

Ecological Drivers of Group Living
in a Facultatively Social Carpenter Bee (*Xylocopa sonorina*)

by

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ABSTRACT

Understanding why animals form social groups is a fundamental aim of sociobiology. To date, the field has been dominated by studies of kin groups, which have emphasized indirect fitness benefits as key drivers of grouping among relatives. Nevertheless, many animal groups are comprised of unrelated individuals. These cases provide unique opportunities to illuminate drivers of social evolution beyond indirect fitness, especially ecological factors. This dissertation combines behavioral, physiological, and ecological approaches to explore the conditions that favor group formation among non-kin, using as a model the facultatively social carpenter bee, *Xylocopa sonorina*. Using behavioral and genetic techniques, I found that nestmates in this species are often unrelated, and that non-kin groups form following extensive inter-nest migration.

Group living may arise as a strategy to mitigate constraints on available breeding space. To test the hypothesis that nest construction is prohibitively costly for carpenter bees, I measured metabolic rates of excavating bees and used imaging techniques to quantify nest volumes. From these measurements, I found that nest construction is highly energetically costly, and that bees who inherit nests through social queuing experience substantial energetic savings. These costs are exacerbated by limitations on the reuse of existing nests. Using repeated CT scans of nesting logs, I examined changes in nest architecture over time and found that repeatedly inherited tunnels become indefensible to intruders, and are subsequently abandoned. Together, these factors underlie intense competition over available breeding space. The imaging analysis of nesting logs additionally revealed strong seasonal effects on social strategy, with social nesting dominating during winter. To test the hypothesis that winter social nesting arises

from intrinsic physiological advantages of grouping, I experimentally manipulated social strategy in overwintering bees. I found that social bees conserve heat and body mass better than solitary bees, suggesting fitness benefits to grouping in cold, resource-scarce conditions. Together, these results suggest that grouping in *X. sonora* arises from dynamic strategies to maximize direct fitness in response to harsh and/or competitive conditions. These studies provide empirical insights into the ecological conditions that favor non-kin grouping, and emphasize the importance of ecology in shaping sociality at its evolutionary origins.

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CHAPTER 1

INTRODUCTION: ECOLOGICAL DRIVERS OF NON-KIN COOPERATION IN THE HYMENOPTERA

Introduction

Social animals represent some of the most ubiquitous and ecologically dominant organisms globally (Hölldobler & Wilson, 1990; Krause & Ruxton, 2002; Ward & Webster, 2016). To date, our understanding of how social groups emerge has been rooted overwhelmingly in the study of family groups. From these groups have emerged useful theoretical frameworks for explaining cooperation in nature, especially kin selection theory, which posits that indirect fitness benefits of helping kin can compensate for direct fitness costs (Abbot et al., 2011; Bourke, 2014; Hamilton, 1964; Trivers & Hare, 1976; West-Eberhard, 1975). Nevertheless, many animals form groups with non-relatives, and in these societies direct fitness gains are generally the major component of inclusive fitness (Clements & Stephens, 1995; Clutton-Brock, 2009; Dugatkin, 2002; Goodnight, 2005; Queller, 2011). These social groups, which exist across diverse animal taxa (Bernasconi & Strassmann, 1999; Brask et al., 2019; Clutton-Brock, 2009; Riehl, 2013; Suarez & Goodisman, 2021; Wilkinson et al., 2016), demonstrate the value of examining the diversity of selection contexts for understanding the evolution of sociality, and provide useful models for examining ecological drivers of social evolution.

Kin selection has proven critically valuable for understanding the evolution of eusociality, especially within the highly related colonies of the social insects (Abbot et al., 2011; Bourke, 2011; Hughes et al., 2008; Linksvayer & Wade, 2011; Queller & Strassmann, 1998; West-Eberhard, 1975). However, eusociality is rare; even among the Hymenoptera; other forms of group living are considerably more common (Fewell & Abbot, 2018; Heinze et al., 2017; Hunt & Toth, 2017; Wcislo & Fewell, 2017). Perhaps

due to the prominence of kin selection as a framework for understanding insect sociality, non-kin groups in insects have received relatively little attention, despite advances in our understanding of non-kin vertebrate groups (Brask et al., 2019; Clutton-Brock, 2009; Riehl, 2013; Wilkinson et al., 2016). Departures from a kin-centric framework for understanding insect social evolution may enable valuable connections to other animal groups, contributing to a broader body of evolutionary theory. Further, these systems may be neglected because interactions among non-kin rarely (if ever) constitute altruism—that is, behavior that reduces the direct fitness of the actor and increases the fitness of the group—which has been a major focus of social evolutionary research in the eusocial Hymenoptera (Foster et al., 2006; Hamilton, 1972; Kennedy et al., 2018; Simon, 1990). Rather, non-kin associations provide examples of cooperation based on mutual benefits of grouping, with or without reproductive division of labor.

I review advances in our understanding of non-kin social groups in the Hymenoptera, with a focus on patterns of diversity in social structure and ecological context. I characterize variation in the organization of these groups and describe the distribution of non-kin sociality across the bees, ants, and wasps. Across these groups, I then highlight common ecological drivers of non-kin sociality, particularly environmental challenges and intra- and inter-specific interactions. Finally, I synthesize insights from the current body of research on non-kin sociality and highlight promising directions for future research. In doing so, I emphasize the role of ecological context in shaping sociality at its evolutionary origins.

Non-Kin Cooperation in the Hymenoptera

Non-kin sociality is found broadly among the social ants, wasps, and bees, and ranges in complexity from simple, facultative nest sharing in primarily solitary populations to

cooperative founding of eusocial colonies (Figure 1.1). For the purposes of this review, I define sociality as any long-term association between conspecifics characterized by mutual tolerance and/or cooperation within shared nesting space (Costa, 2006; Fewell & Abbot, 2018). By “long-term,” I refer to an extended or significant portion of an individual’s lifespan, as opposed to more transient interactions like mating. Further, I emphasize mutual tolerance as a minimum requirement in our definition of sociality for the sake of including even groups characterized by limited cooperative behavior. Mutual tolerance serves as a preadaptation for the evolution of cooperation, by enabling individuals to share nest space and providing opportunities for more complex social interactions (Michener, 1990; Michener, 1974).

Specifically, I examine social interactions in the context of breeding and offspring care, because behavioral decisions in these contexts have important fitness impacts. I emphasize nest sharing to exclude from our definition of sociality those animals living within aggregations of spatially clustered nests, but otherwise living solitarily. Though some Hymenoptera (such as army ants) are non-nesting, nests are used predominantly by this taxon as an essential physical site for the prolonged interactions intrinsic to social living. Additionally, I define sociality as distinct from intraspecific social parasitism, and therefore exclude from our discussion those systems in which non-kin relationships arise through parasitic behavior (Beekman & Oldroyd, 2008), including adoption of unrelated offspring (Klahn, 1988; Nonacs & Reeve, 1993) and cleptoparasitism (Michener, 1974; Rozen, 1991).

Non-kin associations vary considerably in the degree of cooperation, and thus serve as an important counterpoint to vertebrate sociality. However, discussions of cooperation for social insects and social vertebrates have historically been treated separately. For example, cooperation in the social insects is often studied in the context of task allocation and division of labor (Beshers & Fewell, 2001; Hölldobler & Wilson,

1990; Seeley, 1996), while social vertebrate sociality is more often discussed in terms of the costs and benefits of cooperative interactions (Hamilton 1964; Clutton-Brock, 2009; Dugatkin, 2002). Defining cooperation itself has also presented challenges, with debate surrounding the questions of whether cooperative interactions may incur differential costs for actor and recipient, and whether cooperative sociality can be maintained under such conditions without indirect fitness gains (Bergmüller et al., 2009; Lehmann & Keller, 2006; West et al., 2007; West et al., 2006). Within such discussions, however, has emerged a central theme that cooperation broadly entails behaviors that benefit the social group (Clutton-Brock, 2009).

Social evolution in the Hymenoptera

The evolution of cooperative behaviors is shaped by ecological context and by the phylogenetic pathway that group has taken to sociality. The task of categorizing the various forms of sociality and their evolutionary histories has been the subject of considerable debate (Boomsma & Gawne, 2018; Crespi & Yanega, 1995; Michener, 1974; Richards, 2019; Toth et al., 2016; Wilson, 1971). A well-established hypothesis has proposed a stepwise evolutionary progression from simple forms of sociality to complex eusociality (Evans, 1956; Evans & West-Eberhard, 1973; Rehan & Toth, 2015; Wilson, 1971). Recent, renewed discussion of this topic has challenged the theoretical presumption of a “social ladder” in which less complex social forms represent intermediate “levels” along an evolutionary trajectory toward eusociality (Holland & Bloch, 2020; Linksvayer & Johnson, 2019). Accordingly, I consider the diversity of cooperative systems in the social insects not as transitional forms in the evolution of sociality, but instead in terms of their shared cooperative behavioral repertoires that are adaptive in a given ecological context.

One of the simplest forms of sociality, known as communal living, refers to societies in which multiple same-generation females (often unrelated) share nesting space but independently forage and provision their own offspring (Michener, 1974). Communal groups are characteristically casteless: group members are not distinguished behaviorally or morphologically by their capacity for reproduction. Only a subset of tasks—typically nest construction and nest defense—are shared cooperatively. Communal groups often exist among otherwise solitary populations of bees and wasps, and are characterized by behavioral repertoires similar to those of solitary females: they mass-provision brood at the egg stage, and do not engage in further direct parental care (Wcislo & Fewell, 2017; Wcislo & Tierney, 2009). In contrast to mass-provisioners, other social insects, including ants, wasps, and some bee taxa, perform direct parental care in which larvae are fed progressively (Field, 2005). The cooperative repertoire of these groups is similarly expanded. These associations occur when related or unrelated females found nests cooperatively (pleometrosis) by sharing or dividing such tasks as provisioning, nest construction, and defense (Heinze et al., 2017; Ross & Matthews, 1991).

Social diversity in the Hymenoptera

Here I describe the diversity of non-kin sociality defined as long-term adult nest sharing, with groups often characterized by cooperative behaviors and task sharing. Because relatedness is a relative attribute (Pamilo, 1989), I do not strictly define kin vs. non-kin, but rather focus on groups in which individuals may be no more related to their nestmates than they are to non-nestmates. For some of the systems I discuss, non-kinship in social groups has been evaluated with high confidence by inferring relatedness from molecular markers. In many other cases, the presence of non-relatives in social

groups has been inferred from observations of nest-joining behavior, often by individuals from distant nests (in bees and wasps), or of cooperative nest founding by presumed unrelated foundresses (in wasps and ants). Though these observations cannot confirm the degree of relatedness between joiners and their nestmates, they provide suggestions of potential flexibility in tolerance toward unrelated conspecifics. Because the data on kinship in these groups is so incomplete, I highlight these uncertain cases as promising avenues for future genetic investigation.

Within the Hymenoptera, I explore non-kin groups among wasps, bees, and ants, finding limited evidence for true sociality among the sawflies (Hymenoptera: Symphyta), which have short adult lifespans and are non-nesting (Kudo et al., 1998). For each group, I describe patterns and diversity of non-kin social systems. I do not present an exhaustive review of all known non-kin groups in the Hymenoptera, but instead highlight common patterns of social organization across the major suborders.

Wasps: Communal societies and foundress associations

The wasps (Hymenoptera: Apocrita) comprise more than 37 families, among which only three (Aculeata: Pompilidae, Sphecidae, and Vespidae) contain social species (Hunt & Toth, 2017). Non-kin groups are found within all three of these families (Table 1). Communal nesting has been described for several species, and among these, nest-joining by non-relatives is possible, though unconfirmed, for the spider wasp *Auplopus semialatus* (Pompilidae: Pepsinae); (Wcislo et al., 1988), the digger wasp *Crabro cribrellifer* (Crabronidae: Crabroninae); (Wcislo et al., 1985), and the pollen wasp *Trimeria howardii* (Vespidae: Masarinae); (Zucchi et al., 1976). Facultative nest sharing is likewise known among the hover wasps (Vespidae: Stenogastrinae), where unrelated females can join established foundresses (Strassmann et al., 1994; Turillazzi, 2012).

Similarly, among the primitively eusocial paper wasps (Vespidae: Polistinae), foundress associations often form among sisters or other close relatives (Ross & Matthews, 1991; West-Eberhard, 1969), but in many cases may be comprised of non-kin (Hunt, 2007; Mora-Kepfer, 2014; Queller et al., 2000). For the paper wasp *Polistes dominula*, 15-35% of foundress associations consist of unrelated females (Leadbeater et al., 2011; Queller et al., 2000; Zanette & Field, 2008). Co-founding by non-relatives is also known, but uncommon, in *Polistes fuscatus* (Klahn, 1979) and *Polistes exclamans* (MacCormack, 1982). Unlike communal groups, these societies are characterized by high reproductive skew, so unrelated joiners often become subordinate helpers with limited reproductive opportunities (Leadbeater et al., 2010; Mora-Kepfer, 2014; Queller et al., 2000).

Bees: Communal and parasocial societies

Communal nesting occurs across all six major bee families (Kukuk et al., 2005; Wcislo, 1993), and many of these communal groups are known or expected to consist of non-kin. This social strategy is perhaps best known among the sweat bees (Halictidae), which are known for their incredible diversity of social behaviors (Brady et al., 2006; Michener, 1974, 1990, 2007). Halictid communal nesting has been described within the subfamilies Halictinae and Nomiinae; for most of these species, relatedness among communal nestmates is unknown (Michener, 1969; Vogel & Kukuk, 1994; Wcislo, 1993; Wcislo & Engel, 1996), but may be inferred to be low through observations of nest-joining behavior (Abrams & Eickwort, 1981; Michener & Lange, 1958; Richards et al., 2003). Kukuk and Sage analyzed two polymorphic genetic loci among colonies of the sweat bee *Lasioglossum hemichalceum* (Halictidae: Halictinae) and found relatedness within reproductively active nests to be indistinguishable from zero (1994). Communal nesting is present but less common among the Colletid bees (Sakagami & Zucchi, 1978), with low

relatedness ($r = 0.26$) confirmed among nestmates of *Amphylaeus morosus* (Colletidae: Hylaeinae); (Spessa et al., 2000). Similarly, non-kin nesting is possible among the communal Andrenidae (Danforth, 1991; Paxton et al., 1999), and has been confirmed for two species: *Andrena scotica* (formerly *jacobi*); (Andrenidae: Andreninae); (Paxton et al., 1996) and *Macrotera* (formerly *Perdita*) *texana* (Andrenidae: Panurginae); (Danforth et al., 1996).

In other cases, the social organization of some non-kin bee groups is more aptly described by the umbrella term “parasocial,” which includes all associations of same-generation adults, which may be cooperative or non-cooperative, and which may exhibit high or low reproductive skew (Michener, 1974). This is the case for many bees of the family Apidae, which includes both solitary and highly social species. For example, bees in the genus *Exomalopsis* (Apidae: Apinae) form multi-female nests, which may be characterized by cooperative provisioning (Michener, 1966) and even reproductive skew (Raw, 1977). Relatedness in this genus has not been formally investigated, but is likely to be low for many species, considering the high number of females per nest (884 in one nest of *E. aureopilosa*; Rozen, 1984). Non-kin associations could also be found among pleometrotic foundresses of eusocial colonies, though this is rare within the bees. Low relatedness has been described for co-foundresses of the primitively eusocial sweat bee *Halictus ligatus*, likely arising from chance encounters among females emerging from their winter hibernacula (Richards & Packer, 1998).

An interesting case of non-kin sociality exists among the large carpenter bees in the genus *Xylocopa* (Apidae: Xylocopinae). Nest-joining behavior has been observed in several species, in many cases by unrelated bees (Gerling, 1982; Gerling et al., 1983; Hogendoorn & Leys, 1993; Michener, 1990; Peso & Richards, 2011; Velthuis, 1987). However, low relatedness in social groups has only been demonstrated with molecular evidence for two species, *X. sonorina* and *X. virginica* (Ostwald et al., 2021a; Vickruck &

Richards, 2021). Sociality in these groups is not easily classified, given variation and ambiguity in helping behavior, reproductive skew, and generational overlap (Gerling et al., 1989; Hogendoorn & Velthuis, 1993; Michener, 1990). In most cases, a single dominant female per social nest will monopolize egg laying and provisioning behavior, with nestmates potentially contributing to nest guarding (Buchmann & Minckley, 2019; Gerling et al., 1989; Gerling et al., 1983; Hogendoorn & Velthuis, 1999).

Ants: Foundress associations and primary polygyny

In the ants, non-kin sociality through pleometrosis is relatively commonplace in incipient colonies, but usually ends with a queen culling event triggered by worker emergence (Bernasconi & Strassmann, 1999). However, permanent non-kin social groups can form when a pleometrotic queen association extends past worker emergence and into colony maturity. This results in primary polygyny, a group of unrelated worker lineages that share a nest, colony resources, and colony tasks. Importantly, workers in polygynous colonies may be close kin if they were produced by the same queen. Nevertheless, overall worker nestmate relatedness is often low in polygynous colonies (DeHeer & Herbers, 2004; Kellner et al., 2007). More importantly, the queens themselves represent prominent examples of non-kin cooperative behavior, analogous to cooperative breeders in other taxa, regardless of offspring group relatedness. Primary polygyny is generally found interspersed between monogynous colonies or as the majority structure in discrete populations, but has never been documented as the only social structure of an ant species.

Primary polygyny is represented in several ant subfamilies but is especially well documented in the Myrmicinae. Moser & Lewis (1981) first observed multiple unrelated queens in mature colonies of the Texas leaf-cutter ant *Atta texana*. Mintzer and Vinson

subsequently found that these cooperative associations are stable and beneficial to *A. texana* queen survival in the lab (Mintzer, 1987; Mintzer & Vinson, 1985). Shortly afterwards, Rissing et al. (1989) utilized allozyme markers to directly show that cohabiting *Acromyrmex versicolor* queens were not related and also reared stable multi-queen colonies in the lab. There is also genetic evidence, using isoenzymes, that two South American *Acromyrmex* species practice primary polygyny, *A. striatus* and *A. heyeri* (Diehl & Cavalli-Molina, 2001). Multiple, unrelated queens were also found in colonies of *Myrmica gallienii* using enzyme electrophoresis (Seppä, 1996), however colony age was not reported in this study. Primary polygyny may also occur in the fungus growing ant species, *Cyphomyrmex transversus*. Multiple queens were found in 37.7% of colonies examined by Ramos-Lacau et al. (2012) but it is unknown if these queens were related. Within the Myrmicinae, there are also several harvester ant species that practice primary polygyny. *Pogonomyrmex californicus* displays primary polygyny in southern California, as confirmed with field observation (Johnson, 2004), laboratory colonies (Clark & Fewell, 2014; Overson et al., 2014), and microsatellite analysis (Overson et al., 2016). Primary polygyny also occurs in a California population of the seed harvester *Veromessor pergandei*, also confirmed using microsatellites (Helms & Helms Cahan, 2012). Queens of another species in the same genus, *Messor barbarous*, can be induced into stable cooperative associations in the lab, but no polygynous colonies have been found in the field (Provost & Cerdan, 1990).

Within the subfamily Formicinae, the honeypot ant *Myrmecosystus mimicus* also practices primary polygyny in an Arizona population as confirmed by microsatellite analysis by Hölldobler et al. (2011). The mound building ant *Formica podzolica* exhibits primary polygyny in Colorado, as suggested by field excavation (Deslippe & Savolainen, 1995) and confirmed through microsatellite analysis (DeHeer & Herbers, 2004). Finally, multiple unrelated queens have been found in mature colonies of the pleometrotic

weaver ant *Oecophylla smaragdina*, strongly suggesting primary polygyny (Schlüns et al., 2009).

Some of the most detailed genetic and behavioral research has been performed on species in the Ponerinae subfamily. Primary polygyny has been confirmed in *Neoponera inversa* through behavioral observation in the field and lab (D’Ettorre et al., 2005) as well as with multiple microsatellite analyses (Heinze et al., 2001; Kolmer et al., 2002). In a closely related species, *Neoponera villosa*, queen cooperation has been demonstrated in the lab (Trunzer et al., 1998) and unrelated queens have been documented in field colonies (Kellner et al., 2007), strongly suggesting primary polygyny. Mature *Neoponera striata* Smith colonies have also been found with multiple queens, but more work is needed on queen relatedness to confirm primary polygyny (Rodrigues et al., 2011). The arboreal trap jaw ant *Odontomachus hastatus* has been found in colonies containing several queens and workers, but it is unknown if these queens are related (P. S. Oliveira et al., 2011).

Primary polygyny has also been confirmed via microsatellite analysis in two species of the Myrmeciinae: the Australian jumper ant *Myrmecia pilosula* (Qian et al., 2012) and the red ant *Myrmecia rubra* (Pearson, 1982; Barrie Pearson, 1983; Seppä & Walin, 1996).

Finally, in the Dolichoderinae subfamily, Hölldobler and Carlin (1985) found that the Australian meat ant *Iridomyrmex purpureus* is oligogynous, i.e., multiple queens share a nest but do not tolerate each other and relegate themselves to different areas of the nest. Further genetic analysis confirmed that oligogynous *I. purpureus* queens are unrelated and share a workforce (Carew et al., 1997). Oligogyny has also been documented in the subfamily Formicidae (*Camponotus ligniperdus*, Gadau et al., 1998; *Camponotus herculeanus*, Seppä & Gertsch, 1996).



Figure 1.1. Examples of non-kin sociality are widespread across hymenopteran taxa. In the ants, unrelated foundresses may cooperate to rear eusocial colonies, as in the harvester ant *Pogonomyrmex californicus* (top left; photo by Elizabeth Cash). Similarly, foundresses of some wasp species, like the paper wasp *Polistes dominula* (bottom; photo by Meagan Simons), may cooperatively found eusocial nests with non-relatives. Non-kin associations are also found among the communal bees, such as the sweat bee *Agapostemon virescens* (top right; photo by Nicholas Dorian), which shares nest-entrance guarding duties with unrelated nestmates.

Ecological Drivers of Non-Kin Sociality

Group living may have its evolutionary origins across a particular set of ecological conditions that favor nest sharing and/or cooperation (K. E. Arnold & Owens, 1997; Krause & Ruxton, 2002; Rubenstein & Abbot, 2017). For non-kin groups especially, local ecology may be a prominent driver of group formation in the absence of strong indirect fitness benefits. Below, I discuss evidence for the evolution of non-kin sociality in the Hymenoptera as driven by five major ecological conditions/constraints: (1) predator and parasite pressures, (2) intraspecific competition, (3) physiological constraints, (4) productivity constraints, and (5) climatic stressors. Importantly, the distinctions I make between these five factors do not represent mutually exclusive conditions; rather, they are highly interactive and may even represent flip sides of the same environmental selective pressures (e.g., productivity constraints that arise from intense intraspecific competition). Together, these conditions may give rise to fitness differentials between solitary and social individuals when benefits of group living outweigh intrinsic costs of resource sharing.

