, similarly to honey bee propolis, these samples are generally biologically active against gram-positive bacteria at the least (Lokvam and Braddock, 1999; Farnesi et al., 2009). The possibility that resin use as a mechanism of disease resistance is a widespread phenomenon across the social insects is certainly a topic that should warrant future study. It is not known if resin collection evolved several times among the ants and bees. The evolution of these behavioral disease resistance mechanisms is also currently unknown.

There are clearly a multitude of options for future research related to propolis and resin use by bees ranging from the pharmacological opportunities for human health to understanding the individual and colony-level mechanisms of resin foraging to the possible applicability for propolis as a treatment against bee pathogens and diseases. At the least, information on resin use and its incorporation into the honey bee nest architecture is an exciting area of research concerning environmental impacts on disease resistance and social immunity.

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Propolis et santé de l'abeille : l'histoire naturelle et la signification de l'utilisation de résine végétale chez les abeilles.

***Apis mellifera* / immunité sociale / défense antimicrobienne / immunité écologique**

Zusammenfassung – Propolis und Bienengesundheit: Die Naturgeschichte und die Bedeutung des Gebrauchs von Pflanzenharzen durch Bienen. Die „soziale Immunität" als neues Forschungsfeld bei sozialen Insekten beschreibt, wie das individuelle Verhalten von Mitgliedern einer Gruppe wirkungsvoll die Verbreitung von Krankheiten und Parasiten auf der Ebene des Sozialstaates verhindern kann. Ein bisher zwar wenig untersuchtes aber wichtiges Verhaltensmerkmal zur Krankheitsabwehr bei Honigbienen ist die Verwendung von Pflanzenharzen. Honigbienen sammeln Harze mit antimikrobiellen Eigenschaften von verschiedenen Pflanzen, mischen diese dann im Bienenvolk mit unterschiedlichen Mengen von Wachs und benutzen dies als Propolis (Abb. 1–4). Propolis ist demnach der bienenkundliche Begriff für Harze, die im Bienenstock verwendet werden. Während es zahlreiche Untersuchungen zur Verwendung bestimmter Bestandteile des Propolis zur Krankheitsbekämpfung beim Menschen gibt, sind kaum Informationen über die Bedeutung von Propolis für die Bienengesundheit vorhanden.
Dieses Review ist eine Zusammenstellung neuerer Forschungsergebnisse zum Verhalten der Bienen in Bezug auf Harze und Propolis mit dem Schwerpunkt auf die möglichen evolutiven Vorteile des Harzsammelns für die Honigbienen. Die Verwendung von Harzen durch Bienenvölker (*Apis mellifera*) ist weit verbreitet. Während es erhebliche Unterschiede zwischen einzelnen Völkern bzgl. der Menge an gesammelten Harzen und Propolis gibt, scheinen alle – und dabei insbesondere die wildlebenden – Bienenvölker das Propolis zur Auskleidung des gesamten Stockinneren zu benutzen. Es wird angenommen, dass Propolis dazu beiträgt, die Homöostase innerhalb des Bienenstockes aufrecht zu erhalten. Konkret könnte das Propolis dabei das mikrobielle Wachstum an den Beutenwänden reduzieren, unkontrollierten Luftzug ins Beuteninnere verhindern und zusätzlich mechanische Barrieren gegenüber Eindringlingen bilden. Einige Forschungsprojekte zeigen eindeutig, dass Propolis im Bienenstock direkt gegenüber Krankheitserregern (z.B. Amerikanische Faulbrut) und Parasiten (z.B. Kleiner Beutenkäfer, *Varroa destructor*) wirkt. Daneben scheint es aber auch eine subtilere Wirkung über die Unterstützung des individuellen Immunsystems zu geben. Die weiteren Forschungen sollten sich auf das bessere Verständnis der Verwendung von Harzen durch Honigbienen und andere soziale Insekten konzentrieren. Dafür gibt es eine Vielzahl an Forschungsfeldern, von den pharmazeutischen Möglichkeiten des Propolis für die menschliche Gesundheit über die Mechanismen der Sammelstrategie von Propolis auf den Ebenen der Einzelbienen und des Bienenvolkes bis hin zu einer möglichen Anwendung von Propolis als Bekämpfung von Bienenkrankheiten. Schließlich ermöglichen Informationen zur Verwendung von Harzen und

deren Aufnahme in den Bienenstock spannende Forschungsansätze zum Einfluss der Umwelt auf Krankheitsresistenz und soziale Immunität.

***Apis mellifera* / Soziale Immunität / antimikrobielle Abwehr / ökologische Immunität**

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