Predator and parasite pressures

The need for communal defense represents one prominent benefit of nesting with non-kin. In particular, social defensive strategies often arise in contexts where brood is vulnerable to predation or parasitism (Alexander, 1974; Krause & Ruxton, 2002; A. Ward & Webster, 2016a). Importantly, social nest defense can be a passive, emergent property of shared nesting rather than actively cooperative behavior. The presence of multiple females (or even males; Kukuk & Schwarz, 1988) in the nest can deter invaders by decreasing the daily time window in which the nest is unattended (Lin & Michener,

1972; Weislo & Tierney, 2009). In other cases, labor may be divided such that guarding is a functional role of certain group members, often subordinates (Dunn & Richards, 2003; Hogendoorn & Velthuis, 1995). Indeed, task specialization on guarding can even emerge spontaneously among forced, unrelated associations of normally-solitary individuals, suggesting that improved nest defense can arise in communal nests from existing behavioral repertoires. (Holbrook et al., 2013; Jeanson et al., 2005; Holbrook et al., 2009).

Although predator/parasite pressures have been broadly implicated in social evolutionary transitions (Krause & Ruxton, 2002; Lin & Michener, 1972; Michener & Lange, 1958; Wilson & Hölldobler, 2005), empirical demonstrations of the effectiveness of group defense in non-kin systems are sparse. For the sweat bee *Agapostemon virescens*, Abrams and Eickwort (1981) found that communal nests were more effectively defended against the cleptoparasite *Nomada articulata* than were solitary nests. Indeed, Lin and Michener (1972) consider parasite/predator pressures to be the major driver of sociality in the Halictidae (see also Michener & Lange, 1958). Similarly, co-founding wasps may experience reduced predation from birds and mammals relative to solitary foundresses, likely due to more continuous nest guarding (Strassman et al., 1988; Tindo et al., 2008). For other non-kin groups, guarding may function to repel conspecific intruders, but may not be an effective defense against predation and parasitism. For the facultatively social bees *Xylocopa virginica* and *Halictus ligatus*, rates of brood parasitism by Bombyliid flies were found to be no different between solitary and social nests, despite increased guard presence in social nests (Prager, 2014; Richards & Packer, 1998). Similarly, though multiple *Polistes* wasp foundresses may provide effective protection against intraspecific usurpation (Gamboa, 1978; Gamboa et al., 1978; Klahn, 1988), they may be no more effective in guarding against predators (Gamboa, 1978; Gamboa et al., 1978; Gibo, 1978) and parasites (Gamboa et al., 1978) than solitary

foundresses, despite more continuous guard presence (Gamboa et al., 1978). However, co-founding may provide important benefits during recovery from predation attempts (Gibo, 1978; Strassman et al., 1988).

Intraspecific competition and resource limitation

Grouping may arise as a response to limiting resources, especially nesting sites and food (Emlen, 1982; Hatchwell & Komdeur, 2000). Environments characterized by strong intraspecific competition may favor cooperative strategies that allow groups to exploit resources. In many cases grouping occurs in densely populated or saturated environments. Indeed, pleometrosis and primary polygyny in ants have been associated in several species with high population density (Bennet, 1987; Rissing & Pollock, 1991; Trunzer et al., 1998; Tschinkel & Howard, 1983; Rissing & Pollock, 1986; Trunzer et al., 1998). Likewise, for the facultatively polygynous harvester ant, *Pogonomyrmex californicus*, sites dominated by polygyny have higher colony density than primarily monogynous sites (Haney & Fewell, 2018). Further, colonies in the polygynous population have lower reproductive output than colonies from the monogynous population. Experimental food supplementation increased reproductive output of polygynous colonies to that of colonies from the monogynous population, suggesting that competitive, food-scarce conditions drive cooperation in this species (Haney & Fewell, 2018). Similarly, bees may adopt non-kin social strategies under food-scarce conditions, even in the absence of productivity benefits of group living. For the facultatively social carpenter bee *X. pubescens*, solitary nests typically outperform social nests in terms of reproductive output, due to brood mortality that results from dominance competitions in social nests (Hogendoorn, 1996; Hogendoorn, 1991). However, under conditions of food

scarcity, social nesting can provide an important safeguard against pollen robbery, outweighing costs of nest sharing (Hogendoorn, 1991; Hogendoorn & Velthuis, 1993).

Nest sites can also be major limiting resources, favoring social strategies that enable nest sharing and/or increase the likelihood of nest inheritance. Carpenter bees are strongly limited by access to nest sites, creating intense competition for constructed nests that results in frequent supersedure and usurpation (Buchmann & Minckley, 2019; Gerling et al., 1989). Social nesting could feasibly provide an important defense against the threat of nest invasion, but empirical studies have demonstrated that guards of *X. pubescens*, though potentially valuable in preventing pollen robbery, do not effectively defend the nest from usurpers (Hogendoorn & Velthuis, 1995; Hogendoorn & Velthuis, 1993). Instead, subordinate joiners are likely hopeful reproductives that queue for reproductive opportunities upon the death of the dominant bee and subsequent nest inheritance (Hogendoorn & Velthuis, 1995; Richards & Course, 2015; Vickruck & Richards, 2018). Nest inheritance is likewise important for co-founding wasps (Leadbeater et al., 2011; Reeve, 1991), especially for species that reuse old nests (Queller & Strassmann, 1988). Similarly, for many communal bees, group living enables shared exploitation of valuable nest sites (Michener, 1974). In all these cases, intraspecific competition for nests promotes group living and interacts with other ecological constraints, especially energetic and labor constraints on nest construction.

Energetic and physiological constraints

Non-kin groups may also form in contexts that impose steep physiological costs on independent breeders. For example, animals that invest in energetically costly nest building behaviors may experience selection for strategies that reduce founding costs, such as cooperative building and/or nest inheritance (Hansell, 1987). Cooperative nest

building has been documented broadly across Hymenopteran non-kin groups (Bartz & Hölldobler, 1982; Bernasconi & Strassmann, 1999; Danforth, 1991; Hunt & Toth, 2017; Peeters & Andersen, 1989; Rissing & Pollock, 1986; Tschinkel & Howard, 1983; West-Eberhard, 1969). In some cases, these benefits have been linked to ecological conditions and energetic constraints. The ground-nesting communal bee *Perdita portalis* excavates nests through a dense, clay layer of soil, prompting Danforth (1991) to propose energetic costs of nest construction as a major driver of sociality in this environment. Challenging excavation through hard soil may likewise favor cooperative nest construction strategies in the communal bee *Macrotera texana* (Danforth et al., 1996). Carpenter bees may also face particularly high energetic costs of nest building, due to the tendency of many *Xylocopa* species to nest in dense wood substrate. For the carpenter bee *X. sonorina*, the energetic cost of new nest construction is higher on average than the cost of nest inheritance, even accounting for the potential cost of renovating overused tunnels (Ostwald et al., 2021b). In this group, and more broadly, high costs of nest building can underlie intraspecific competition for existing nests. These costs may incentivize social strategies such as reproductive queueing or communal nesting, even at the expense of uncertain reproductive opportunities.

Beyond energetic costs, nest building behavior can impose physiological wear and damage. In arid habitats, nest construction behaviors could be constrained more by desiccation risk than by energetic costs. For many desert ants, nest excavation causes cuticular abrasion that increases water loss rates (Johnson, 2000), exacerbating desiccation risk, which is a major cause of foundress mortality (Johnson, 1998). Cooperative nest excavation during founding poses an important possible solution to this challenge. However, the physiological costs of excavation may not be shared equally among co-foundresses (Fewell & Page, 1999). Cahan and Fewell (2004) measured excavation task specialization in experimental pairs of the facultatively polygynous

Pogonomyrmex californicus, with foundresses collected either from a typically group-founding or typically solitary-founding population. For both populations, more than half of foundress pairs divided excavation labor asymmetrically, with one foundress emerging as an excavation specialist. However, pairs from the group-founding population showed smaller asymmetries in excavation performance (Cahan & Fewell, 2004). These findings suggest that while some foundresses may experience disproportionate costs of excavation, cooperative strategies overall can reduce physiological costs of excavation for a significant portion of the population. Cooperative nest excavation and maintenance may likewise be important for some ground-nesting social bees (Danforth, 1991), but the extent to which nest excavation behavior is physiologically constrained in these groups is still unclear.

Productivity constraints

Cooperation among non-kin can also improve productivity under harsh or competitive conditions. In particular, cooperative founding may provide competitive advantages in conditions that favor rapid nest establishment via worker production. Group founding in ants has been associated both with faster initial worker production and accelerated colony growth (Deslippe & Savolainen, 1995; Eriksson et al., 2019; Ostwald et al., 2021c; Rissing & Pollock, 1987; Tschinkel & Howard, 1983). Rapid production of a large workforce may beneficially accelerate incipient groups through the vulnerable founding period, providing a critical survival advantage for cooperatively founded colonies (Clark & Fewell, 2013; Ostwald et al., 2021c). These advantages may be especially important for colonies vulnerable to intraspecific brood raiding. Cooperative founding has been shown to improve colony survival and success during brood raiding, likely due to the protective effect of larger colony sizes (Bartz & Hölldobler, 1982; Eriksson et al., 2019; Rissing &

Pollock, 1991; Rissing & Pollock, 1987). Increased colony size in multi-foundress nests is also associated with reduced colony failure rates for the paper wasp *Polistes dominula* (Tibbetts & Reeve, 2003). Importantly, cooperative foundresses may experience enhanced colony growth without increasing costly individual investment in sterile worker production. Multi-queen colonies of the harvester ant *P. californicus* experience faster colony growth than single queen colonies, but lower per-queen worker production (Ostwald et al., 2021c). The ability to assemble a large workforce while minimizing individual investment in non-reproductive offspring may represent an important physiological benefit of cooperation with non-relatives.

Specifically, individuals may face productivity constraints associated with resource exploitation. For example, the communal bee *Macrotera texana* faces severe reproductive time constraints due to its foraging dependence on *Opuntia* flowers that bloom for only 2-3 weeks per year (Danforth et al., 1996). Cooperative nest excavation likely enables females to exploit this time-limited resource by accelerating nest founding (Danforth et al., 1996). Similarly, increased colony activity levels in polygynous *P. californicus* colonies suggests both increased worker production and corresponding enhanced efforts to capitalize upon limiting food resources (Haney & Fewell, 2018). In this way, productivity constraints interact strongly with resource limitation and intraspecific competition.

Importantly, worker production benefits may not translate to enhanced production of reproductives. For *P. californicus* as well as for the sweat bee, *Halictus ligatus*, group-founding nests produce more workers but fewer reproductive offspring than solitary-foundress nests (Haney & Fewell, 2018; Richards & Packer, 1998). *Polistes* foundress associations are likewise associated with reduced per-capita reproductive output (Queller & Strassmann, 1988; Reeve, 1991), despite increased worker production in some species (Tibbetts & Reeve, 2003). These cases suggest that cooperation often

functions not as a means to enhance reproductive output under ideal conditions, but rather as a strategy to minimize losses under constraining or challenging environmental conditions.

Climatic stressors

Climatic factors represent fundamental ecological drivers of group living across animal taxa. In particular, cooperation may be favored in harsh or stochastic climates (K. E. Arnold & Owens, 1997; Griesser et al., 2017; Jetz & Rubenstein, 2011; Kennedy et al., 2018; Lukas & Clutton-Brock, 2017; Rubenstein, 2011). In insects, climate likewise mediates the expression of social behavior, especially through impacts on development time and seasonal activity windows, which affect the available time for rearing workers and therefore the potential for colony life to emerge (Eickwort et al., 1996; Fucini et al., 2009; Hirata & Higashi, 2008; Hunt & Amdam, 2005). These factors may be important in the evolution of eusociality by promoting generation overlap in the nest. For non-kin groups, however, that arise from stable cooperative relationships between unrelated individuals, the effects of climate on group formation are relatively unexplored.

Nevertheless, several studies point to prominent roles for climatic conditions, especially environmental temperatures, in facilitating non-kin cooperation. Among *Polistes* paper wasps, which can found nests with non-relatives, cooperative nest founding is associated with high temperature variability, perhaps due to buffering cooperation of sociality in unpredictable environments (Sheehan et al., 2015). Polygyny in ants has also been associated with harsh thermal environments (Heinze & Hölldobler, 1994; Heinze & Rüppel, 2014; Heinze, 1993) and with success of invasive species in their introduced environments (Holway et al., 2002; Tsutsui & Suarez, 2003). Future work

should clarify mechanisms underlying this link between cooperation and success in harsh, variable, or novel thermal environments.

Precipitation can also influence the relative costs and benefits of grouping. Arid environments and drought conditions can increase soil hardness, potentially increasing excavation costs and exacerbating nest limitation for ground nesting bees, ants, and wasps (Michener, 2007; Purcell, 2011; Wcislo, 1997). Under drought conditions, Bohart and Youssef (1976) found that 30% of nests of the normally solitary sweat bee *Lasioglossum galpinsiae* were provisioned by multiple females. In desert ants, group founding may be a by-product of the tendency to seek refuge from desiccating conditions in shared belowground spaces (Pfennig, 1995). Under desiccating conditions, group-founding by the desert seed-harvester ant *Veromessor pergandei* enhanced queen survival and water content relative to solitary queens, though the mechanism for this advantage is unclear (Johnson, 2021). Shared foraging duties could feasibly reduce risk of desiccation in desert habitats. Cahan and Fewell (2004) suggest that a group-founding population of the harvester ant *P. californicus* occupies a habitat with lower and less predictable summer precipitation than sites occupied by solitary founding populations, suggesting possible desiccation constraints. In less arid habitats, extended periods of rain can cause nest failure for ground-nesting species. For the sweat bee *Halictus ligatus*, foundress cooperation may provide protection against rain-induced nest failure through enhanced nest maintenance (Richards & Packer, 1998). As such, like environmental temperature, precipitation can alternately promote or constrain cooperative behavior among non-relatives.

Discussion

Sociality can be understood as an adaptive response to ecological conditions. Non-kin groups present valuable test cases for hypotheses about the ecological drivers of group formation, in particular, because communal and co-founding strategies are nearly always facultative at the individual or population level (Heinze et al., 2017; Michener, 2007; Ross & Matthews, 1991). Studying non-kin groups usefully controls for indirect fitness benefits, thus enhancing our understanding of other, relatively neglected drivers of group formation. These systems have yielded important intraspecific demonstrations of the role of ecology in determining the adaptive value of grouping behavior. Here, I have explored five central ecological factors expected to interact with the expression of social behavior: interspecific pressures from predators and parasites, intraspecific pressures over limited resources, environmental constraints on individual physiology and productivity, and stressors associated with climate. Evidence from across Hymenopteran systems indicates that these conditions play a pivotal role in shaping non-kin social strategies.

Importantly, these ecological drivers of sociality are highly interactive. Efforts to understand sociality across a single environmental axis are limiting and often yield contradictory results (e.g., sociality alternately increasing and decreasing with latitude; Purcell, 2011). Instead, integrative approaches that accommodate these interactions can provide important insights into the complex conditions underlying grouping responses. Studies in Hymenopteran systems have emphasized interactions among intraspecific, interspecific, and abiotic selective pressures. For example, sociality can be a response to intraspecific competition for access to nests (Gerling et al., 1989; Leadbeater et al., 2011). This competition is often a direct product of physiological constraints associated with nest construction behavior (Johnson, 2000; Ostwald, et al. 2021b), which can be

exacerbated by climatic stressors such as low precipitation (Purcell, 2011; Wcislo, 1997). This particular nexus of challenges is an important driver of group formation among the communal and parasocial bees and polygynous ants (Cahan & Fewell, 2004; Danforth, 1991; Danforth et al., 1996). Highly competitive environments can also give rise to cooperative strategies that mitigate worker production constraints experienced by solitary foundresses. Accelerated worker production is a major benefit of cooperation among ant foundresses vulnerable to brood raiding in contexts dominated by intraspecific competition (Bartz & Hölldobler, 1982; Eriksson et al., 2019; Rissing & Pollock, 1991; Rissing & Pollock, 1987). Productivity constraints may also be important drivers of grouping in environments dominated by predation pressures; for group-founding wasps, increased colony sizes can provide essential resilience following predation attempts (Strassman et al., 1988). Together, these examples suggest shared sets of ecological conditions that favor cooperative behavior even when relatedness is low or absent among group members. Importantly, these conditions are not restricted geographically but instead occur at intersections of particular selective pressures that can occur across a wide variety of habitat types.

These findings in non-kin groups of ants, bees, and wasps parallel known drivers of social evolution in non-insect social systems, both kin and non-kin. Ecological constraints are prominent, known drivers of cooperative breeding in birds and mammals (Arnold & Owens, 1997; Emlen, 1984; Emlen, 1982; Hatchwell & Komdeur, 2000; Shen et al., 2017). Inheritance tactics in nest-limiting environments may favor delayed dispersal and nest joining (Emlen, 1984; Woolfenden & Fitzpatrick, 1978). As with the ground-nesting ants and bees, nesting constraints may be physiological, and can be exacerbated by climatic conditions: nest excavation costs in arid conditions have been proposed as a major driver of sociality in the African mole-rats (Faulkes et al., 1997; Hansell, 2005; Jarvis et al., 1994). More broadly, low and unpredictable rainfall has been

associated with the global biogeography of cooperatively breeding mammals (Lukas & Clutton-Brock, 2017). Environmental stochasticity has also been implicated in the global distribution of cooperative breeding in birds (Jetz & Rubenstein, 2011), suggesting important links between cooperation and environmental uncertainty that parallel trends described in *Polistes* foundress associations (Sheehan et al., 2015).

Strengthening the conceptual links among Hymenopteran and vertebrate sociality has great potential for the development of broader evolutionary frameworks explaining non-kin cooperation. Vertebrate research has benefited from a more comprehensive understanding of the taxonomic distribution of kin and non-kin sociality, especially among the cooperatively breeding birds. This knowledge base has enabled valuable phylogenetic studies highlighting the roles of environmental and life history factors in shaping social organization (Cornwallis et al., 2017; Downing et al., 2015, 2020; Riehl, 2013). The social Hymenoptera likewise present special opportunities to study non-kin sociality because it occurs frequently across closely related lineages. To our knowledge, this comparative approach has not yet been applied to the Hymenoptera in the context of kin vs. non-kin social evolution, but may be feasible for those taxa in which non-kin sociality is better documented, especially the polygynous ants.

Beyond this comparative framework, the literature on vertebrate social systems can provide social insect researchers with valuable approaches for studying direct benefits of cooperation. The social vertebrate literature is rich in explorations of the costs and benefits of well-defined cooperative behaviors, from hunting and defending food (Lucas & Brodeur, 2001; Packer & Ruttan, 1988) to detecting and repelling predators (Foster & Treherne, 1981; Hamilton, 1971) or successfully rearing offspring (Ebensperger et al., 2007; Hodge et al., 2009). Likewise, studies should investigate direct benefits of cooperative behaviors in Hymenopteran societies, for example, the effectiveness of nest defense in social vs. solitary bee nests (as in Hogendoorn & Velthuis, 1993; Prager,

2014), or the consequences of shared foraging duties in ant and wasp foundress associations (Cahan & Fewell, 2004). Importantly, the exchange of theories and ideas between vertebrate and invertebrate sociality research should be bi-directional. Insights from Hymenopteran systems have the potential to overcome many of the limitations of work with vertebrate systems. Especially given their short generation times and experimental tractability in lab settings, insect systems have the potential to fill gaps in our broader understanding of the long-term direct fitness outcomes of cooperation over multiple generations.

Current understanding of social evolution among unrelated individuals is constrained by limited knowledge of the full diversity of Hymenopteran taxa that form non-kin groups. The incidence of non-kin cooperation is likely to be greatly underestimated due to the tendency of non-kin groups to occur within otherwise solitary populations (Heinze et al., 2017; Michener, 2007; Ross & Matthews, 1991), and due to limitations associated with quantifying relatedness in some species. This knowledge gap can be addressed with simple behavioral techniques (e.g. mark-recapture or observations of nest joining; Abrams & Eickwort, 1981; Peso & Richards, 2011) and inexpensive genotyping methods (e.g. microsatellites; Moore & Kukuk, 2002). Other techniques, like radio-frequency tracking (Kissling et al., 2014; Sumner et al., 2007), have the potential to reveal nest switching patterns that maintain low relatedness in some insect groups. A first priority in future research on non-kin sociality should be to expand our understanding of the diversity of non-kin systems via integrated behavioral and molecular research. Many of the species highlighted in Table 1 currently possess incomplete evidence for non-kin sociality, especially among the wasps and bees. It is likely that non-kin groups form among many other, related species for which kinship has not yet been quantified. The same may be true for similarly structured social groups outside the Hymenoptera, especially among the termites, which can form polygynous

colonies through colony fusion (Deheer & Vargo, 2008; DeHeer & Vargo, 2004; Korb & Roux, 2012).

Beyond characterizing the organization and formation of these groups, studies that relate social founding strategies to ecological conditions or compare social and solitary strategies in sympatry represent promising directions for future research. Particularly illuminating would be controlled experimental studies relating social condition to ecological conditions and, especially, to fitness outcomes. The abundance of facultatively social non-kin groups provides diverse, experimentally tractable systems in which social condition can be observed and even manipulated within a single species, thus avoiding the pitfalls of comparisons across species with very different evolutionary histories. Manipulative studies such as these could rigorously test hypotheses about proposed drivers of sociality, providing insights into the ecological conditions at the origins of group living.

Conclusions

The ecological drivers of non-kin cooperation represent a highly overlapping suite of conditions that interact to constrain solitary reproductive opportunities. Integrative research that accommodates these interactions has the potential to reveal common principles underlying social evolution broadly across animal taxa and across kin and non-kin groups. Our current understanding of the full diversity of non-kin sociality in the Hymenoptera is highly limited, but existing analyses suggest that groups containing non-relatives are more widespread than previously acknowledged. Future work should quantify relatedness across a diversity of species, and leverage these systems as models for evaluating the ecological conditions that favor group formation. Studies of known non-kin groups in the Hymenoptera have emphasized the role of harsh, competitive

environments in selecting for cooperative strategies even in the absence of indirect fitness benefits. These findings parallel patterns more broadly across animal groups that indicate a major role for ecological constraints in shaping diverse forms of sociality.

Table 1.1. Hymenopteran species with the strongest evidence for non-kin associations.

Where available, I report r values for comparisons among adult female nestmates, often foundresses.

	Taxon	Social Organization	Evidence for Non-Kin Sociality	Within-group r	References
Wasps	Vespidae Stenogastrinae <i>Liostenogaster flavolineata</i>	Primitively eusocial	Allozyme analysis	Not reported for foundresses	Strassman 1994
	Polistinae <i>Mischocyttarus mexicanus</i>	Primitively eusocial	Behavioral observations	NA	Mora-Kepfer 2014
	<i>Polistes exclamans</i>	Primitively eusocial	Behavioral observations	NA	MacCormack 1981
	<i>Polistes fuscatus</i>	Primitively eusocial	Behavioral observations	NA	Klahn 1979
	<i>Polistes dominula</i>	Primitively eusocial	Microsatellite analysis	~0.1 (for 15% of population)	Queller et al. 2000, Zanette and Field 2008
Bees	Andrenidae Panurginae <i>Macrotera texana</i>	Communal	DNA fingerprinting	0.008	Danforth 1996
	Andreninae <i>Andrena scotica</i>	Communal	Microsatellite analysis	~0	Paxton et al. 1996
	Halictidae Halictinae <i>Lasioglossum hemichalceum</i>	Communal	Allozyme analysis	0.07	Kukuk and Sage 1994
	<i>Halictus sexcinctus</i>	Communal or primitively eusocial	Behavioral observations	NA	Richards et al. 2003
	<i>Halictus ligatus</i>	Primitively eusocial	Allozyme analysis	-0.18	Richards and Packer 1998
	<i>Agapostemon virescens</i>	Communal	Behavioral observations	NA	Abrams and Eickwort 1981
	<i>Pseudagapostemon divaricatus</i>	Communal	Behavioral observations	NA	Michener and Lange 1958
	Colletidae Hylaeinae <i>Amphylaeus morosus</i>	Communal	Allozyme analysis	0.26	Spessa et al. 2000
	Apidae Xylocopinae				

	<i>Xylocopa virginica</i>	Parasocial	Microsatellite analysis	0.09 – 0.30	Vickruck and Richards, 2021
	<i>Xylocopa sonorina</i>	Parasocial	Microsatellite analysis	-0.09 – 0.35	Ostwald et al. 2021a
	<i>Xylocopa sulcatipes</i>	Parasocial or semisocial	Behavioral observations	NA	Velthuis 1987
	<i>Xylocopa pubescens</i>	Parasocial or semisocial	Behavioral observations	NA	Gerling et al. 1983, Hogendoorn and Leys 1993
<i>Ants</i>	Formicidae				
	Myrmecinae				
	<i>Atta texana</i>	Eusocial	Behavioral observations	NA	Moser and Lewis 1981
	<i>Acromyrmex versicolor</i>	Eusocial	Allozyme analysis	-0.12	Rissing et al. 1989
	<i>Acromyrmex heyeri</i>	Eusocial	Isozyme analysis	Not reported	Diehl and Cavalli-Molina 2001
	<i>Acromyrmex striati</i>	Eusocial	Isozyme analysis	Not reported	Diehl and Cavalli-Molina 2001
	<i>Myrmica gallienii</i>	Eusocial	Isozyme analysis	0.01	Seppä 1996
	<i>Pogonomyrmex californicus</i>	Eusocial	Microsatellite analysis	0.059	Overson et al. 2016
	<i>Messor pergandei</i>	Eusocial	Microsatellite analysis	~ 0	Helms and Helms Cahan 2012
	<i>Camponotus ligniperdus</i>	Eusocial	Microsatellite analysis; DNA fingerprinting	Not reported	Gadau et al. 1998
	Formicinae				
	<i>Myrmecocystus mimicus</i>	Eusocial	Microsatellite analysis	0.03 – 0.11	Hölldobler et al. 2012
	<i>Formica podzolica</i>	Eusocial	Microsatellite analysis	0.156	DeHeer and Herbers 2004
	<i>Oecophylla smaragdina</i>	Eusocial	Microsatellite analysis	0.08	Schlüns et al. 2009
	Ponerinae				
	<i>Neoponera inversa</i>	Eusocial	Microsatellite analysis	-0.036 (2007)	Heinze et al. 2001; Kolmer et al. 2002; Kellner et al. 2007
	<i>Neoponera villosa</i>	Eusocial	Microsatellite analysis	0.024	Kellner et al. 2007
	Myrmeciinae				
	<i>Myrmecia pilosula</i>	Eusocial	Microsatellite analysis	0.088	Qian et al. 2012
	<i>Myrmecia rubra</i>	Eusocial	Microsatellite analysis; Isozyme analysis	0.041 (1982)	Pearson 1982, 1983, Seppä and Walin 1996
	Dolichonderinae				
	<i>Iridomyrmex purpureus</i>	Eusocial	mtDNA analysis	Not reported	Carew et al. 1997

CHAPTER 2

FLUID NEST MEMBERSHIP DILUTES RELATEDNESS IN CARPENTER BEE GROUPS

Introduction

For many animal groups, kin selection theory has served as the central paradigm for understanding the evolution of social behavior (Hamilton, 1964; Trivers & Hare, 1976; West-Eberhard, 1975). Nevertheless, many animals form social groups with non-kin, and gain little to no indirect fitness benefits from cooperation (Bernasconi & Strassmann, 1999; T. Clutton-Brock, 2009; Riehl, 2013). Particularly within the Hymenoptera, these groups tend to be understudied relative to kin groups, but offer valuable opportunities to test hypotheses about drivers of social evolution while controlling for indirect fitness benefits. However, the extent to which animals form alliances with non-relatives and the mechanisms by which these groups arise remain unknown for many social taxa.

Non-kin groups may arise through shared exploitation of limiting resources, especially nesting sites. These conditions may prompt individuals to disperse and seek reproductive opportunities by joining established groups or constructed nests. Nest joining by non-relatives is common within the cooperatively breeding birds, which may gain direct fitness benefits of cooperation even when relatedness is low (Baglione et al., 2002; Piper et al., 1995; Riehl, 2011; Young, 1998). Likewise, among the communal and polygynous wasps and bees, females may join nests established by non-relatives, where they may benefit from reduced costs of guarding, provisioning, and/or nest construction (Danforth et al., 1996; Johnson, 2004; Mora-Kepfer, 2014; Wcislo & Tierney, 2009; Ostwald et al. 2022).

The large carpenter bees (genus *Xylocopa*) represent useful candidates for testing hypotheses about social evolution, particularly in the context of non-kin sociality.

Carpenter bees may be solitary or may form small, fluid societies in which a single dominant female performs all or most of the egg laying, provisioning, and nest construction/maintenance (Buchmann & Minckley, 2019; Gerling et al., 1989; Richards & Course, 2015). Subordinate females may perform guarding duties but otherwise contribute little to the productivity of the nest (Hogendoorn & Velthuis, 1993; Prager, 2014; Richards, 2011). Instead, subordinates are likely waiting for opportunities to inherit existing nests (Richards, 2011; Schwarz et al., 2011; Velthuis & Gerling, 1983; Vickruck & Richards, 2018), which can be less costly than new-nest construction (Ostwald et al., 2021b).

Because nests are costly and valuable resources, most females will breed in existing nests rather than undertaking new nest construction (Peso & Richards, 2011), which is energetically expensive (Ostwald et al., 2021b). This limitation creates a shortage of available breeding space that can give rise to intense intraspecific competition for reproductive opportunities (Buchmann & Minckley, 2019; Gerling et al., 1989). Following emergence, adult *Xylocopa* often (but not always—see Gerling, 1982; Velthuis, 1987) overwinter with siblings in the natal nest in mutually tolerant pre-reproductive assemblages (Michener, 1990a). These family groups become aggressive at the onset of the reproductive season, prompting dispersal and the formation of dominance hierarchies (Michener, 1990; Richards & Course, 2015; Velthuis, 1987). To secure reproductive opportunities, females may compete for dominance in their natal nests or may attempt to usurp reproductives in nearby nests (Hogendoorn, 1996; Hogendoorn & Leys, 1993; Richards, 2011). Alternatively, females may disperse from their natal nests to join neighboring nests, perhaps seeking to advance their position in a reproductive queue or to minimize competition with close kin (Vickruck & Richards, 2018, 2021).

Nest joining behavior has been observed in several *Xylocopa* species and is expected to create opportunities for association among non-relatives (Camilo & Garofalo,

1989; Gerling, 1982; Peso & Richards, 2011; Velthuis, 1987). Peso and Richards (2010) used mark-recapture techniques to examine the extent of nest joining in the eastern carpenter bee, *Xylocopa virginica*, and found that roughly half of recaptured females were found at a different nest from the one at which they were originally marked. The high rate of relocation can explain low within group relatedness in social groups of this species (Vickruck & Richards, 2021). Aside from this study, genetic relatedness of nesting groups is unknown for any other species of carpenter bee, despite ample behavioral observations indicating that carpenter bees tolerate non-relatives in their nests.

I examined nest joining behavior and relatedness in the facultatively social valley carpenter bee, *Xylocopa sonorina*. Like most carpenter bees, this species is characterized by high reproductive skew and intense nest-site competition (Gerling, 1982). Gerling (1982) observed adult females joining active nests during the reproductive season, and also found that some recently emerged offspring dispersed from their natal nests soon after emergence. I predicted that dispersal and nest relocation may dilute relatedness within nests, leading to mixed associations of kin and non-kin. Using complementary behavioral and genetic approaches, I characterized the dynamic group membership of *X. sonorina* and the consequences of these behaviors for relatedness within and among nesting groups. In doing so, I aim to highlight mechanisms of group formation that can evolve in the absence of helping behavior and indirect fitness returns.

Methods

Study design and X. sonorina seasonal activity

To characterize nest relocation and relatedness patterns in *X. sonorina*, I collected genetic and behavioral data from a single nesting aggregation (an occupied log of Goodding's

willow, *Salix goodingii*, 206 cm length x 23 cm diameter) sourced from a riparian area in Phoenix, AZ, USA (33.41988 N, -112.07062 W). In central/southern Arizona, winter quiescence for *X. sonora* typically ceases in March (Minckley, 1987). Mating activity occurs in March and April (Minckley & Buchmann, 1990), and female reproductive activities, including nest construction/renovation, egg laying, and offspring provisioning, occur primarily in April and May (Minckley, 1987; Ostwald et al., 2020), and offspring emerge in late May to June (Minckley, 1987; Ostwald et al., 2020). This species is univoltine and produces an average of 11.5 brood per nest (Ostwald et al., 2020), laid by a single reproductive female. Nests may be solitary or may contain as many as 9 adults during the spring (Ostwald et al., 2020), though the distribution of group sizes is expected to depend strongly on local factors such as nesting density.

To capture dynamic nest movement behavior across the reproductive season but prior to offspring emergence, I conducted behavioral observations from mid-March to early May of 2021. Likewise, to capture group relatedness at the onset of dispersal and reproductive activity I collected genetic samples in late March to early April of 2019 and 2020.

Behavioral observations of dispersal and nest joining

I used mark-recapture techniques to examine dispersal and nest joining by female bees over the course of the reproductive season. During spring 2021, I caught and/or observed bees entering and departing nest entrances in our focal log (as in Peso & Richards, 2011; Peso & Richards, 2010). Upon first capture of an individual, I recorded the nest of origin and marked each bee with a unique two-color paint marking on the thorax and abdomen using Testors enamel paint (Testors, Vernon Hills, IL). For all subsequent observations I recorded the identity of the bee and the nest of departure or arrival. These nests may have

been natal nests or non-natal nests to which they had dispersed. To estimate total population size, I extrapolated from counts of the number of marked and unmarked female bees entering and exiting the log over the course of one hour at the end of the spring, after all focal bees had been marked.

I observed nest entry and departure activity for 17 days between March 18 and May 2, 2021. On each sampling day I observed bees for 1-4 hrs within the daily window of peak flight activity, for a total observation period of 30 hrs 25 min over the course of the spring. I observed all entries and exits during these observation periods, and recorded the nests visited. Ambient temperatures at the time of observation ranged from approximately 20°C to 34°C.

Genetic analysis

I collected genetic samples by capturing bees upon departure from their nests. I anaesthetized all females on ice then removed the most distal tarsal segment from one metathoracic leg using a sterile razor blade. Removal of this tarsal segment is not known or expected to significantly impair mobility (Vickruck & Richards, 2017). Tarsal samples were stored in ethanol at -20°C for later genetic analysis.

I extracted DNA from all tarsal samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). I amplified DNA at 6 microsatellite regions characterized for the congener *X. virginica* (Vickruck, 2015: XV7, XV9, XV27, XV28, XV30, XV42), having previously confirmed the presence of these loci in *X. sonora* using gel electrophoresis of amplified PCR products. Loci were amplified in three 12.5 µl PCR multiplex reactions of two or three primers per multiplex. Forward primers were tagged with a fluorescent probe (6FAM, TET, PET, HEX, or VIC) for fragment identification (Table 2.1). Genotypes were

analyzed by fragment analysis and scored by visual inspection of the tracefiles using Geneious R8 (Kearse et al., 2012).

Relatedness calculation and statistical analysis

I estimated relative relatedness of sampled individuals using methods developed by Queller & Goodnight (1989), using the R package *related* (Pew et al., 2015). Data are presented as pairwise comparisons of the relative relatedness of all possible pairs of individual female bees in the sample. I used Wilcoxon tests to compare relative relatedness of nestmates (within nest comparison) versus non-nestmates (between nest comparison) within each year of collection. I excluded from analysis any individuals that were missing genotype information at two or more loci ($N = 6$). In addition, I tested for adherence to Hardy-Weinberg equilibrium at all loci, and estimated F_{ST} and F_{IS} between collection years, using the *genepop* package (Rousset, 2008). All statistical analyses were conducted in R 4.1.9 using the *base* and *stats* packages (R Core Team, 2021).

Results

Fluid group membership

Over the course of spring 2021, I marked a total of 75 unique female bees at 25 focal nests. I estimate that there were approximately 147 female bees residing in the log at this time based on the estimated ratio of marked to unmarked bees. Further, I estimate that there were approximately 40 active nests over the spring observation period. Of the 75 marked female bees, I observed 47 bees on more than one occasion, with an average of 2.57 ± 0.22

(range: 1–12) observations per individual. The bees that were only observed once may have dispersed to nests other than our 25 focal nests (including nests in other, distant aggregations) or may not have left the nest during our chosen sampling times. Of the 47 bees observed more than once, 16 (34.04%) were observed only at a single nest. The remaining 31 bees (66.96%) were observed at multiple nests: 19 bees (40.43%) were observed at 2 different nests, 7 bees (14.89%) were observed at 3 different nests, 3 were bees (6.38%) observed at 4 different nests, and 2 bees (4.26%) were observed at 5 different nests (Figure 2.1). No bee was observed re-visiting a nest she had previously occupied.

Importantly, the number of nests visited by each bee is likely to be greater than what I was able to observe during this limited observation period. Many of the 28 bees I marked but did not recapture may have relocated to non-focal nests. For all bees observed more than once, I observed a significant correlation between number of observations of each bee and the observed number of nests visited ($r = 0.49$; $df = 44$, $P < 0.001$). This correlation suggests that more intensive sampling would reveal even lower nest fidelity.

Relatedness within and between groups

I sampled genetic material from 68 adult females in spring 2019 and 2020. In 2019 I sampled 29 females from 12 nests and in 2020 I sampled 39 females from 18 nests. I sampled between 1 and 7 females per nest (mean = 2.27, S.E. = 0.28). Population genetic analyses showed little genetic differentiation between years (across loci $F_{ST} = 0.0488$; Table 2.2). Hardy-Weinberg equilibrium was verified for all but two loci (XS7 and XS30; Table 2.2). In XS7 F_{IS} analysis showed an extreme overabundance of heterozygotes ($F_{IS} = -0.97$). I calculated the relative relatedness of all pairwise comparisons of individuals

sampled (Queller & Goodnight, 1989). In this metric of relatedness, a value of 0 refers to the average relatedness of all individuals sampled. Positive values refer to above-average relatedness and negative values refer to below-average relatedness. A relative relatedness value of 1 indicates that the two individuals share alleles at all six loci tested. In 2019, the estimated relatedness (r) of nestmates (mean = -0.09, SE = 0.15, median = 0.10) was indistinguishable from the relatedness of non-nestmates (mean = 0.10, S.E. = 0.03, median = -0.023; Wilcoxon test: $P = 0.500$); (Figure 2.2). In 2020, the relatedness of nestmates (mean = 0.35, S.E. = 0.07, median = 0.336) was significantly higher than the relatedness of non-nestmates (mean = -0.05, S.E. = 0.02, median = -0.01; Wilcoxon test: $P < 0.001$); (Figure 2.2).

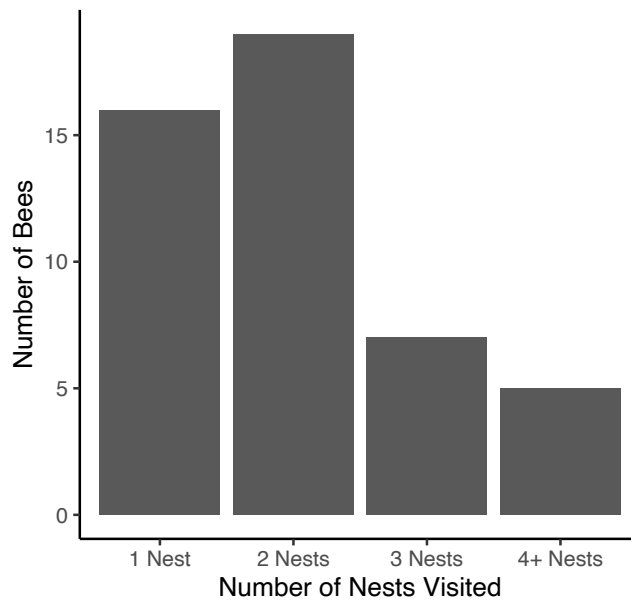


Figure 2.1. Counts of uniquely identified bees observed at 1, 2, 3, or ≥ 4 nests over the course of the 2021 reproductive season.

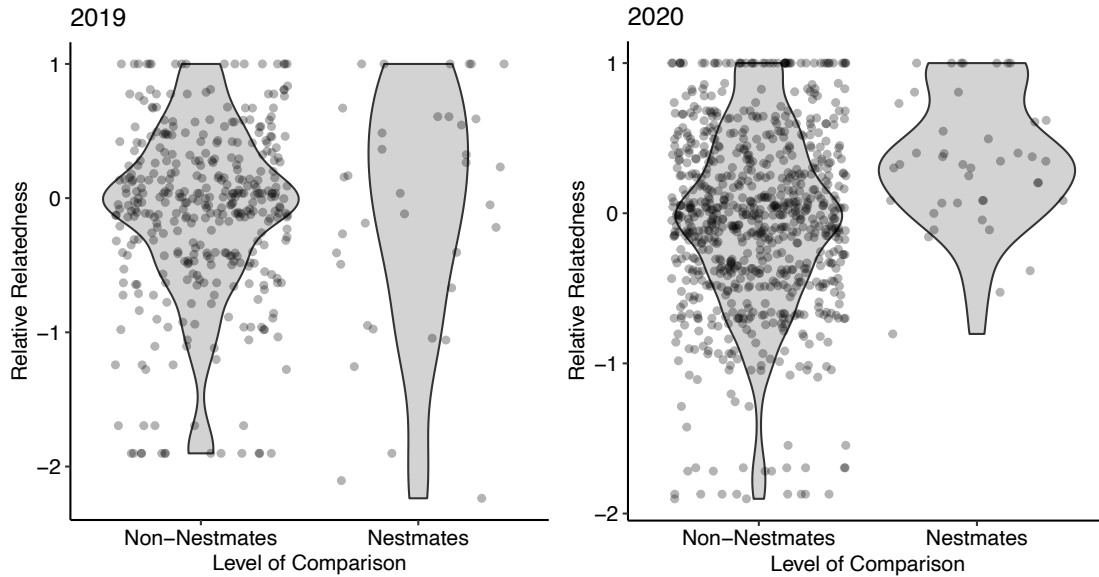


Figure 2.2. Estimated relative relatedness (r) of nestmate vs. non-nestmate females in a single nesting aggregation. Each point represents a single pairwise comparison between two unique individuals in the sample. In 2019 (left), nestmates were no more related than non-nestmates (Wilcoxon test: $P = 0.500$). In 2020, nestmates were significantly more related than non-nestmates (Wilcoxon test: $P < 0.001$). Shaded areas indicate probability density.

Discussion

Choosing whom to live with is one of the most consequential social decisions animals make. Kinship can factor strongly into this decision when individuals receive indirect fitness benefits from helping relatives reproduce. When helping behavior is limited, as for many carpenter bees (Gerling et al., 1989; Prager, 2014; Richards, 2011), incentives for nesting with kin may be likewise minimal. I explored nesting decisions in the valley carpenter bee (*X. sonorina*), which face severe intraspecific competition over nesting opportunities (Gerling, 1982; Ostwald et al., 2021b). I demonstrated that nest joining is common throughout the reproductive season in this species. Joining behavior may prompt

associations between non-relatives. Indeed, I present genetic evidence suggesting a mix of kin and non-kin in nesting groups, with many close relatives nesting apart, and many unrelated individuals nesting together. These highly dynamic social groups raise important questions about the costs and benefits of group living in different social contexts.

Inter-nest migration creates opportunities for individuals to associate with non-relatives. Often, these movements reflect adaptive strategies to access limited reproductive opportunities through resource sharing or cooperation, as in many communal birds and insects (Abrams & Eickwort, 1981; Riehl, 2011; Vehrencamp, 2000; W. Wcislo, 1993; W. Wcislo & Tierney, 2009). In other cases, relocation may reflect usurpation (Hogendoorn & Leys, 1993; Klahn, 1988). In our study, a majority of bees (67%) relocated from the nests at which they were originally captured (similar to rates observed in other Hymenopteran species; (*Megachile rotundata*, Goerzen et al., 1995; *X. virginica*, Peso & Richards, 2011; *Polistes canadensis*, Sumner et al., 2007), and 27% relocated more than once. These moves may be temporary or permanent, reflecting both relocation and perhaps inspection of possible nesting sites. Notably, however, I never observed a bee relocate and then return to her previous nest, suggesting that relocations are often long-term.

The high rates of nest relocation in our study likely represent attempts to seek out reproductive opportunities within saturated nesting space. Bees that relocate may be attempting to usurp dominant reproductives in nearby nests (Hogendoorn, 1996; Hogendoorn & Leys, 1993; Richards, 2011). Alternatively, they may join existing groups as subordinates, but perhaps with a greater chance of nest inheritance than they had in their natal nests (Richards & Course, 2015). Notably, nest relocation was common despite the fact that intruders are usually treated aggressively by resident bees (Hogendoorn & Velthuis, 1995; Velthuis & Gerling, 1983), suggesting that the potential benefits of

relocation can outweigh the costs of physical conflict. The fitness outcomes of the relocation strategy compared with remaining in the natal nest remain to be investigated.

Often termed “drifting,” nest relocation behavior should not be conflated with navigational errors. For example, navigational errors are a well-documented apicultural phenomenon in which honey bees enter unfamiliar hives in crowded apiaries (Free, 1958; R. C. Oliveira et al., 2021; Pfeiffer & Crailsheim, 1998). Studies of inter-nest movements in the paper wasp *Polistes canadensis* and the eastern carpenter bee *X. virginica* found evidence that nest relocation in these species was not merely the result of navigational errors (Peso & Richards, 2011; Sumner et al., 2007). In a study of the navigational abilities of *X. sonorina*, females made very few navigational errors, even in treatments designed to disrupt homing cues (Ostwald et al., 2019). These observations, coupled with the high incidence (67%) of nest relocation in our study, suggest that nest relocation here represents an active strategy rather than simply a consequence of navigational errors.

Our behavioral data support the results of our genetic analysis, which suggests that nestmates are not always close relatives. In 2019, I found that nestmates were no more related to one another than they were to non-nestmates. Frequent inter-nest migration, as observed in our mark-recapture data, was likely to be the mechanism diluting relatedness in these nestmate groups. In 2020, however, I found nestmates to be significantly more related than non-nestmates, despite sampling at the same time of year across sampling years. This suggests that levels of relatedness vary, across years and likely seasonally. If females overwinter with siblings, then I would expect relatedness to progressively decrease over the reproductive season, as bees disperse and are driven from the nest by dominant bees (Richards & Course, 2015; Velthuis, 1987; Vickruck and Richards in review, this issue). Also, annual variation in the timing of environmental cues regulating carpenter bee social phenology (Minckley, 1987; Ostwald et al., 2020) could account for observed

differences across years if, for example, bees began foraging and dispersing later in 2020 than in 2019.

Alternatively, the extent of dispersal and nest relocation across years may depend on factors such as population density and the degree of intraspecific competition. Further sampling throughout the year and across years would usefully clarify the extent to which relatedness changes over time and how these patterns are shaped by environmental factors. Our observed differences in relatedness may be, in part, an issue of limitations on genetic markers. I examined genetic loci characterized for another species, *X. virginica* (Vickruck, 2015), which I demonstrated to be present and variable in *X. sonorina*. However, developing species-specific genetic markers will enable greater resolution of relatedness estimates in future studies.

Why might bees leave their natal nests to join individuals to which they are not closely related? Many non-kin groups benefit from task sharing that improves survival or fitness by reducing the individual labor burden (Bernasconi & Strassmann, 1999; Cahan & Fewell, 2004; Tibbetts & Reeve, 2003; Wilkinson et al., 2016). Carpenter bees, however, do not share the labor costs of reproduction, with only the reproductively active female contributing meaningfully to foraging and nest construction (Richards & Course, 2015; Richards, 2011). As such, additional group members may not improve the productivity of the nest (Buchmann & Minckley, 2019; Prager, 2014). In the absence of helping behavior, the indirect fitness benefits of remaining in the natal nest with relatives are likely to be low or absent. Instead, females may prioritize seeking direct fitness opportunities wherever they may be available, with kin or non-kin.

At the same time, social decisions may not necessarily be made irrespective of kinship. Temporary matrilineal societies may arise from generation overlap between mothers and recently emerged offspring, in which offspring may guard the nest and receive food from their mother (Gerling, 1982; Gerling et al., 1983; Velthuis & Gerling,

1983). Conversely, non-kin nesting may actually represent a strategy to maximize reproductive opportunities among kin. Data from *X. virginica* even suggest that females may actively avoid nesting with relatives during the reproductive season to reduce kin competition (Vickruck and Richards in review, this issue). Indeed, our data show many instances of closely related non-nestmates. Further study is needed to determine whether females can benefit from associating with relatives, and if so, what conditions and life history stages favor these associations.

In conclusion, I found evidence for variable relatedness within carpenter bee nesting groups, suggesting that groups can consist of a dynamic mix of kin and non-kin nestmates. This study represents one of only two to quantify genetic relatedness in *Xylocopa* groups (Vickruck and Richards in review, this issue). Observations of nest relocation in an additional two *Xylocopa* species suggests that low relatedness may be common among the social species in the genus (*X. pubescens*, Gerling et al., 1983; *X. sulcatipes*, Velthuis, 1987). Nest membership in our study was highly fluid, with most females spending time in multiple nests over the course of the reproductive season. This nest relocation strategy likely reflects attempts to secure reproductive opportunities among strongly limited nest sites. Changes in nest membership demonstrate that relatedness is not a fixed condition, but rather may shift with seasonal and social variables. Instances of low relatedness among our sampled bees suggest limited indirect fitness benefits for nestmates, and instead emphasize the importance of ecological factors, especially nesting constraints, in facilitating sociality in *X. sonora* (Ostwald et al., 2020; Ostwald et al., 2021b). Though sociality is often interpreted through the lens of kin selection, systems such as these with low and dynamic relatedness highlight the complexity of social decisions beyond the role of kinship.

Table 2.1. Amplified microsatellite loci with sequence, dye, and multiplex information.

Loci were characterized for *X. virginica* in Vickruck, 2015.

<i>Locus</i>	<i>Primer sequence 5'-3'</i>	<i>Dye</i>	<i>Multiplex</i>
<i>XV7</i>	F: GCTCGACGTACCCTTGCG R: GTGGCAGTGACGTGGTGG	6-FAM	A
<i>XV9</i>	F: ACTCTATTATTCTACATTAGTACGGTTCGC R: TTCGATTTCTGGCCTCTTCG	VIC	A
<i>XV27</i>	F: GAACAAGAGGACGGCAGAGG R: CCAGCACTGCAGACAGTGTACC	PET	A
<i>XV28</i>	F: CCGAGCTTCTGCTCTTCTGC R: CCTACCACCGTCCGATCTCC	6-FAM	B
<i>XV30</i>	F: TTGATATAGCGCCGACCTCC R: TCCTCTCGCCAAGTCTCCC	PET	B
<i>XV42</i>	F: CAACGAATACAAACACCAGGTAGG R: AACCTGCATTCTTGATACGG	HEX	C

Table 2.2. Summarized population genetic information across microsatellite loci, including F_{ST} , F_{IS} , X^2 test for Hardy-Weinberg Equilibrium with associated DF and P-values.

<i>Locus</i>	F_{ST}	F_{IS}	X^2	<i>DF</i>	<i>P</i>
<i>XV7</i>	-0.000178	-0.977047	> 82.1902	6	< 0.0001
<i>XV9</i>	0.042962	-0.240289	5.1609	6	0.5233
<i>XV27</i>	0.039322	-0.044913	5.6141	6	0.4678
<i>XV28</i>	0.041916	0.059614	4.7835	6	0.5719
<i>XV30</i>	0.016704	0.225506	15.4552	6	0.0170
<i>XV42</i>	0.028394	-0.115763	10.2598	6	0.1141

CHAPTER 3
SEASONAL AND NEST ARCHITECTURAL FACTORS INFLUENCE SOCIAL
STRATEGY

Introduction

The initial transitions from solitary to group living are likely facultative, with some individuals in a solitary population adopting a social lifestyle (W. Wcislo & Fewell, 2017; West-Eberhard, 1987). As such, facultatively social species, found among diverse taxa including birds, mammals, and insects, provide valuable insights into the conditions promoting the evolution of social behavior. Studies of the drivers of flexible sociality have variously highlighted ecological factors (Field et al., 2010; Haney & Fewell, 2018), social factors (Kapheim et al., 2015; Lawson et al., 2016), and indirect fitness effects (Reyer, 1984; Yagi & Hasegawa, 2012). Within these broader contexts, individuals must weigh the fundamental considerations of *when* and *where* it is adaptive to be social. In this study, I use CT imaging to characterize a facultative social organization in the carpenter bee *Xylocopa sonorina*, for which both seasonal and spatial selective pressures likely play roles in the formation of social groups.

Complex social behavior often has its evolutionary origins in ecological and spatial constraints that compel organisms to forego independent reproductive opportunities. In particular, environmental factors that change seasonally can drive cyclical variation in social behavior. For example, temporary grouping behavior can maximize seasonal opportunities for resource exploitation (Bos et al., 2004; A. Smith et al., 2019; Watanuki et al., 2004). Additionally, climatic stressors can drive periods of mutual tolerance between conspecifics seeking shared refugia. This is especially the case for temperate species with limited ability to withstand winter conditions outside of hibernacula or aggregations (W. Arnold, 1988; Dapporto & Palagi, 2006).

Habitat saturation and costly nest founding can also encourage social nesting (Barve et al., 2019; Emlen, 1982; Hatchwell & Komdeur, 2000). Inheritance of nesting structures by descendants is a common adaptation to environments lacking in opportunity for dispersers (M. A. Harris & Murie, 1984; Leadbeater et al., 2011; Myles, 1988). Extended multi-generational use of inherited nests may have implicit limitations, however, when nest structures decline in quality over time (Holmes et al., 2003; Moller & Erritzoe, 2006). Furthermore, these changes in nest architecture can shape interactions within societies. Effects of the built environment on social behavior are well established in human societies (Allen, 2000; Baum & Valins, 1977; J. Williams, 2005) and increasingly in the eusocial insects (Pinter-Wollman, 2015; Pinter-Wollman et al., 2017), but spatial determinants of social complexity are underexplored in discussions of social evolution (He et al., 2019).

Here I explore the nest as the interface at which organisms shape their environment and which in turn shapes the social interactions of its inhabitants. To examine interactions among sociality, ecological factors, and the built environment, I observed seasonal changes in the social behavior of the carpenter bee *X. sonora* and correlated impacts on nest architecture. Carpenter bees are known for their diversity of social behaviors (Michener, 1990a; Rehan et al., 2012), with a number of species expressing intra-population social polymorphism (Gerling et al., 1981; Gerling & Hermann, 1978). Nest site limitation has often been invoked as a primary ecological driver of carpenter bee social nesting (Gerling et al., 1989; Gerling & Hermann, 1978). Likely due to this limitation, carpenter bees are strongly philopatric, inheriting and renovating nests from older generations (Michener, 1990a). The ultimate abandonment of these nesting tunnels, however, suggests an eventual functional expiration of inherited nests. One possible mechanism by which nest quality may decline over time is through tunnel widening that accompanies brood rearing activities (Gerling et al., 1981). Wider tunnels require the construction of larger-diameter nest partitions, which may increase the cost of

constructing these partitions. More importantly, however, the diameter of nest tunnels has important implications for within-nest behaviors, particularly dominance interactions, which often involve physically blocking a rival's passage through the nest (Gerling et al., 1981; Hogendoorn & Velthuis, 1993; M. Richards & Course, 2015)/ As such, important behaviors mediating social organization would be compromised by tunnels that are wide enough to permit non-consensual passing.

I conducted a year-long series of CT scans of carpenter bee (*X. sonorina*) nesting logs to test the hypothesis that social organization responds dynamically to seasonal and architectural changes. The destructive nest surveys that are typically used to characterize insect social structure fail to capture important structural changes caused by long-term interactions between the social group and its extended phenotype, the nest. For many taxa, the nest is the site of interactions that are essential for understanding group dynamics; however, it is often inaccessible to non-destructive observation. Recent work has made use of imaging technology to visualize ant and termite nests (Fuchs et al., 2004; Halley et al., 2005; Perna et al., 2008; Varoudis et al., 2018), but these studies have focused on characterizing nest construction by large eusocial colonies rather than examining the ways in which the use of space may shape sociality at its evolutionary origins. Some of the foundational work on carpenter bee sociality has made use of two-dimensional, field X-ray views of nest structures in thin wooden boards (Gerling et al., 1981; Gerling & Hermann, 1978; Hogendoorn & Velthuis, 1993). Our study builds on this foundation by using CT techniques to produce a three-dimensional view of nest architecture and social strategies in natural nesting logs. This approach allows visualization of social structure over time and demonstrates flexible matching of social strategy with seasonal and spatial conditions.

Methods

Population, nesting logs, and X. sonorina life history

To track the social dynamics of naturally nesting carpenter bees, I established a population of *X. sonorina* in a desert riparian research area at Arizona State University (33.42°N, -111.93°W). I collected and relocated two logs (Log A: *Prosopis* sp. wood, 51 cm long, and Log B: *Salix gooddingi* wood, 107 cm long) containing multiple *X. sonorina* nests from local residential and park areas in December 2017. A third log, Log C (*Prosopis* sp. wood, 135 cm long), was used for dissection in February 2018 immediately after it was removed from its original location in a nearby residential area. Each log contained over a dozen previously constructed nest structures in varying stages of decay, only a fraction of which were occupied at a given time. Logs in the courtyard experienced ambient temperature and humidity, and received morning shade.

In the desert Southwest, *X. sonorina* spend the winter inactive in their nests, before emerging in early March (Minckley, 1987). Mating activity generally occurs between March and May (Alcock & Johnson, 1990). Beginning in March, females provision brood and perform any necessary excavation of nesting tunnels (Minckley, 1987). Emergence of adult offspring generally begins in June, after which activity levels taper through the fall into November and December, by which time adults enter winter-quiescence (Robert Lynn Minckley, 1987; personal obs.).

CT scanning

To estimate frequencies of social and solitary nesting strategies, nesting logs were scanned once every two months between January 2018 and March 2019, for a total of eight scans

per log, using a CT machine (General Electric, LightSpeed VCT) at St. Joseph's Hospital in Phoenix, AZ. In January 2018 only Log B was scanned, and in March 2018 only Log A was scanned; for all subsequent scan dates, both logs were scanned together. Scans took place between 1:00AM and 6:00AM to ensure that all bees were inactive inside their nests during scanning. To prevent escape, nest entrances were plugged with cotton prior to transport to the hospital. Scan image slices were 2.5 mm thick and provided in sagittal and coronal views relative to the long axis of the log. Following scanning, logs were placed in their original locations at the research site.

3D reconstructions: Social organization and nest architecture analysis

To assign bees in scan images to their particular nests, I manually reconstructed the 3D structure of nests and visually identified all bees, brood, and pollen inside the nest tunnels (Figure 3.1). 3D images were constructed from scans using Avizo™ version 9.0 (Thermo Scientific™); (Figure 3.2). For each 3D nest structure, I counted the number of unique tunnel branches per nest.

To determine the effect of brood cell construction on tunnel diameter, I measured the diameter of tunnels at their widest point within three brood-cell lengths of the terminal end, in spaces where brood cells had been or would be constructed. I measured diameters of tunnels occupied by brood in the May 2018 scans (for both logs) and compared them to the diameter of these same tunnels before the brood cells were constructed (Log A: March 2018 scan; Log B: January 2018 scan). I also measured tunnel diameter of abandoned nests in both logs in the May 2018 scans. Tunnel diameter was measured using ImageJ version 2.0.0 (National Institutes of Health).

The 3D reconstructions of nests and bees in scan images allowed us to estimate the prevalence of social (multi-female) and solitary (single female) nesting at different times

throughout the year. It is important to note that I was unable to distinguish female from male bees on the CT images. Due to this limitation, any nests that may have been comprised of a single female and one or more males would have been counted as a possible multi-female nest, causing us to *underestimate* the number of solitary nests. Conversely, any nests consisting of a single male would have been counted as a possible solitary female nest, causing us to *overestimate* the number of solitary nests. However, I do not expect these counterbalancing potentials for over- and underestimation to affect observed ratios of solitary to social nests throughout the year. Males are active in the spring through May, when defending mating territories (Alcock & Johnson, 1990; Minckley & Buchmann, 1990), before dying and being replaced by the new generation of males in June (personal obs.). Due to this relative continuity of male presence in nests, I do not expect significant seasonal shifts in sex ratio that would impact our social nest frequency estimates.

To additionally overcome the limitation associated with not distinguishing males and females in scan images, I dissected nesting logs and censused occupied nests near the beginning and the end of the 14-month study. In February 2018, a month after the first scan, I dissected Log C, which was not used for any scans. In March 2019, after all scans are completed, I dissected Logs A and B, the subjects of our series of eight scans. During log dissection, I exposed nesting tunnels with a hammer and chisel, and censused bees in each nest to categorize it as social or solitary. I also measured head widths of females using digital calipers to contextualize our measurements of tunnel diameter.

Statistical analysis

I evaluated the effect of season on social strategy (solitary or social) with a Fisher's exact test. I used Pearson's correlation analysis to test for a relationship between the number of tunnel branches in a nest and the number of adults per nest in July 2018. For our analysis

of nest tunnel diameter, I confirmed normality and homoscedasticity of data with Shapiro tests and Levene's tests, respectively. To determine the effect of brood cell construction on tunnel diameter, I compared tunnel diameter measurements before and after brood cells had been constructed, using a paired *t*-test with a Bonferroni correction for multiple pairwise comparisons. To compare these data to the diameters of tunnels in abandoned nests as well as to the doubled head width of females (a measure of the ease of passing other adults in the nest), I conducted *t*-tests as appropriate and Mann-Whitney U tests when assumptions were violated. *P*-values are reported with Bonferroni corrections for multiple pairwise comparisons. Results are presented as mean \pm standard error. All statistical analyses were performed in R version 3.4.2 (R Development Core Team, 2017).

Results

Phenology of social organization

The total population (Logs A & B) numbered 29 bees when logs were CT scanned in May 2018, during early brood provisioning and before the emergence of the first brood (Figure 3.3). The adult population more than quadrupled to 129 bees after the emergence of brood in the July 2018 CT scans. However, this increase was still less than half the potential population increase suggested by the emergence of 245 brood between May and July. By September 2018, the within-log population had fallen to below 40 adults, probably due to a combination of mortality and dispersal, where it remained until the following year. Overwintering mortality, estimated as the decrease in the number of adults present in scans between November and January, was relatively minimal at 13% (4 bees).

Social and solitary nests co-occurred throughout the year, demonstrating facultative sociality. The number of active nests in the two nests fluctuated throughout the

year between a minimum of 7 and a maximum of 13, due to migration, new nest construction, and nest abandonment. There was a significant effect of time of year on the frequency of social nesting in this population (Fisher's exact: $P < 0.001$); (Figure 3.3). Specifically, social nesting was common (50% of nests or more) throughout the year, except when solitary nesting predominated in May (10 of 11 nests), when females were provisioning their first brood. In July 10 of 13 nests were social, likely because young adults remained in the nest after emergence. Social nests at this time contained 6.50 ± 1.12 adults. In September, nests were split evenly between solitary and social strategies, and social nesting predominated through the winter (Figure 3.3).

Nest censuses from our log dissections indicated that our estimates of social and solitary nesting frequencies were roughly consistent with actual proportions of social and solitary nests, and that error resulting from ambiguous social states was minimal. Of 18 total nests dissected, four contained a solitary female and one or more males (which would have caused an *underestimate* of the number of solitary nests) and two contained a single male (which would have caused an *overestimate* of the number of solitary nests). The counterbalancing effects of these two types of potential error help mitigate the effects of ambiguity in social strategy assignment using CT scans.

Nest architecture

Our 3D reconstructions of nests revealed a diversity of nest structures (Figure 3.4), ranging from a single linear tunnel to large tunnel networks with more than fifteen branches. Additionally, the reconstructions suggested that multiple nests had become connected such that a continuous tunnel structure contained multiple adjacent entrances, probably from formerly independent nests (see blue tunnel network, Log B, Figure 3.2). For those nests occupied when the population reached its peak size in July, I found no

relationship between the number of adults in a nest and the number of branches ($r < 0.01$, $n = 12$, $P = 0.98$).

The construction of brood cells significantly increased tunnel diameter, from 15.06 ± 0.16 mm before brood to 16.80 ± 0.24 after brood cell construction (Paired t -test: $t = -7.43$, $n = 24$, Bonferroni-adjusted $P < 10^{-6}$); (Figure 3.5). Abandoned tunnels (16.87 ± 0.23 mm, $n = 24$) were also significantly wider than tunnels before brood cell construction (Welch's t -test: Bonferroni-adjusted $P < 10^{-6}$), and similar to those after brood cell construction (Welch's t -test: Bonferroni-adjusted $P = 1$); (Figure 3.5). Because measurements of tunnel diameter before brood laying were taken before nest excavation activity had begun, I assume that these tunnels had been constructed the prior season (spring 2017) or earlier. Therefore, it is likely that the tunnels measured in this study had housed brood for at least two consecutive seasons.

The doubled head width of females (a measure of ease of passing in tunnels) (15.27 ± 0.03 mm; $n = 141$) was significantly less than the diameter of abandoned tunnels (Mann-Whitney U test: Bonferroni-adjusted $P < 10^{-7}$) and the diameter of tunnels after brood cell construction (Mann-Whitney U test: Bonferroni-adjusted $P < 10^{-7}$), but no different from the diameter of tunnels before brood cell construction (Mann-Whitney U test: Bonferroni-adjusted $P = 0.93$); (Figure 3.5).

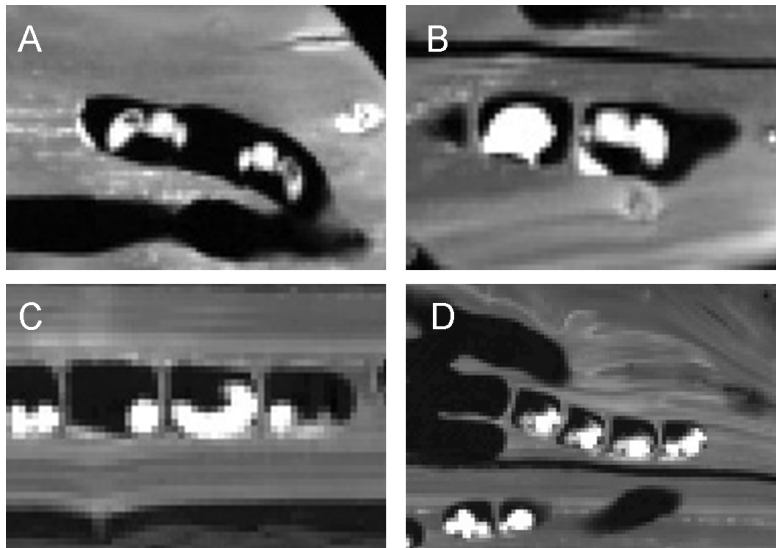


Figure 3.1. Scan images showing nesting tunnels with (a) adult bees, (b) a full pollen provision and a “pollen slant” or in-progress pollen provision tended by an adult bee, (c) brood cells containing larvae, and (d) brood cells containing pupae.

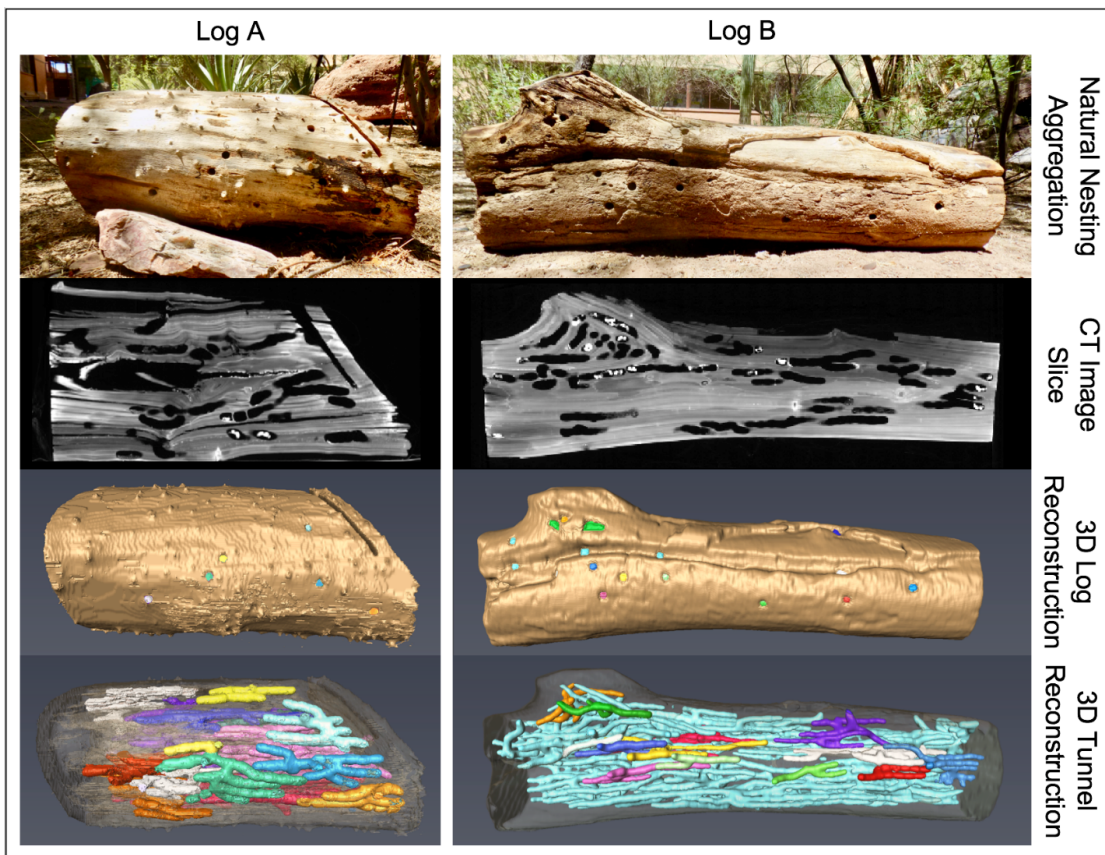


Figure 3.2. Images showing nesting logs, CT scan image slices (July 2018), and 3D reconstructions of logs and nest tunnels. Tunnels and nest entrances shown in different colors represent separate, unconnected nests. The blue tunnel network in Log B consists most likely of a series of passively merged nests.

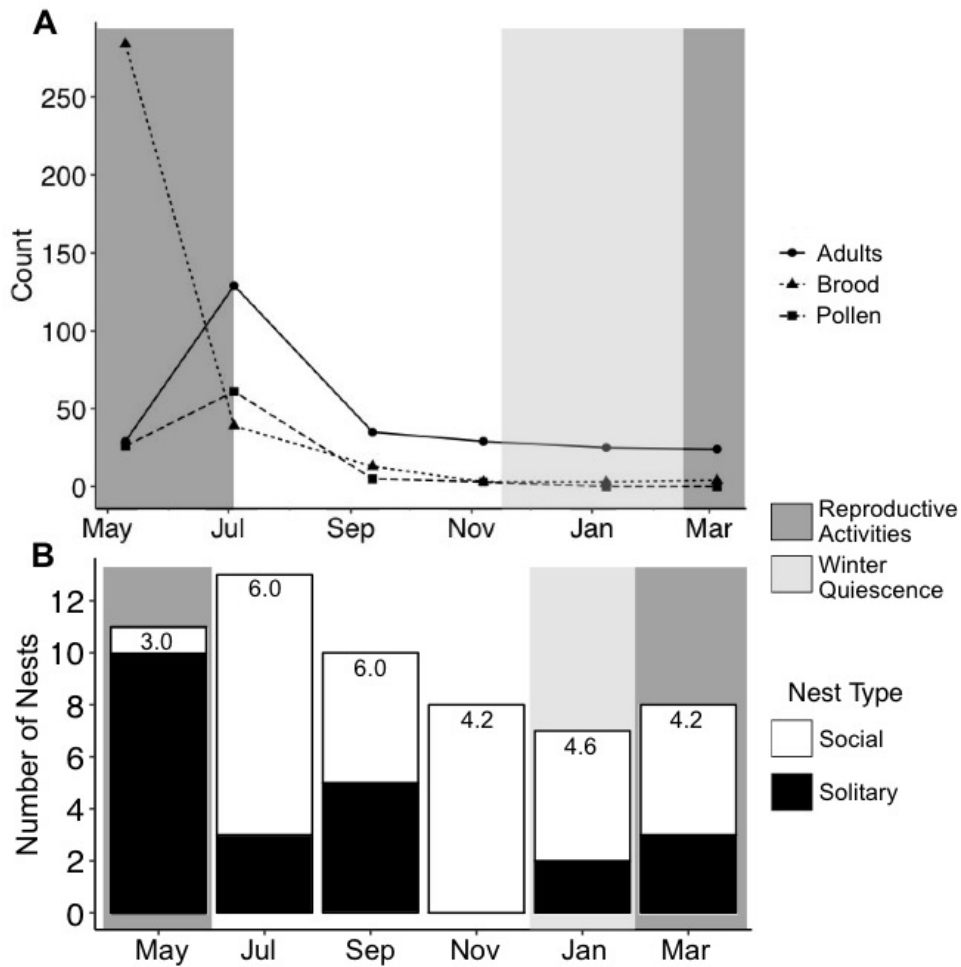


Figure 3.3. Phenology of carpenter bee sociality, represented as (a) total counts across both logs of brood cells including eggs, larvae, or pupae, and “pollen slants” representing the early stage of provisioning future brood. Times shaded orange represent the general period of activity devoted to reproductive activities such as mating, nest construction, and brood provisioning. Times shaded blue represent the period of winter quiescence. (b) Frequencies of solitary and social nests in Logs A and B combined, between May 2018 and

March 2019. Numbers in white bars represent mean numbers of adult bees in social nests. Total nest number changed throughout the year as nests were variously occupied and abandoned. Time of year had a significant effect on the frequency of social nesting ($P < 0.001$).

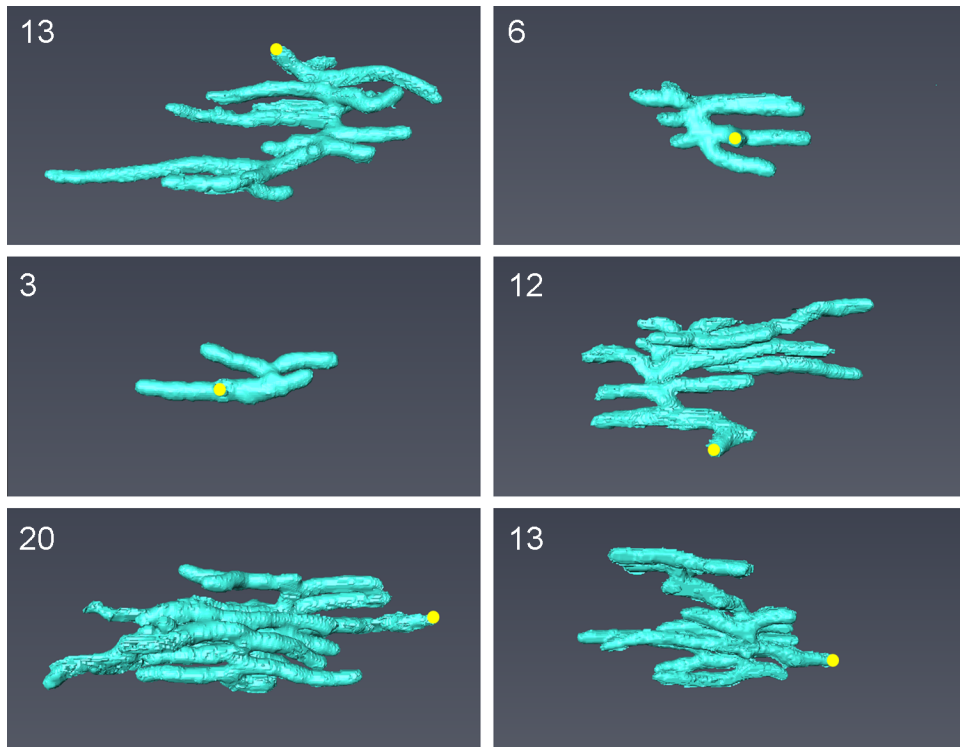


Figure 3.4. Sample of 3D nest reconstructions with number of branches per nest (top left of boxes). Yellow dots indicate the position of nest entrances.

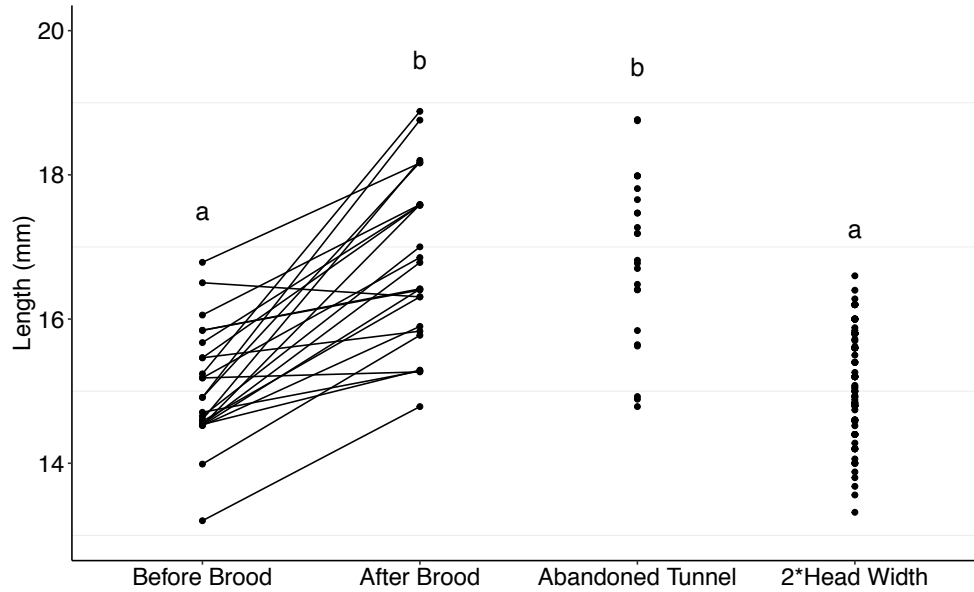


Figure 3.5. Comparison of tunnel diameters before and after brood cell construction ($N = 24$), diameters of abandoned tunnels ($N = 24$), and the doubled head width of females ($N = 141$, from Log C) as a contextualizing measure of ease of passing in tunnels.

Discussion

Plasticity in social behavior allows animals to adaptively match life history strategy to changing environmental conditions. In this study, I used non-invasive, repeated CT imaging of carpenter bee nesting logs to demonstrate flexible sociality strongly influenced by seasonal effects. I also examined the interplay between nest use and nest architecture, and found that reused nests change in a way that has implications for within-nest social interactions. Our results suggest important effects of phenology and nest site limitation on the incidence of social nesting, and capture the fluidity of social behavior in a facultatively social species.

Facultative sociality and social phenology

Alternative social strategies within populations often arise from complex interactions between organisms and their environment. Plastic responses to environmental variables are likely to underlie the evolutionary transition from solitary to group living (Michener, 1974; W. Wcislo & Fewell, 2017; West-Eberhard, 1987). Social organization may shift according to seasonal changes in ecological and behavioral variables, particularly those affecting resource availability, social competition, and climatic challenges. Our results reveal a dynamic social organization likely driven by seasonally fluctuating costs and benefits of group nesting.

Seasonal periods of increased competition can constrain sociality, as in red deer stags, which form bachelor groups for 10 months of the year that break up during the mating season (*Cervus elphaus*; Lincoln et al., 1972), or in female wild sheep, which group when food resources are plentiful and disperse in summer when meadows are impoverished (*Ovis orientalis*; Bon et al., 1990). Social groups of carpenter bees likewise disband when within-group competition increases, as mutually tolerant overwintering groups become competitive and produce dispersers in the spring (Gerling et al., 1989; Hogendoorn & Velthuis, 1993). Our results are consistent with this typical behavioral pattern, with a high incidence of social nests found in winter and predominantly solitary nests found in spring. Seasonal factors are likely to impact important differences in the nature of group living, whether as family-based pre-reproductive assemblages or as female-based reproductive social groups. Because subordinate females in *Xylocopa* nests contribute only minimally, if at all, to brood provisioning (Gerling et al., 1989; M. Richards & Course, 2015), females may prefer to nest solitarily in the spring to minimize reproductive competition and resource sharing.

Social groups may also form according to the seasonality of certain adaptive benefits of sociality. Adaptive group thermoregulation, for example, explains temporary social behavior in winter groups of taiga voles (*Microtus xanthognathus*; Wolff & Lidicker, 1981), sea snails (*Nerita atramentosa*; Chapperon & Seuront, 2012), and night lizards (*Xantusia vigilis*; Rabosky et al., 2012). For the facultatively eusocial sweat bee, *Megalopta genalis*, tropical seasonal variation drives fitness effects of alternative social strategies: social nests reap productivity benefits in the dry season, when resources are abundant, and survival benefits in the wet season, when risk of nest failure is high (A. Smith et al., 2019). Likewise, it may be that seasonal physiological demands encourage group nesting by carpenter bees. In our study, socially nesting bees in the January CT scans appeared in densely packed groups at the terminal ends of tunnels, suggesting potential thermoregulatory grouping. Future studies should examine the effect of social strategy on potential adaptive winter behaviors such as thermoregulation and water conservation. Additionally, in some species, subordinate females play an important role in guarding the nest against pollen robbers when floral resources are limiting (Hogendoorn & Velthuis, 1993). As such, the benefits of tolerating potential rivals in the nest vary with season.

Importantly, a persistent minority of individuals nested socially in the spring and solitarily in the winter. This evidence of persistently facultative sociality indicates that season alone does not govern social behavior. Within-population variation in social behavior has been broadly interpreted as a plastic behavioral response to environmental and social cues (Field et al., 2010; Schradin et al., 2012; Shell & Rehan, 2017b). Flexible social behavior in carpenter bees could allow for matching of life history strategy with variable ecological and social conditions. Furthermore, the mechanisms underlying this plasticity may be central to the evolution of more complex forms of sociality.

Nest architecture and implications for social behavior

Across taxa, passive architectural features of nesting structures are known to influence behavior (Collias & Collias, 1984; Dawkins, 1982; Pinter-Wollman et al., 2017). Human social behavior is often shaped by architecture; connectivity in workspace layouts can influence levels of innovation (Wineman & Davis, 2009) and scientific collaboration (Kabo et al., 2014). More fundamentally, dwelling structural complexity may have been an important precursor to the development of complex hominid societies (Jaubert et al., 2016). Likewise, the relationship between structural and social complexity in insects is becoming increasingly clear (Pinter-Wollman, 2015; Pinter-Wollman et al., 2017; Theraulaz et al., 1998). Our study situates this discussion at the evolutionary origins of sociality.

Our use of non-destructive CT imaging techniques allowed us to track dynamic architectural features and their associated social effects. Like many animals, carpenter bees construct nests that can be inherited and modified by subsequent generations (Laland et al., 2003; Prager & Hunter, 2011; Rau, 1933). As such, I did not observe a relationship between the number of adults in a nest and the number of tunnel branches per nest, since only a fraction of tunnels in large nests is actively used for provisioning brood at a given time. Conversely, Prager and Hunter (2011) found a positive association between foundress number and number of tunnel branches in *X. virginica*, but this species generally constructs smaller, less branched nests than *X. sonorina* (Gerling & Hermann, 1978). Our results suggest that tunnels, and even whole nests, may become abandoned when their over-use leads to tunnel widening. Carpenter bees lay eggs in linear sequence along tunnels, with each egg separated by partitions made of wood pulp. Females gather raw materials for this pulp by chewing wood from the sides of the tunnel (Gerling et al., 1981), such that the annual need for new partition material may cause tunnels to

progressively widen over years of reuse (Gerling et al., 1983). The preference for newer, narrower tunnels likely motivates nest renovation and produces larger nest structures with many branches.

The width of tunnels shapes social interactions because it determines bees' ability to effectively defend valuable food provisions and vulnerable offspring, both from invaders and from rival nestmates. When a pair of bees meet in a nest tunnel and one attempts to pass by, the other has the option to either block her, back up, or manoeuvre her body such that both can move past one another, ventral side to ventral side (Brothers & Michener, 1974). As such, successful passing is usually interpreted as a tolerant behavior, because it entails mutual maneuvering in a position that exposes each bee to the other's mandibles and stinger (Arneson & Wcislo, 2003; Breed et al., 1978; Peso & Richards, 2010). Spatial effects on the ease of passing nestmates thus can influence within- and between-group social dynamics (Jeanson et al., 2005).

Prevention of passing is associated with social dominance in Xylocopine bees (Michener, 1990a). Dominant *X. pubescens* females prevent subordinates from entering nest tunnels used for provisioning brood, both because adults may consume pollen from growing "pollen slants" intended for larvae, and because usurpation events involve destruction of the dominant's brood (Hogendoorn & Velthuis, 1993). Females are likely reluctant to lay brood in wide tunnels that they are unable to defend. Furthermore, tunnel blocking is an important behavior mediating food sharing. Mother bees of *X. pubescens* are accosted by hungry progeny upon return from foraging trips and are prevented from passing until they offer a food concession to their offspring, who jockey for positions nearest the nest entrance in order to receive the largest share of food (Gerling et al., 1981, 1989). Wider than average tunnels would permit non-consensual passing that would neutralize the effectiveness of behaviors maintaining dominance hierarchies and nest defense. Thus, the architecture of nest tunnels and their progressive restructuring via

repeated use may impact nest utility over time, intensifying the competition over already scarce nesting resources.

Conclusions

Environmental factors are known to shape social behavior (Emlen, 1982; Lion & Gandon, 2009), and, conversely, social interactions can dictate the spatial use of the environment (Pinter-Wollman et al., 2017; M. L. Smith et al., 2015; Theraulaz et al., 1998). Our study provides evidence for the reciprocity of these effects by characterizing a seasonal effect on social behavior and an effect of nest reuse that may influence social interactions. I found that group nesting is common throughout the year, but disfavored during brood provisioning when reproductive competition encourages dispersal from the natal nest. Furthermore, the functional deterioration of nests caused by multi-generational reuse leads to nest abandonment and exacerbates nest site competition. These interactions between spatial constraints and social competition are likely to underlie plasticity in social behavior more broadly.

CHAPTER 4

ENERGETICALLY COSTLY NEST CONSTRUCTION DISINCENTIVIZES SOLITARY NESTING

Introduction

Sociality can arise as an adaptive strategy to mitigate the costs of independent breeding [1,2]. Especially in risky, unpredictable, or harsh environments, individuals may have little chance of success without the buffering advantages supplied by the social group (Cahan & Julian, 1999; Kennedy et al., 2018). In particular, group living can provide shared access to limiting resources, especially habitat space (Koenig et al., 1992; Lion & Gandon, 2009). As such, environments lacking in opportunities for dispersers can provide fitness incentives for offspring to remain at the natal nest (Hansell, 1987; Myles, 1988), or for individuals to form cooperative alliances (Barve et al., 2019; Haney & Fewell, 2018). Thus, evolutionary transitions from solitary to group living may be facilitated by adaptive avoidance of costly life histories.

These costs of independent breeding may be energetic in nature. Relative to group-living animals, independent breeders may make greater personal investments in energetically costly behaviors such as dispersal, foraging, and nest founding (Benoit et al., 2019; Cahan & Fewell, 2004; Slobodcickoff, 1984). Nest building behavior in particular has been implicated as a context favoring the evolution of sociality (Hansell, 2005). Animals living and breeding in complex nests often need to expend considerable energy gathering building materials and then constructing and maintaining these structures. The substantial energetic expense associated with nest construction has given rise to various strategies that circumvent or minimize such costs. Nest inheritance is one such tactic common across diverse cooperative taxa (Emlen, 1984; Myles, 1988; Woolfenden & Fitzpatrick, 1978). Inheritance strategies can result in group living if offspring delay

dispersal or fail to disperse from the natal nest (M. A. Harris & Murie, 1984; Koenig et al., 1992; Schwarz et al., 2011). Alternatively, social groups may form when individuals seek access to non-natal nests via usurpation or nest joining, often delaying reproduction until inheriting the nest (Hogendoorn & Leys, 1993; Keller & Reeve, 1994; Young, 1998). High nest building costs thus favor sociality as competitors saturate low-cost nesting opportunities.

The social implications of founding costs are particularly observable in facultatively social animals, which plastically express social behavior in response to ecological and social variables. Because initial transitions to group living are likely to be facultative (W. Weislo & Fewell, 2017), these systems provide important insights into conditions shaping sociality at its evolutionary origins. Studies of facultatively social animals have emphasized the roles of genetic, ecological, and social factors in shaping sociality (Kapheim, 2017; Montero et al., 2020; Schradin et al., 2012; Shell & Rehan, 2017a, 2019). However, the evolution of sociality in these groups has rarely been explored from an energetic perspective. Energetic considerations are likely to factor strongly into the context-dependent behavioral decisions underlying facultative sociality.

With their flexible sociality and impressive nest construction behaviors (Buchmann & Minckley, 2019; Gerling et al., 1983, 1989; M. H. Richards, 2011), the large carpenter bees (genus *Xylocopa*) represent ideal candidates for examining the influence of energetic costs on social evolution. Unlike bees that nest in hollow cavities or soft piths (Michener, 1974), many carpenter bees construct their nests by boring tunnels into wood with their mandibles, a process that is predicted to entail significant time and energetic costs (Gerling et al., 1989; Louw & Nicolson, 1983). Perhaps due to these costs, some carpenter bees seek reproductive opportunities in already-constructed nests rather than undertaking the construction of a new nest (Hogendoorn & Velthuis, 1993; M. H. Richards, 2011; Vickruck & Richards, 2018). This nest inheritance strategy leads to the

formation of fluid, often ephemeral nesting groups comprised of kin and/or non-kin, often with overlapping generations (Gerling et al., 1989; Hogendoorn & Velthuis, 1993). The carpenter bee *X. sonorina* nests either solitarily or in small groups, in which only a single female dominates egg laying, foraging, and excavating behaviors at a given time [35,36; note that *X. varipuncta* has been synonymized with *X. sonorina*, 37]. As such, additional group members do not strongly reduce the individual labor burden for dominant bees, aside from potential shared nest guarding [36, but see 34,39]. In the absence of strong helping behavior, sociality may be influenced more strongly by nesting constraints (Gerling et al., 1989; Michener, 1990a). While nesting substrate is not expected to be limiting in our study population, costs of exploiting available substrate may constrain founding behavior. Nest inheritors could reap substantial energetic savings if metabolic costs of construction are high. These savings are not guaranteed, however, and benefits could vary considerably across nests when inheritance entails addition or expansion of tunnels to replace over-used structures (Ostwald et al., 2020).

In this study, I investigate the energetic costs underlying social and life history strategies in *X. sonorina*. As with many nest-building species, sociality in carpenter bees has been widely attributed to the costs of new nest construction (Gerling et al., 1989; Louw & Nicolson, 1983; Michener, 1990a); however, the energetic costs of constructing a nest have been difficult to quantify empirically. Using respirometric techniques coupled with 3D structural analysis enabled by computerized tomography (CT) imaging, I directly measured metabolic costs during nest construction. From these data, I estimate the metabolic cost of nest excavation per offspring provisioned, providing a fitness-relevant estimate of this neglected component of offspring production costs. In doing so, I provide quantitative support for the claim that high excavation costs underlie nest limitation. Further, I quantify costs of nest renovation behavior to understand the range of costs

incurred by nest inheritors. Our results demonstrate that avoidance of costly nest building may provide an important fitness benefit of sociality.

Methods

To assess metabolic costs of various behaviors associated with reproduction, I measured the metabolic rates of female *X. sonora* during excavation, flight, and resting behaviors. Experiments were conducted between March and July 2019 (the season of peak nest excavation activity) using free-living bees nesting at Arizona State University in Tempe, Arizona (33°25'12" N, 111°55'48" W). Bees were captured at dawn upon their first departure from the nest to ensure that none had fed that day, because feeding status influences metabolic rate (Gmeinbauer & Crailsheim, 1993). Bees were weighed upon capture (A&D GR-200; repeatability 0.0001 g).

Respirometry

To measure metabolic rates of excavating bees (*Xylocopa sonora*, female mass = 0.95 ± 0.02 g), I passed dry, CO₂-free air through a 15 mL syringe containing a bee and a small (8 cm³) cube of wood. Dry, CO₂-free air was supplied at 500 mL/min by a FTIR Purge Gas Generator (Parker-Balston, Palmer, MD) connected to 1-L columns of cobalt-doped copper (II) sulfate (Drierite, W.A. Hammond Drierite Co Ltd, Xenia, OH, USA) and NaOH (Ascarite II, Sigma-Aldrich, St. Louis, MO, USA). Each bee was given either balsa (*Ochroma pyramidale*), a soft wood (density = 0.089 g/cm³, $N = 18$) or Goodding's willow (*Salix gooddingii*), a hard wood species (density = 0.539 g/cm³, $N = 15$) that I sampled from a log occupied by nesting *X. sonora*. These wood species are both known to be used by *X. sonora*, and span much of the range of densities of known nesting substrates for

this species (Hurd, 1978; Ostwald et al., 2020). The respirometry traces were closely matched to behaviors; I only reported CO₂ emission rates for bouts of digging behavior that were longer than 5 s, which is greater than the 95% washout time for our system (time constant 0.03 min, 2.5 s for 95% washout).

For comparison to excavation metabolic rate, I measured metabolic rates of resting bees and bees in flight, using flow-through respirometry as above. For quantification of resting metabolic rate, I only used bees that exhibited no locomotion or activity during measurement ($N = 10$). For flight metabolic measurements, I only included bees that achieved sustained hovering behavior for a minimum of 10 s and recorded steady-state CO₂ emission during this flight period ($N = 17$). Bees were motivated to fly by agitation of the chamber prior to the 10 s hovering period and orientation of the chamber toward a lit window in a darkened room. Resting bees were measured in 15 mL syringes with air supplied at 500 mL/min, and flying bees were measured in a 465 mL glass chamber with air supplied at 1850 mL/min.

Flow rate (STP) was regulated using a Flowbar-8 Mass Flow Meter System (Sable Systems International, Las Vegas, NV). Excurrent CO₂ was measured using a Li-Cor 6252 CO₂ infrared gas analyzer (Li-Cor, Lincoln, NE, USA). Measurements were digitized (UI-2, SSI) then recorded and analyzed using the ExpeData data acquisition software, version 1.9.13 (SSI). Mean VCO₂ (mL/min) was calculated using the equation:

$$VCO_2 = FCO_2 \cdot STP \text{ Flow rate}_{mL/min}$$

where FCO₂ represents the fractional content of CO₂ in the excurrent stream (Lighton, 2008). Conversions from VCO₂ to watts assumed a respiratory quotient (RQ) of one for metabolism of simple carbohydrates (Gäde & Auerswald, 1999). All measurements were taken at $25 \pm 1^\circ\text{C}$ to control for temperature effects on metabolic rate; further, I observed that bees initiated daily flight activity at ambient temperatures roughly near this temperature range.

Behavioral analysis

I continuously observed each bee in the respirometry chamber for 30 minutes and recorded the time periods spent excavating. To calculate average excavation rate, I weighed wood cubes before and after the sampling period to measure total mass of wood excavated, and divided by the total time spent excavating.

CT scans and nest volume reconstructions

To measure nest structure volumes and assess the amount of nest excavation that occurs within a single breeding season, I CT (computerized tomography) scanned a nesting log occupied by *X. sonorina* at three different time points: 1) during winter quiescence, prior to the breeding season (Jan 2018), 2) during peak brood production activity (May 2018), and 3) after the end of the breeding season, by which time nest excavation activity has ceased (Sept 2018). The scanned log also provided the hard willow wood used in the respirometry trials, to ensure equivalent wood density in our estimations. Scans were conducted using a CT machine (General Electric, LightSpeed VCT) at St. Joseph's Hospital in Phoenix, AZ, with 2.5 mm-thick scan image slices provided in sagittal and coronal views relative to the long axis of the log. I manually reconstructed the 3D structure of nests in scans and calculated their volumes using the imaging software Avizo™ version 9.0 (Thermo Scientific™); (Ostwald et al., 2020). Additionally, I measured brood production rates by counting the number of occupied brood cells per nest on the May scans. This population of *X. sonorina* is primarily univoltine, with most brood emerging between late May and early June (Ostwald et al., 2020).

Estimations of energetic costs of nest construction and renovation

I used nest volume measurements and metabolic data to calculate 1) costs of nest excavation per offspring, and 2) costs of nest renovation. I chose eight focal nests that were occupied during the period of active brood production (May), and measured their volumes prior to (Jan), during (May), and after (Sept) the breeding season. The change in nest volume over the course of the entire breeding season (Jan – Sept) provided us with an estimate of the potential renovations necessary for a female breeding in an existing nest. Additionally, I measured the volume of the tunnel space used to house a single developing offspring (a “brood cell”); ($N = 30$). The majority of occupied nest volumes consist of brood cells, but additional space is constructed at entrances, to join adjacent nest tunnels, and to house adult bees. As such, brood cells represent only a portion of total excavation costs, and a conservative estimate of per-offspring excavation costs.

These measured volumes, the average metabolic rate of hard wood excavation, and the rate of hard wood excavation were used to calculate the total energetic expenditure associated with excavation of different nest volumes, according to the following equation: energetic cost (J) = metabolic rate ($W = J/s$) x volume excavated (cm^3) ÷ rate of excavation (cm^3/s). I used hard rather than soft wood metabolic rate in this calculation because the wood excavated in hard wood trials was sourced from the same log I CT scanned for nest volume analysis. In this way, I controlled for potential interactions between wood hardness and nest volume. Finally, to contextualize the measurements of metabolic costs, I calculated a flight-time equivalent of these energy costs using the measurements of flight metabolic rate. Flight provides relevant energetic context as it is a necessary provisioning behavior for reproductive *Xylocopa* (Gerling, 1982; Gerling et al., 1989; M. H. Richards, 2011), and because insect flight is among the costliest animal behaviors (Feuerbacher et al., 2003; R. K. Suarez, 2000).

Statistical analysis

To assess differences in mass-specific metabolic rate among resting, flying, and excavating bees, I used a Kruskal-Wallis test. I ruled out analysis of variance due to departures from normality and homoscedasticity revealed by Shapiro's tests and Levene's tests, respectively. I performed pairwise Wilcoxon tests on treatment pairs for post-hoc analysis, because individual bees were measured in all three behavioral activities: excavating, flying, and resting. Similarly, I used Mann-Whitney U-tests to assess differences in excavation rate and excavation rate-adjusted metabolic rates. I used linear regression to examine the relationship between metabolic rate and excavation rate, and analysis of covariance (ANCOVA) to assess the nature of this relationship with respect to wood hardness. Samples for this analysis were independent; individual bees were either measured excavating hard wood or excavating soft wood, but not both. Results are presented as mean \pm standard error. All statistical analyses were performed in R v 3.4.2 (R Development Core Team, 2017).

Results

Metabolic rate during nest excavation, resting, and flight

Metabolic rate during nest excavation (0.016 ± 0.002 W, $N = 33$) was significantly higher than the metabolic rate of resting bees (0.005 ± 0.001 W $N = 10$, Wilcoxon rank sum test: $P < 0.001$), but significantly lower than the metabolic rate of bees in flight (0.179 ± 0.008 W, $N = 17$, Wilcoxon rank sum test: $P < 0.001$); (Figure 4.1). This flight metabolic rate was lower than previous estimates for *Xylocopa* (Chappell, 1982; Gäde & Auerswald, 1999;

Nicolson & Louw, 1982; Roberts et al., 2004), such that the estimates of flight-time equivalents for excavation behavior may be conservative. Resting metabolic rate was similar to that recorded for *X. capitata* (Gäde & Auerswald, 1999). My results indicated a significant positive linear relationship between metabolic rate and excavation rate both for the soft wood ($P = 0.037$, $N = 18$, adjusted $R^2 = 0.196$) and the hard wood ($P = 0.008$, adjusted $R^2 = 0.391$, $N = 15$) Regression lines did not differ in slope (ANCOVA: $P = 0.750$) or intercept (ANCOVA: $P = 0.245$). Wood hardness did not significantly affect metabolic rates of excavators (Mann-Whitney U-test, $P = 0.34$), but bees excavating soft wood excavated at a significantly faster rate (soft wood: 1.62 ± 0.12 cm³/hr, hard wood: 0.12 ± 0.02 cm³/hr, Mann-Whitney U-test : $P < 0.001$). As such, when mass-specific metabolic rate is adjusted for excavation rate, excavation of hard wood (621 ± 181 J cm⁻³) is significantly more energetically costly than excavation of soft wood (42.1 ± 3.96 J cm⁻³; Mann-Whitney U-test: $P < 0.001$); (Figure 4.1).

Energetic costs of nest construction and renovation

To estimate the energetic costs of nest construction and renovation, I repeatedly measured the volumes of nest structures across a single breeding season (Figure 4.2). Whole nest structures measured prior to the breeding season (Jan) averaged 257 ± 41.2 cm³. By the time brood production was underway (May), these nests had increased in volume by $47.7 \pm 23.8\%$ to a total volume of 353 ± 57.3 cm³. Between May and the end of the season (Sept), nests were further expanded by $10.2 \pm 4.52\%$ to a final average volume of 380 ± 53.5 cm³, for a total expansion of $63.1 \pm 28.2\%$ or 122 ± 32.8 cm³ across the entire breeding season. The age and full history of these nests prior to this year of observation is unknown. As such, nest measurements taken in January may include older nests that had undergone some expansion in previous years.

I used these volume measurements to estimate energy investment associated with brood production and nest renovation (Figure 4.2). Spring CT scans indicated that bees in this population provisioned 11.5 ± 1.81 offspring per nest (therefore, per reproductive female) over the reproductive season. To excavate the space necessary to rear a single offspring (a brood cell; $11.9 \pm 0.21 \text{ cm}^3$) within a nest would require $4.3 \pm 0.1 \text{ kJ}$ of energy. A female bee using an existing nest and undertaking nest renovations of the average volume observed in this study would require $44.8 \pm 1.2 \text{ kJ}$ of energy, (range: $7.08 \text{ kJ} - 89.1 \text{ kJ}$).

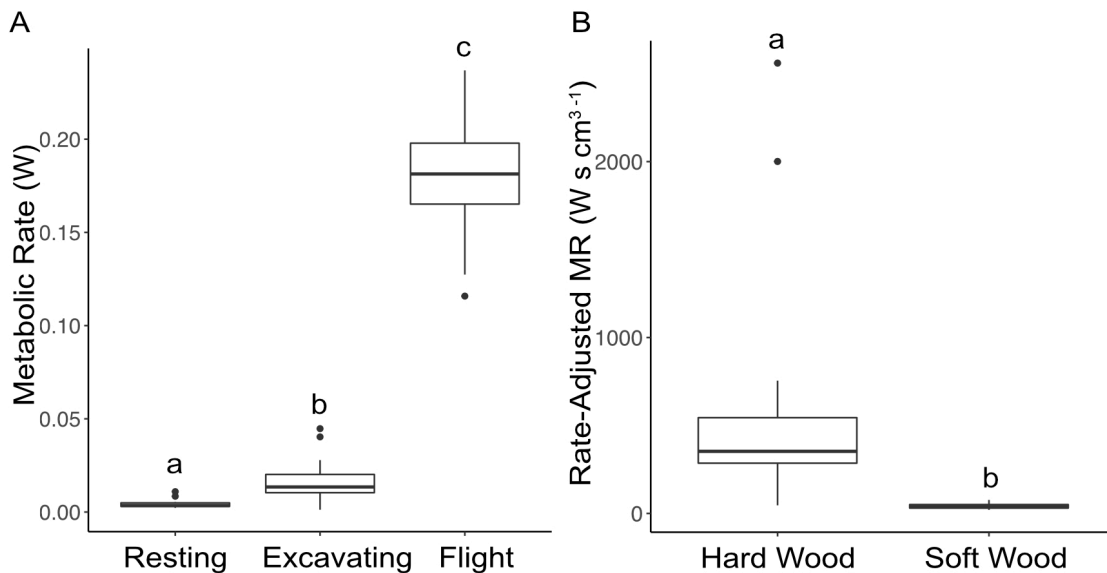


Figure 4.1. (a) Metabolic rates (W) of bees during resting, excavation, and flight. The metabolic rate of bees during wood excavation was significantly higher than that of resting bees ($P < 0.001$), but lower than that of flying bees ($P < 0.001$). (b) Cost of excavation (J cm^{-3}) is significantly higher for excavation of hard wood than for soft wood ($P < 0.001$).

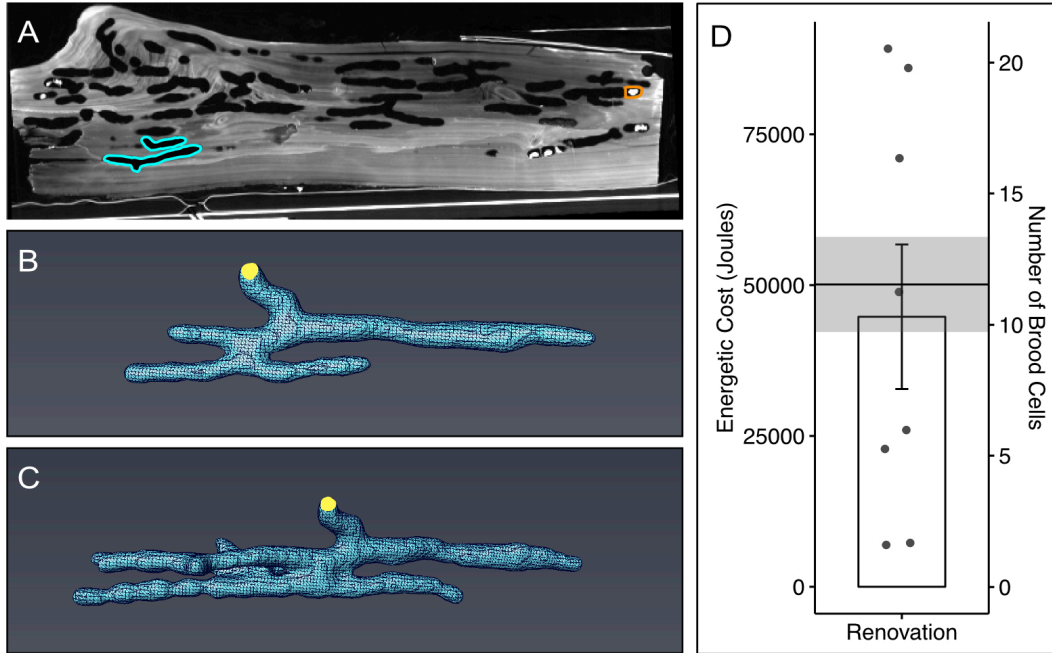


Figure 4.2. (a) CT scan image slice of an occupied nesting log, showing one focal nest, highlighted blue, and one brood cell, highlighted orange. (b) Three-dimensional reconstruction of focal nest prior to the breeding season (January) (c) Three-dimensional reconstruction of the same focal nest after the breeding season (September). The change in tunnel volume from (b) to (c) represents the renovation volume excavated by the female who inherited this nest. Yellow dots indicate the position of nest entrances. (d) Estimates of energy costs (J) to renovate a nest (left axis; $n = 8$ nests). The axis on the right corresponds to estimated energy costs associated with excavating brood cells (i.e., one brood cell = space to provision a single offspring). The horizontal line and grey region represent the observed mean \pm s.e. number of offspring provisioned per nest in this population.

Discussion

Group living can provide immediate opportunities for individuals to increase fitness by minimizing energetic costs. For animals that build costly nests, group living can arise when individuals compete for access to existing nest space (Hansell, 2005; Myles, 1988; Woolfenden & Fitzpatrick, 1978). These findings support the assertion that high energetic savings of nest inheritance can favor group living even in the absence of strong cooperative behavior among group members. For the carpenter bee *X. sonorina*, females that opt out of nest excavation activity by joining an existing nest, often with other females (Gerling, 1982), may bypass significant energetic costs if they inherit the nest. I used metabolic rate measurements in combination with CT imaging to demonstrate high costs of nest excavation by the carpenter bee *X. sonorina*. These costs favor the reuse of existing nests, perhaps even at the expense of delayed or uncertain reproduction. The effects of this energetic investment represent a significant but neglected component of reproductive fitness, and one with important implications for the formation of social groups.

Nest construction as a context for the evolution of sociality

Life histories involving costly building behaviors may facilitate social evolution by placing a premium on constructed nest habitats (Hansell, 2005). In these cases, the risks and/or costs associated with nest founding can outweigh the potential benefits of independent breeding, encouraging offspring to remain in the natal nest. Nest inheritance has been implicated as an important selective pressure in the evolution of sociality in the Hymenoptera (Hansell, 1987; Leadbeater et al., 2011), colonial rodents (M. A. Harris & Murie, 1984; Jarvis et al., 1994; Wallace & Bennett, 1998), and cooperatively breeding birds (Cockburn, 1998; Emlen, 1984; Woolfenden & Fitzpatrick, 1978). Fewer studies,

however, have directly quantified the energetic costs of nest building (Collias & Collias, 1984; Mainwaring & Hartley, 2013). This evidence can provide essential empirical support for hypotheses that seek to explain the origins of social nesting strategies.

In *X. sonora*, as in many *Xylocopa*, only a single female per nest reproduces at a given time, though females may join established, occupied nests throughout the reproductive season [26, personal obs.]. In addition to monopolizing reproduction, dominant bees perform most or all of the foraging and nest excavation, such that non-reproductive subordinates contribute little, if at all, to the productivity of the nest, aside from possible nest guarding duties (Gerling, 1982; Gerling et al., 1989). Why, then, do females join or invade nests rather than constructing their own? Nest limitation has been widely proposed as the major driving force in the evolution of carpenter bee sociality, but this hypothesis rests on the assumption that nest construction is challenging, costly, or otherwise prohibitive (Gerling et al., 1989; Gerling & Hermann, 1978; Michener, 1990a).

These results validate the assertion that nest construction entails high metabolic costs. Per offspring produced, I conservatively equate this expense to that of approximately 7 hours of bee flight, one of the most energetically demanding behaviors that animals perform (Feuerbacher et al., 2003; R. K. Suarez, 2000). For context, the carpenter bee *X. capitata* spends 3.5 hours flying among flowers to provision a single offspring ((Louw & Nicolson, 1983); note that this estimate includes flower handling time and excludes commuting time), suggesting that nest building ranks among the principal energetic investments associated with offspring production. A female excavating the volume necessary to rear the average brood size (11.5) in this population would expend just under 50 kJ of energy, which is equivalent to nearly 80 hours of flight. Further, foundresses must expend additional energy to excavate non-brood rearing space in the nest. Together, this amounts to an expense exceeding the average cost of nest renovation (about 45 kJ). Importantly, the large variation in renovation costs observed in this study,

ranging more than twelve-fold from about 7 to 90 kJ, suggests that nests inherited in good condition (requiring little renovation) provide even greater savings. In other scenarios, inheriting females may invest as much in renovation as they may have in new nest construction. As such, the advantages of nest inheritance can vary widely, likely depending strongly on inherited nest quality. On average, however, the high energetic cost of excavation in this species should incentivize social strategies that rely on nest inheritance.

With finite energy budgets, nest inheritors could feasibly reinvest energy savings into other reproductive efforts, such as egg production and foraging, to produce more or higher-quality offspring than they might following new-nest excavation. For *Xylocopa*, which lay massive eggs relative to their body size (Iwata, 1964; Iwata & Sakagami, 1966), egg production is a high but unavoidable cost of reproduction; potentially avoidable reproductive behaviors such as nest construction may therefore provide important opportunities for reproductive energy savings. Nest inheritance may also provide important time savings. Gerling (Gerling, 1982) observed one *X. sonora* female spend 14 days excavating a single tunnel, of which there can be several per nest. Avoidance of this considerable time investment could therefore have important fitness implications by increasing available time for provisioning. The average time spent waiting for nest inheritance in this species is unknown, but is likely to vary widely based on local conditions, the degree of competition, and individual competitive ability, among other factors. Females may acquire nests immediately through usurpation, or may delay reproduction until a nest becomes available; subordinates of the carpenter bee *X. virginica* may even postpone reproduction for as long as a year (Vickruck & Richards, 2018). Future studies should aim to clarify fitness consequences of nest inheritance, quantifying both time and energetic costs of all reproductive behaviors to more thoroughly contextualize the energetic trade-offs underlying patterns of social nesting.

Interactions between nest properties and social strategies

Social strategy decisions involving nest inheritance are likely made in consideration of the quality of existing nest structures. For most animals, nest sites are a finitely renewable resource. Many birds, for example, experience a trade-off between the advantages of nest inheritance and the potential for exposure to parasites in older nests (Martin, 1995; Tomas et al., 2007). Carpenter bees, likewise, tend to cease reuse of nest tunnels once they have grown past a certain threshold diameter (Ostwald et al., 2020). With each successive season of nest reuse, the re-construction of brood cell partitions progressively widens tunnels to the point where they may be indefensible to usurpers (Ostwald et al., 2020). As nest tunnels decline in quality, bees may construct additional, new tunnels off of existing nest structures. In this way, even bees that acquire nests through inheritance may be forced to undertake some degree of nest excavation, depending on the state of the nest at the time of inheritance. The extent of necessary renovation can vary substantially, ranging in this study by a factor of more than ten. This wide variation implies that some inheritors may experience significant energetic savings relative to foundresses, while others invest substantially in renovation, perhaps losing this advantage.

The properties of available nesting substrate likewise may influence nesting decisions. I measured excavation costs for bees using two wood species spanning much of the range of wood hardness available to *X. sonorina* (Hurd, 1978), and measured lower metabolic rates for bees excavating softer wood, when adjusted for excavation rate. However, these immediate energy savings may be counter-balanced by long-term disadvantages of nesting in soft wood, such as reduced durability and vulnerability to weathering and predators. Several species are known to be vulnerable to predation by woodpeckers (Buchmann & Minckley, 2019; Gerling et al., 1989; Watmough, 1983), and may benefit from nesting in more protective, harder wood, despite initial time costs

associated with nest excavation. Furthermore, nesting in hard wood could increase the longevity of the nesting log, which may be continuously occupied for as long as fifteen years (S.L. Buchmann, pers. comm.). If the aggregation occupying the log is closely related, long-term success of descendants may be important. *X. sonora* may invest more in nest excavation than carpenter bee species that nest principally in stalks or culms, taking advantage of the plant material's soft pith or hollow interior to minimize construction costs (Gerling et al., 1989; Hurd, 1978). Indeed, of the eight social *Xylocopa* species noted in Gerling *et al.*'s review of the genus (Gerling et al., 1989), seven species nest in solid wood (Ben Mordechai et al., 1978; Bonelli, 1976; Camillo et al., 1986; Camilo & Garofalo, 1982; Gerling, 1982; Gerling & Hermann, 1978) and just one nests in stems (Stark, 1992). Variation in nesting ecologies may therefore represent an important determinant of social organization within and across species.

Conclusions

Energetic considerations underlie life history trade-offs across taxa (Schwenke et al., 2016; Stearns, 1989), yet have remained underexplored in the context of social evolution. Efforts to describe comprehensive energy budgets encompassing a range of relevant behaviors could provide important insights into the fitness outcomes of alternative social strategies. These findings support a critical role for energetically costly nest excavation as a driver of carpenter bee sociality, providing quantitative support for a widely cited hypothesis (Gerling & Hermann, 1978; Hogendoorn & Velthuis, 1993). These results form an important mechanistic link between behavioral decisions and ecological conditions, and emphasize the significance of energetic trade-offs at the origins of group living.

CHAPTER 5

WINTER CONDITIONS FAVOR GROUP LIVING THROUGH IMPROVED CONSERVATION OF HEAT AND BODY MASS

Introduction

Social animals are often abundant in harsh or unpredictable environments, suggesting a role for sociality in coping with environmental stressors (Emlen, 1982; Faulkes et al., 1997; Hatchwell & Komdeur, 2000; Jetz & Rubenstein, 2011; Kennedy et al., 2018; Lukas & Clutton-Brock, 2017; Rubenstein, 2011). However, evidence for this relationship between sociality and harsh environments is typically correlative and/or relies on potentially problematic comparisons across species with different evolutionary histories. Facultatively social species offer solutions to these limitations, by allowing for direct comparison of sympatric solitary and social individuals within a single species (Kapheim et al., 2012; Montero et al., 2020; Randall et al., 2005; Shell & Rehan, 2017b). Here, I manipulate social strategy in a facultatively social carpenter bee and measure effects of sociality on survival, nest temperature, and body condition in winter to assess potential advantages of sociality under environmental challenges.

Challenging environmental conditions may arise during seasonal periods of extreme temperatures and resource scarcity. Winter conditions, especially, can compel individuals to seek shared refugia for insulation against low temperatures (W. Arnold, 1990; Dapporto & Palagi, 2006; Wang et al., 2011). Facultative grouping or huddling behavior can elevate individual body temperatures, and has been documented across many taxa, famously in penguins (Gilbert et al., 2006) and small mammals (Nowack & Geiser, 2016; Sukhchuluun et al., 2018; Yahav & Buffenstein, 1991), but also in reptiles (Shah et al., 2003; White & Lasiewski, 1971) and insects (Dapporto & Palagi, 2006;

Fahrenholz et al., 1989; Wang et al., 2011). In both endotherms and ectotherms, social thermoregulatory strategies often function principally through the reduction of the group's surface area-to-volume ratio, which minimizes heat lost to the environment (Canals et al., 1997; Contreras, 1984; Gilbert et al., 2008). Winter grouping strategies such as these may provide critical protection against the lethal and sub-lethal effects of cold exposure.

These same grouping strategies may additionally help conserve body mass during seasonal periods of resource scarcity, during which animals can be deprived of food and water for months at a time. Group living may mitigate these challenges by providing novel or enhanced strategies for management of energetic and water reserves. For example, collective thermoregulation may reduce individual investment in energetically costly self-warming behaviors (Andrews & Belknap, 1986; Gilbert et al., 2008; Nuñez-Villegas et al., 2014; Perret, 1998). Grouping can also facilitate water conservation through increased local humidity and/or reduced surface area-to-volume ratios, as described in aggregations of bats (Boratynski et al., 2015), slugs (Cook, 1981), and caterpillars (Klok & Chown, 1999). Together, these benefits of sociality may improve winter survival by reducing the depletion of important resources. Further, social strategies that reduce body mass loss in the winter may translate to fitness gains in the reproductive season if larger animals have greater reproductive output, via social dominance or enhanced fecundity (Honek, 1993; Thornhill & Alcock, 1983)

Animals that group facultatively in winter provide useful test cases for understanding the role of sociality in mitigating seasonal challenges. One such species, the large carpenter bee *Xylocopa sonorina* Smith (formerly *X. varipuncta*; Sheffield et al., 2020), nests either solitarily or in small social groups, with strong seasonal effects on social strategy (Ostwald *et al.*, 2020). In the winter, social nesting is predominant: adult bees remain inactive in tight huddles at the terminal ends of their nests in tunneled logs

(Ostwald *et al.*, 2020). Social overwintering is standard among temperate *Xylocopa*. Typically, same-generation adults spend the winter months in mutually tolerant pre-reproductive assemblages. These groups become antagonistic at the onset of the reproductive season, when bees compete for dominance of the natal nest and/or disperse to seek alternative nesting opportunities (Gerling *et al.*, 1989; Michener, 1990; Richards & Course, 2015; Velthuis, 1987). In social *Xylocopa* nests, a single female monopolizes reproduction at a given time, while subordinate non-reproductives perform little, if any, foraging and nest construction (Hogendoorn & Velthuis, 1993; Richards, 2011). Because subordinates take on minimal labor, social nests may be no more productive than solitary ones (Prager, 2014). Rather, groups may form in the reproductive season due to nesting limitations that arise from the high cost of nest construction (Ostwald *et al.*, 2021a). The drivers of group living in winter, however, remain unexplored, and are likely to differ from those driving group formation during the reproductive season.

I investigated the role of environmental challenges as drivers of group living in winter nests of *X. sonorina*. Bees in this study population experience Sonoran Desert winter conditions, where freezing and below-freezing night-time temperatures are possible but not common. Compared to higher-latitude *Xylocopa* populations which experience regular extreme cold conditions, this population presents interesting opportunities to ask whether even mild environmental challenges are sufficient to favor sociality in this flexibly social species. By experimentally manipulating social condition, I tested the hypothesis that social bees experience physiological advantages over solitary bees in winter. I housed bees in the field in ambient desert winter conditions, and measured nest temperatures, survival, and changes in body mass. In doing so, I compared the body condition of social and solitary bees to assess effects of social strategy on overwintering success. Manipulative studies such as these provide some of the most robust evidence for environmental drivers of the transition from solitary to group living.

Methods

Field site, observation nests, and social condition manipulations

To examine the effects of social strategy on the physiological condition of bees in winter, I artificially manipulated the social condition (social vs. solitary) of bees housed at ambient winter temperatures, then assessed their conservation of heat, water, and energy. I removed female and male adult bees from their winter nests by splitting occupied nesting logs in the early morning January 2, 2020. Working in a 4°C environmental chamber to approximate early morning outdoor temperatures, I measured live mass (A&D GR-200; repeatability 0.0001 g) and tagged all bees with unique numbered plastic discs, glued to the thorax, for individual identification. Bees were then assigned to one of three conditions: 1) a solitary condition, with single bees housed individually ($N = 30$ bees in 30 nests), 2) a social condition, with bees housed in groups of five individuals ($N = 30$ bees in 6 nests), or 3) a baseline condition with which I assessed physiological condition of bees just prior to the start of the experimental period ($N = 38$ bees). The experimental social condition was set to five bees to approximate the average number of adults in naturally occurring social nests in January in this region (Ostwald et al., 2020). Bees were assigned to conditions haphazardly with respect to nest of origin to control for potential genetic effects on physiological status. *X. sonorina* is known tolerate non-relatives as nestmates (Ostwald et al., 2021b). Additionally, I preserved the approximate observed sex ratio (7F:3M) across treatments. Baseline bees were immediately freeze-killed after weighing and tagging and stored at -80°C for future analysis.

Social and solitary bees were installed into artificial wooden observation nests covered with clear plastic viewing windows (for observation nest design, see Vickruck &

Richards, 2017). I placed these nests into a protected outdoor research area at Arizona State University in Tempe, AZ (33.420°, -111.933°) where they were exposed to ambient conditions. All nests were covered with plywood to reduce heat loss through the plastic observation window. Nest entrances were plugged with cotton to enable air flow but restrict movement of bees between nests. We left nests undisturbed in these conditions for four weeks (28 days) of the coldest month of the year. On January 30, 2020, we removed nests from the field site and assessed survival and final live mass in the 4°C environmental chamber. All social and solitary bees were then freeze-killed and stored at -80°C for future analysis.

Temperature measurements

To assess potential effects of group living on conservation of heat during the winter, I recorded nest temperatures for social and solitary nesting conditions ($N = 6$ focal social and 6 focal solitary nests). Between 5:30 and 6:30 AM (approximately the coldest time of day) I removed the plywood nest covering and inserted a wire thermocouple under the nests' plastic observation window to record the temperature in two locations: 1) the air immediately surrounding the bee (within 2 mm of the thorax), and 2) the air in the empty, distal tunnel area away from the bees. For social nests, I recorded the air temperature surrounding the central bee in the linear cluster. I conducted these measurements sparingly, on January 6, 8, and 13, 2020, to minimize disruption of winter quiescence. Simultaneously, I continuously recorded ambient temperature and humidity at the nest site using an iButton® temperature/humidity logger (DS1923).

Water storage assay

To examine potential effects of social condition on conservation of water stores, I measured whole-body water content of social, solitary, and baseline bees. All frozen bee samples were removed from -80°C storage exactly 32 days after their initial sampling date, to control for potential dehydrating effects of long-term cold storage. Frozen bees were reweighed then placed in a drying oven set to 55°C for 4 days, then weighed once dry. Preliminary analysis confirmed that this protocol is sufficient to dehydrate bees. The difference in pre- and post-drying mass provided the estimate of stored water mass.

Lipid storage assay

Similarly, to assess effects of social condition on conservation of lipid stores, I measured whole-body neutral lipid content of social, solitary, and baseline bees, as this typically represents the principal form of energy storage in diapausing insects (Hahn & Denlinger, 2011; C. M. Williams et al., 2011). I used a gravimetric lipid assay with a diethyl ether solvent on dried, homogenized whole-body bee tissue (C. M. Williams et al., 2011). I conducted a series of 4 24-hour washes in diethyl ether. Preliminary analysis confirmed that 4 washes are sufficient to dissolve triacylglycerides in *X. sonora*. The difference in pre- and post-assay mass provided the estimate of stored lipid mass.

Metabolic rate measurements

I measured metabolic rates of overwintering bees ($N = 14$) using stop-flow respirometry. In January 2021 I extracted adult females from their nesting logs at dawn and weighed them (A&D GR-200; repeatability 0.0001 g). Bees were placed in 20-mL syringes and

acclimated for one hour in the dark at 3.2°C, a temperature that represents an extreme but realistic winter low temperature for this population. At this temperature, bees showed no activity and so I was confident that measured metabolic rates reflected a resting state. I flushed syringes with dry, CO₂-free air supplied at 500 mL min⁻¹ by a FTIR Purge Gas Generator (Parker-Balston, Palmer, MD, USA) connected to 1 L columns of cobalt-doped copper (II) sulphate (Drierite, W.A. Hammond Drierite Co Ltd, Xenia, OH, USA) and NaOH (Ascarite II, Sigma-Aldrich, St Louis, MO, USA). I sealed flushed syringes for one hour and then injected 2 mL air from the chamber into a Li-Cor 6252 CO₂ infrared gas analyzer (Li-Cor, Lincoln, NE, USA). I regulated flow rate using a Flowbar-8 Mass Flow Meter System (Sable Systems International, Las Vegas, NV, USA). I digitized measurements (UI-2, SSI) and recorded and analyzed data using the ExpeData data acquisition software, v. 1.9.13 (SSI). I calculated VCO₂ by integrating baseline-corrected CO₂ concentration readings and adjusting for flow rate (500 mL min⁻¹), sealing time (60 min), and the fraction of chamber air analyzed (2 mL of 20 mL syringe occupied by 1 mL bee). I also calculated the temperature coefficient (Q₁₀) value according to the following equation,

$$Q_{10} = (MR_2 / MR_1)^{(10/T_2 - T_1)}$$

where MR₁ represents the measured resting metabolic rates at T₁ = 3.2°C and MR₂ represents the resting metabolic rate of female *X. sonorina* at T₂ = 25°C (previously measured in Ostwald et al., 2021a).

Statistical analysis

To compare temperatures experienced by social and solitary bees I used a *t*-test after confirming normality and homoscedasticity of data with Shapiro tests and Levene's tests, respectively. Adding sampling date as a random effect to this analysis did not significantly

affect the model, so I removed the random term and performed the *t*-test. To compare temperatures experienced by social and solitary bees to the temperatures of the empty regions of their nests, I used a paired Wilcoxon test, as data did not meet assumptions of normality and homoscedasticity. I used a chi-square test to assess differences in survival across social conditions. To evaluate changes in mass at the beginning and end of the experiment I used a paired Wilcoxon signed rank test. To compare proportions of body water and lipid across baseline, solitary, and social treatments, I used Kruskal-Wallis tests followed by pairwise Wilcoxon post-hoc tests. Similar to the analysis of temperature, this analysis was enabled by the fact that including nest identity as a random effect did not significantly affect the models. I further used a Wilcoxon test to assess a possible effect of sex on body mass loss. All results are presented as mean \pm standard error.

Results

Thermal differences between social and solitary nesting contexts

Throughout the four-week experiment, bees experienced typical ambient winter conditions for this site: temperatures ranged from 6.6°C to 23.6°C, with daily mean high temperatures of $19.7 \pm 0.04^\circ\text{C}$ and low temperatures of $10.0 \pm 0.04^\circ\text{C}$, and relative humidity of $40.5 \pm 0.1\%$. When we conducted early-morning thermocouple measurements of in-nest temperatures, ambient temperatures measured $7.5 \pm 0.5^\circ\text{C}$. Social bees experienced significantly warmer body surface temperatures than did solitary bees (10.4°C vs. 9.0°C , respectively; *t*-test: $P < 0.001$); (Figure 5.1) as measured by air temperature 2 mm away from the bee. Furthermore, social bees experienced temperatures significantly warmer than those of the empty regions of their nest ($+1.3^\circ\text{C}$; Paired

Wilcoxon test: $P < 0.001$), while solitary bees were no different in temperature from their empty nest tunnels (Paired Wilcoxon test: $P = 0.575$); (Figure 5.1).

Winter survival and body mass loss

Thirteen of 60 bees (21.6%) did not survive the duration of the 28-day experimental period, including 7 solitary bees and 6 social bees. Survival was no different between social and solitary nests (Chi-Square test: $X^2 = 0.098$, $P = 0.754$). Two dead bees in social nests had visible injuries, suggesting possible physical conflict when winter quiescence paused on warm days toward the end of January (Vickruck and Richards, 2017). Surviving bees across both social and solitary treatments experienced significant reductions in body mass, losing 0.164 g (12.34% of initial body mass; Paired Wilcoxon test: $P < 0.001$). However, social bees experienced a lower reduction in whole-body mass than did solitary bees. Solitary bees lost 0.188 ± 0.016 g over the course of the experiment, or 14.04% of initial body mass, while social bees lost only 0.139 ± 0.016 g, or 10.60% of initial body mass (Wilcoxon test: $P = 0.009$); (Figure 5.2). Mass loss did not differ by sex (Wilcoxon test: $P = 0.523$).

Metabolic rate, lipid storage, and water storage

I measured the metabolic rate of overwintering bees at 3.2°C as 44.7 ± 5.7 $\mu\text{L/hr g}$. At 25°C, the metabolic rate of *X. sonorina* has been measured as 368.3 ± 59.4 $\mu\text{L/hr g}$ (Ostwald et al., 2021b). From these two measurements, I calculate the temperature coefficient (Q_{10}) as 2.6.

Bees sampled at the beginning of the experimental period (baseline bees) contained 0.612 ± 0.015 g of water, or $50.48 \pm 0.65\%$ of body mass. I found no significant

difference in proportional water content among baseline bees and social and solitary bees at the end of the experiment (Kruskal-Wallis test: $P = 0.499$); (Figure 5.3), nor between baseline bees and pooled social and solitary bees (Wilcoxon test: $P = 0.303$). Social bees sampled at the end of the experiment contained 0.565 ± 0.021 g of water ($50.70 \pm 0.37\%$ of body mass), and solitary bees contained 0.557 ± 0.019 g of water ($51.26 \pm 0.61\%$ of body mass).

The lipid assays indicated that baseline bees sampled at the beginning of the experiment contained 0.174 ± 0.010 g of triacylglycerides, or $28.14 \pm 1.23\%$ of body mass. Similar to water content, the proportional content of lipids did not differ between bees sampled before and after the experiment (Wilcoxon test: $P = 0.159$), nor between social, solitary, and baseline bees (Kruskal-Wallis test: $P = 0.280$); (Figure 5.3). Social bees sampled at the end of the experiment contained 0.142 ± 0.011 g lipid ($25.76 \pm 1.61\%$ of body mass), while solitary bees contained 0.141 ± 0.010 g lipid ($26.12 \pm 1.28\%$ of body mass).

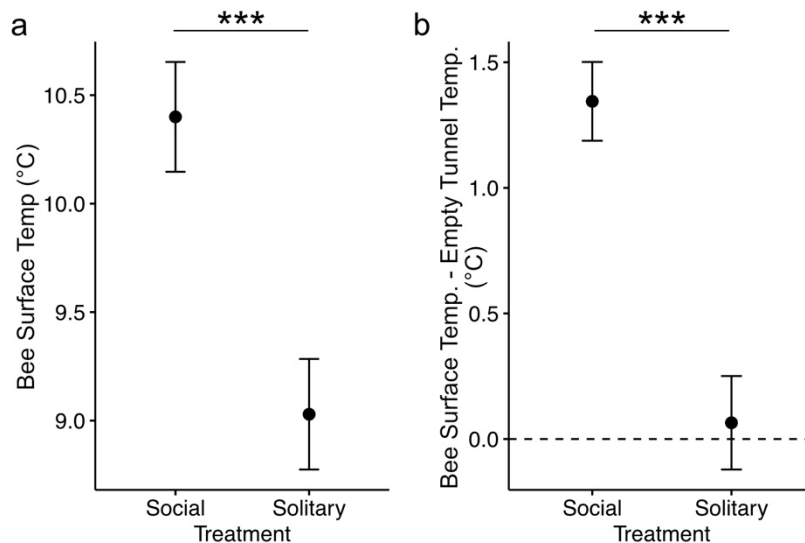


Figure 5.1. (a) Temperatures experienced by social bees during winter are significantly greater than those experienced by social bees (t -test: $P < 0.001$) during the coldest time of

day (ambient temp: $7.5 \pm 0.5^{\circ}\text{C}$). “Bee Surface Temps” represent boundary air temperatures within 2 mm of the thorax. (b) Social bees experience temperatures significantly warmer than the unoccupied tunnels of their nests (Paired Wilcoxon test: $P < 0.001$), whereas solitary bees experience temperatures no different from the empty regions of their nests (Paired Wilcoxon test: $P = 0.5752$).

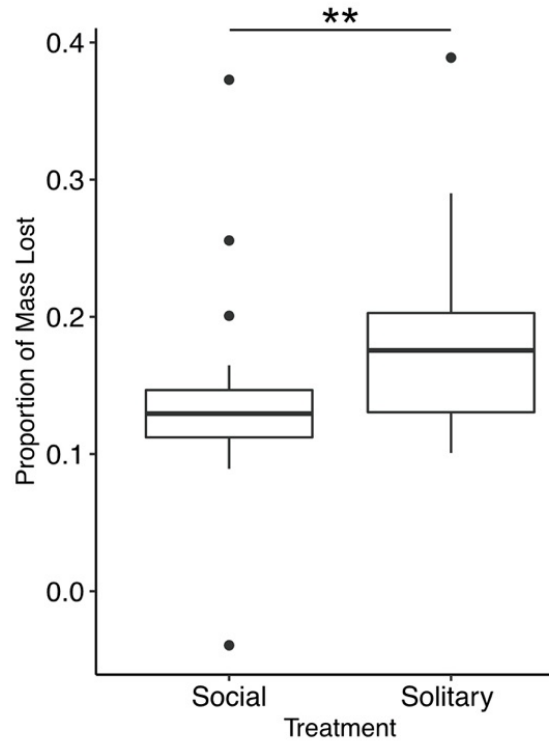


Figure 5.2. Social bees lost a significantly lower proportion of their total body mass over the course of the 28-day experiment than did solitary bees (Paired Wilcoxon test: $P = 0.009$).

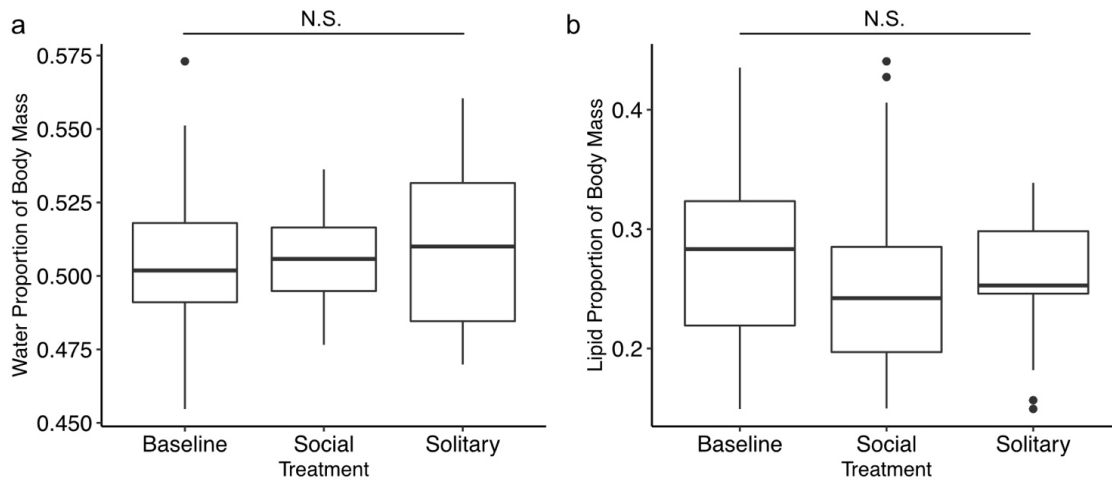


Figure 5.3. I detected no differences among baseline, social, and solitary bees in the proportion of total body mass represented by a) water (Kruskal-Wallis test: $P = 0.530$) or b) triacylglycerides (Kruskal-Wallis test: $P = 0.475$).

Discussion

Evolutionary transitions from solitary to group living may arise under environmental challenges that favor cooperative or tolerant behavior. However, understanding environmental conditions at the origins of sociality can be challenging, especially for taxa in which sociality evolved millions of years ago. Facultatively social animals provide tractable empirical test cases for exploring the drivers of this social evolutionary transition (Kapheim et al., 2012; Montero et al., 2020; Randall et al., 2005; Shell & Rehan, 2017b). In this study, I experimentally manipulated social condition and demonstrated significant physiological advantages of winter group living for the facultatively social carpenter bee, *X. sonorina*. These advantages of grouping behavior emphasize the utility of group living as a strategy for coping with environmental stressors.

Effects of winter group living on survival and body condition

Mutual tolerance among individuals sharing nesting or hibernating space can provide important benefits by buffering against extreme or variable environments (Dapporto & Palagi, 2006; Morton, 1978; Wolff & Lidicker, 1981). During the reproductive season, carpenter bee social groups likely arise in large part from constraints on independent nesting opportunities (Ostwald et al., 2021a). In winter, however, alternative selective pressures may favor sociality. Carpenter bees in temperate climates typically spend the winter months in pre-reproductive assemblages of adult relatives and/or non-relatives (Gerling et al., 1989; Michener, 1990; Mikát & Straka, 2021). In this study population, solitary nesting was most common in the early reproductive season, but in winter, nearly all nests were social (Ostwald et al., 2020). The results of this present study suggest that social advantages may explain the prevalence of winter group living in this species.

Overwintering bees face survival challenges associated with low temperatures and food and water deprivation (T. Seeley, 1985; Vesterlund et al., 2014). Over the 28-day experimental period in this study, I observed a mortality rate of approximately 20% for both social and solitary bees. Importantly, I expect mortality in this study to be higher than mortality of bees left undisturbed in natural nests (estimated 13% in Ostwald et al., 2020), due to stress associated with transplant into artificial nests. Regardless, I observed no differences in mortality across social and solitary treatments. Solitary winter nesting appears to be a viable strategy in this population, which experiences relatively mild Sonoran Desert winters. In other populations or species that experience harsher, longer winters, social overwintering may be more common or even obligate (Peso & Richards, 2011; Vickruck & Richards, 2021). Future studies manipulating social condition in these populations could reveal a strong relationship between social strategy and overwintering survival.

Even if social and solitary *X. sonorina* have similar survival outcomes in winter, social nesting may still confer important fitness benefits through improved body condition. Conservation of body mass is a critical challenge of overwintering with implications for body condition in the reproductive season (Hahn & Denlinger, 2011; Hodges et al., 2006; Lyman et al., 1982). In this study, bees lost about 12% of their body mass in just four weeks. A study of the eastern carpenter bee, *X. virginica*, found that bees collected in winter were anywhere from 4 to 40% heavier than bees collected in summer, though this study did not measure mass losses of individuals (Skandalis et al., 2009). Solitary bees experienced the most substantial mass losses: about 35% greater than the average mass loss of bees in the social treatment. Among social bees, female body mass and/or body size are associated with reproductive dominance, where it may aid in physical dominance interactions (Michener & Brothers, 1974; Richards & Packer, 1998; Richards, 2011; Smith et al., 2008, 2009; Vickruck & Richards, 2018). Likewise, social nesting could confer fitness gains if there is a relationship between body mass and fecundity (Alcock, 1979; Freeman, 1981; Leather, 1988; Sugiura & Maeta, 1989). Females that overwinter in groups and experience lower reductions in body mass may be better able to establish dominance and reproduce in the spring. Likewise for males, which perform extensive hovering flight behavior at mating territories (Alcock & Johnson, 1990; Marshall & Alcock, 1981; Minckley & Buchmann, 1990), social overwintering may be favored if larger individuals have better flight endurance or improved territory defense (Alcock & Kemp, 2006; Coelho & Holliday, 2001; Kenna et al., 2021; Thornhill & Alcock, 1983). These prolonged consequences of winter behavioral decisions suggest important fitness benefits of social overwintering.

The physiological mechanisms underlying overwintering mass loss in *X. sonorina* remain to be explored. In this study I observed no significant changes in lipid or water content that could sufficiently explain observed body mass losses. Based on the measured

metabolic rate and Q_{10} estimates, at the average temperature observed in this study, I estimate that bees would burn 0.097 g of lipid over a 28-day period, under the assumption that bees are metabolizing primarily lipids (respiratory quotient = 0.7). This estimate is many times the measured non-significant difference in lipid mass between baseline bees and bees at the end of the experiment (0.001 g). It could be that metabolic rates of bees in nests are even lower than what I measured in the lab, due to the stress of nest extraction. Further, the diethyl ether assay I used has accuracy limitations when differences between treatment groups are small (Williams et al., 2011). Alternatively—or additionally—this discrepancy could suggest that overwintering *X. sonora* are metabolizing other macromolecules, such as carbohydrates and protein. Carbohydrates, especially, play important roles in energy metabolism of diapausing insects (Adedokun & Denlinger, 1985; Marron et al., 2003; Yocum et al., 2005). It may also be that lipid and water losses were minor and not detectable in this study over this short time frame (28 days) in mild winter conditions. Importantly, unlike the mass measurements, the destructive nature of these lipid and water assays precludes repeated measures of the same individuals, decreasing my ability to detect small changes. Future studies observing longer overwintering periods, or in cooler climates, could provide further insights into the mechanisms underlying changes in body mass in overwintering bees. Regardless of mechanism, improved mass conservation by social bees in winter may translate to later fitness advantages during the reproductive season.

Thermal benefits of group living in winter

Survival and body condition during winter are strongly linked to thermal conditions (Hahn & Denlinger, 2011; Humphries et al., 2002; Storey & Storey, 1988). By grouping, bees may increase their collective thermal inertia (Chappon & Seuront, 2012; Helmuth,

1998; Reiserer et al., 2008; Shah et al., 2003), such that they reduce heat loss as ambient temperatures drop. At the coldest time of day, solitary bees in this study experienced temperatures no higher than their nest temperatures, whereas social bees were 1.4°C warmer than their nests. Though these observation nests were designed to approximate natural nests (Vickruck & Richards, 2017), there may be important differences in their insulating properties, especially for *X. sonora*, which excavate elaborate nests deep into logs (Ostwald et al., 2020). Further, I expect the quality of insulation to vary substantially with nest properties such as wood substrate, nest architecture, and nesting site. Importantly, I standardized the artificial nests to remove this source of variation from the measurements. Therefore, though nest temperatures in this study may differ from natural nest temperatures, the controlled design allows us to attribute any differences in nest temperatures to social condition alone. This observed 1.4°C difference could have important survival and fitness implications on nights that fall below freezing, which are not uncommon in this and other parts of *X. sonora*'s range. Improved avoidance of the lethal and sub-lethal effects of cold exposure (Lee & Denlinger, 1991; Marshall & Sinclair, 2011, 2018; Sinclair et al., 2003) could thus favor group living strategies in this species.

Temperature during overwintering also has important implications for consumption of energetic stores (Andrews & Belknap, 1986; Gilbert et al., 2008; Hahn & Denlinger, 2011). For overwintering poikilotherms, whose body temperatures vary with environmental temperatures, warmer temperatures increase metabolic rates and therefore increase energy drain (Williams et al., 2015). Why, then, did social bees in this study experience both warmer temperatures and reduced losses in body mass? It could be that the higher thermal inertia of bee groups buffered against temperature variability, which is known to exacerbate energy drain (Ruel & Ayres, 1999; C. M. Williams et al., 2012, 2015). Further, improved heat conservation in social nests could reduce the demand for energetically costly self-warming behaviors such as shivering thermogenesis (Harrison,

1987; Stabentheiner et al., 2003). It is unknown whether thermogenic behaviors occur in overwintering carpenter bees, though it seems plausible that these could occur on warm afternoons that stimulate activity, leading to energy drain.

To minimize disruption of winter quiescence, I measured nest temperatures only at the coldest time of day; future work that continuously tracks nest and body temperatures throughout winter would inform a more comprehensive understanding of the thermal experiences of overwintering social and solitary bees. Similarly, to minimize disturbance, this study focused solely on temperatures at the center of the bee cluster. How temperature is distributed throughout the cluster and whether bees shift positions within the cluster (as in honeybees, Heinrich, 1981; Seeley, 1985) are important questions for future study that could reveal whether thermal and energetic benefits of group living are experienced equally or asymmetrically. These results also raise important, unanswered questions about the effects of winter climate change on social strategy decisions. I demonstrated that social nesting improves heat conservation for *X. sonorina*; as such, warmer winters could feasibly relax selective pressures favoring winter group living. Alternatively, social strategies could be favored under more extreme/variable winter conditions, if these strategies more effectively buffer against extreme temperatures. Future studies should investigate the relationship between winter conditions and the ratio of solitary to social nesting over time, with the aim of clarifying the extent to which this behavioral plasticity could mitigate range shifts and other consequences of winter climate change.

Conclusions

Abiotic conditions represent key selective pressures in the evolution of social behavior. In this study, I demonstrated physiological benefits of group living in *X. sonorina*

overwintering groups. These seasonal benefits may additionally translate to improved fitness outcomes for during the reproductive season. Importantly, I used a flexibly social system situated in mild winter conditions that could favor but not require group living, to gain unique insights into the conditions that can promote cooperative behaviors in typically solitary animals. Furthermore, these benefits occurred independently of kinship, highlighting a possible environmental driver of grouping among non-kin. Social advantages in these contexts provide compelling support for the role of environmental challenges in shaping the evolutionary origins of group living.

CHAPTER 6
MULTIMODAL CUES FACILITATE NEST RECOGNITION WITHIN NESTING
AGGREGATIONS

Introduction

Social organisms experience a trade-off between benefits of group living and the potential fitness costs generated by spatial crowding. To mitigate these costs, individuals must adaptively respond to the cognitive challenges imposed by frequent social contacts. Group-living organisms must navigate densely occupied social landscapes and minimize within-group conflict by maintaining social boundaries. Densely-nesting individuals also face the particular challenge of reliably recognizing their nest and distinguishing it from those in the immediate vicinity. The potential consequences of inaccurate nest identification can be significant. These repercussions include robbery or aggressive interactions for the resident individual (Hogendoorn & Velthuis, 1995), loss of contact with vulnerable offspring for the drifting individual (Hogendoorn & Velthuis, 1993), and potential pathogen spread for both individuals (Forfert et al., 2015). Given these costs, aggregative nesting is likely maintained by effective nest recognition mechanisms.

The sensory cues animals rely on for nest localization vary with environmental conditions and with the sensory capabilities of the animal. Acoustic communication, for example, is particularly important for nest recognition in birds, and can facilitate breeding-site localization when the use of olfactory and visual cues is impractical, as in colonial penguins (Jouventin, 1982). Similarly, nocturnal activity of bats precludes meaningful use of visual cues, so Mexican free-tailed bats use acoustic cues to locate their pups within colonies that can contain millions of individuals (Balcombe, 1990). Most insects, however, do not possess hearing, and therefore rely on alternative cues for

nest localization (Gopfert & Hennig, 2016). Niko Tinbergen's landmark study of the homing behavior of the digger wasp *Philanthus triangulum* elegantly demonstrated the importance of visual cues for nest localization (1972). Other studies have likewise emphasized the significance of visual cues for insect navigation, particularly landmark cues and cues associated with the panoramic view associated with the nest (M. Collett et al., 2013; T. S. Collett et al., 2006; R. A. Harris et al., 2007; Hoinville & Wehner, 2018; Mandal, 2018; von Frisch, 1967; Wehner & Raber, 1979). Beyond the use of visual cues, insects are known to recognize nests at close range using olfactory cues (Buehlmann & Hansson, 2012; Butler et al., 1969; Huber & Knaden, 2018; Steck et al., 2009). Bees, in particular, have been shown to orient to specific chemical cues associated with nest entrances (R. L. Foster & Gamboa, 1989; Guédot et al., 2007; W. Wcislo, 1992).

To provide redundancy and aid recognition under a variety of conditions, nest recognition mechanisms may integrate cues across multiple sensory modalities. Several hypotheses have emerged to explain the complexity of signals and cues, which may vary in their intensity or persistence in the environment, in their ease of detection, or in the information contained. First, the use of multiple modalities may provide "back-ups" that either reinforce or conflict with the original message (Johnstone, 1996). This redundancy can improve accuracy in decision making. Mosquitofish, for example, are better able to locate conspecifics and avoid predators when provided with visual and chemical cues in tandem, rather than either cue separately (A. J. W. Ward & Mehner, 2010). Second, different modalities can convey different types of information, or have modulatory effects on the primary message, as in human speech perception, which is modulated by visual cues (McGurk & MacDonald, 1976; Partan & Marler, 2005). Furthermore, the use of multiple cues or signals can specify context. Female red-winged blackbirds and Cuban grassquits sing different songs to clarify the meaning of a single visual display that can signal either aggression or courtship (Baptista, 1978; Beletsky, 1983). Due to this

functional variation, the weights accorded to different cues may vary, depending on their information content in a given context.

I investigated the importance of visual and olfactory cues in the nest recognition ability of an aggregating carpenter bee, *Xylocopa sonorina*. This species nests primarily in decaying stumps and logs, forming aggregations of up to several dozen nests. To maximize use of this nesting substrate, which is rare in their desert habitat, *X. sonorina* nest densely, with nest entrances often only centimeters apart. As such, *X. sonorina* likely process multiple cues at long- and short-range to avoid entering a neighbor's nest. Previous studies have found evidence for visual and olfactory nest localization in African *Xylocopa* species, but differ in their interpretation of the relative importance of these cues. Anzenberger (1986) suggested that olfactory but not visual cues are important for homing, while Hefetz (1992) found that both play a role, with olfactory cues useful at very close range. Wcislo (1992) demonstrated the importance of olfactory cues for nest localization in the tropical sweat bee *Lasioglossum figuresi* by removing chemical cues at nest entrances as well as supplementing with odor cues from foreign conspecific bees, both of which delayed nest entry by returning foragers. In the present study, I distinguish between the effects of proximal and distal visual landmarks in visual homing, and between the presence and absence of scent cues in olfactory homing. This experimental design allows us to explore the relevant sensory cues *X. sonorina* uses to function in a high-density nest site.

Methods

Experiments were performed from June 2018 through August 2018 at Arizona State University in Tempe, AZ (33.42°N, -111.93°W). Four logs, each containing approximately 5 to 20 active nests of *X. sonorina*, were transferred to the university campus from

nearby park and residential areas in the spring of 2018. Over the course of the experiment, bees abandoned certain nests and colonized others. The number of active nests—and therefore the number of replicates per treatment—varied over the course of the season as bees emerged, dispersed, and experienced mortality and usurpation.

I performed four treatments that manipulated visual or olfactory cues at nest entrances: (1) rotating the log 180° about its vertical axis, (2) moving colored shapes placed next to nest entrances, (3) rinsing entrances with hexane to remove olfactory cues, and (4) applying cuticular hydrocarbon (CHC) extracts from non-resident bees to nest entrances. I compared the results of these treatments to an unmanipulated baseline condition. A period of at least one week was allowed between each treatment to allow bees to adjust to the changing conditions.

To determine the effect of each treatment on a returning forager's ability to find her nest, I measured search time and search error rate during peak foraging time (5:30AM-8:30AM). To standardize measurements of search time, logs were surrounded by 3 to 6 curved rebar poles, 85 cm in height, that were placed 90 cm from the central axis of the log (see Figure 6.1). The population was given five days to adjust to the presence of the rebar poles before measurement began, and did not appear to be disturbed by their introduction.

Measurement of search time began when a returning bee passed into the region defined by the poles, and ended when she entered her home nest. Nest recognition errors were quantified as the number of nests a bee entered and exited before returning to her home nest. Bees were unmarked, so I did not determine nest ownership through monitoring of individually marked bees. Rather, I defined search success as entering a nest and remaining there for at least 30 s without leaving to search nearby nests. Any time spent in the wrong nest was not included in search time. Orientation flights, characterized by slow, nestward-facing circling of the logs (Zeil et al., 1996), were

observed across treatments, but rarely. I collected data from one foraging female per nest, and I used each nest a maximum of once per treatment. Because male *Xylocopa* have been shown to have lower nest fidelity than females, only female bees were included in this study (Peso & Richards, 2011). Females were distinguished by their coloration (black integument, whereas males have yellow integument).

For each log, I chose focal nests that existed in the same face of the log, considering that these nests could reasonably be confused with neighboring nests in that same region. Nests on the ends of the logs, or on opposite faces of the log, were excluded from the study because I expected that bees orient to these major features of the log.

Log rotation: To determine whether bees use distal visual cues to orient to their nests, I rotated logs 180° around their vertical axes and measured search time and the number of errors made. This manipulation altered in tandem various distal cues known to be used in insect navigation, including both celestial and terrestrial cues, notably the position of the sun relative to the nest and the panoramic view associated with it, respectively (Mandal, 2018; Wystrach et al., 2011). These measurements were compared to baseline search times and error counts for unmanipulated logs. If bees make use of distal cues, I reasoned that rotation would increase search times and errors, by altering the apparent relationship between visual cues and nest location. Logs were rotated a single time on the morning of August 14. Observations took place that morning and the following morning during periods of peak foraging activity, in an attempt to observe each bee's first return trip following the manipulation. It is possible that some bees observed on the second day had foraged the previous day after the end of the observation period. In this case, the measurement of search time would be conservative, given the additional opportunity for the observed bee to learn the new orientation of the log. However, foraging activity past early morning is extremely low at this time of year, and it is likely

that most, if not all bees spent the hottest part of the day inside the nest. Additionally, some bees in this treatment had an opportunity to learn the new orientation of the log when they departed it, potentially through orientation flights. As such, my measurement of search time is a conservative estimate of the effect of the disturbance on homing.

Movement of visual symbols peripheral to the nest entrance: I also assessed the importance for nest localization of close-range visual features in the periphery of nest entrances. One week before data collection, I placed artificial visual symbols (colored geometric shapes) approximately 1-2 cm from each nest entrance on the focal side of the log. There is strong evidence for color and shape learning in honey bees (Gould, 1984; Leonard & Masek, 2014). Colors chosen for the shapes were within the visual spectrum for bees (Chittka & Waser, 1997; von Frisch, 1956). Immediately before recording search times and error rate, I laterally shifted the symbols, moving each marker to the periphery of a neighboring nest entrance (see Figure 6.2). Because data collection occurred over multiple days during peak foraging time, I returned the symbols to their original position after each observation period, then shifted them identically before the next data collection bout. In this way, any bees I had not observed that day had not left the nest during the observation period, and remained naïve to the new symbol configuration.

Removal of chemical cues: To determine whether chemical cues on the inside of the nest entrance influence a forager's ability to find her nest, I washed the inside of the nest entrance with hexane immediately after a bee left, and recorded search time and accuracy upon her return. I did this by applying approximately 2 mL hexane with a cotton swab on the walls of the nest gallery, a short 1-2 cm entrance tunnel that leads into the main nesting tunnels. Protocols for the chemical removal treatment and the

chemical addition treatment (below) followed those of Wcislo (1992), which produced positive behavioral results for nest localization in *L. figueresi*.

Addition of foreign-bee chemical cues: To determine whether the presence of chemical cues from a foreign bee influences a bee's homing ability, I collected bees from a distant (2.54 km) aggregation of *X. sonorina* and extracted cuticular hydrocarbons by rinsing chilled bees in 10 mL hexane for 60 s each. After the departure of each forager, I applied these extracts to the inner wall of nest galleries in the same manner as in the chemical removal treatment, recording search time and accuracy upon return.

Olfactory control: Hexane is highly volatile, with 2 mL spread over the area of the nest gallery fully evaporating in under 7 minutes (Braun & Caplan, 1989). Average summer foraging durations are highly variable but have been recorded as 47.2 min in *Xylocopa virginica* (M. Richards & Course, 2015), and in this study were no shorter than approximately 15 minutes. As such, the hexane likely had sufficient time to evaporate fully by the time foragers returned to the nest. Nevertheless, to additionally control for any potential deterrent effects of the hexane itself, I applied hexane in a ring around the outside of the nest entrance, where I did not expect there to be any chemical cues deposited (as in Wcislo 1992). I then recorded search time and error rate and compared these to the results from both olfactory treatments.

Statistical analysis: I used a Kruskal-Wallis test to detect differences in search time among the four treatments, the baseline, and the olfactory control. I ruled out analysis of variance due to departures from normality and equal variance shown by normal QQ plots and Levene's tests for untransformed data as well as log-transformed data. For

post-hoc tests I performed Wilcoxon tests on treatment pairs. Results are reported as mean \pm standard error. To assess differences across treatments in the tendency of bees to make nest recognition errors, I performed Fisher's exact tests on the number of bees making one or more errors across treatment pairs. All statistical analyses were performed in R version 3.4.2 (R Development Core Team, 2017).



Figure 6.1. Example of experimental set-up including rebar poles surrounding a nesting log to create a standard space in which to measure search time. Circular holes are nest entrances.

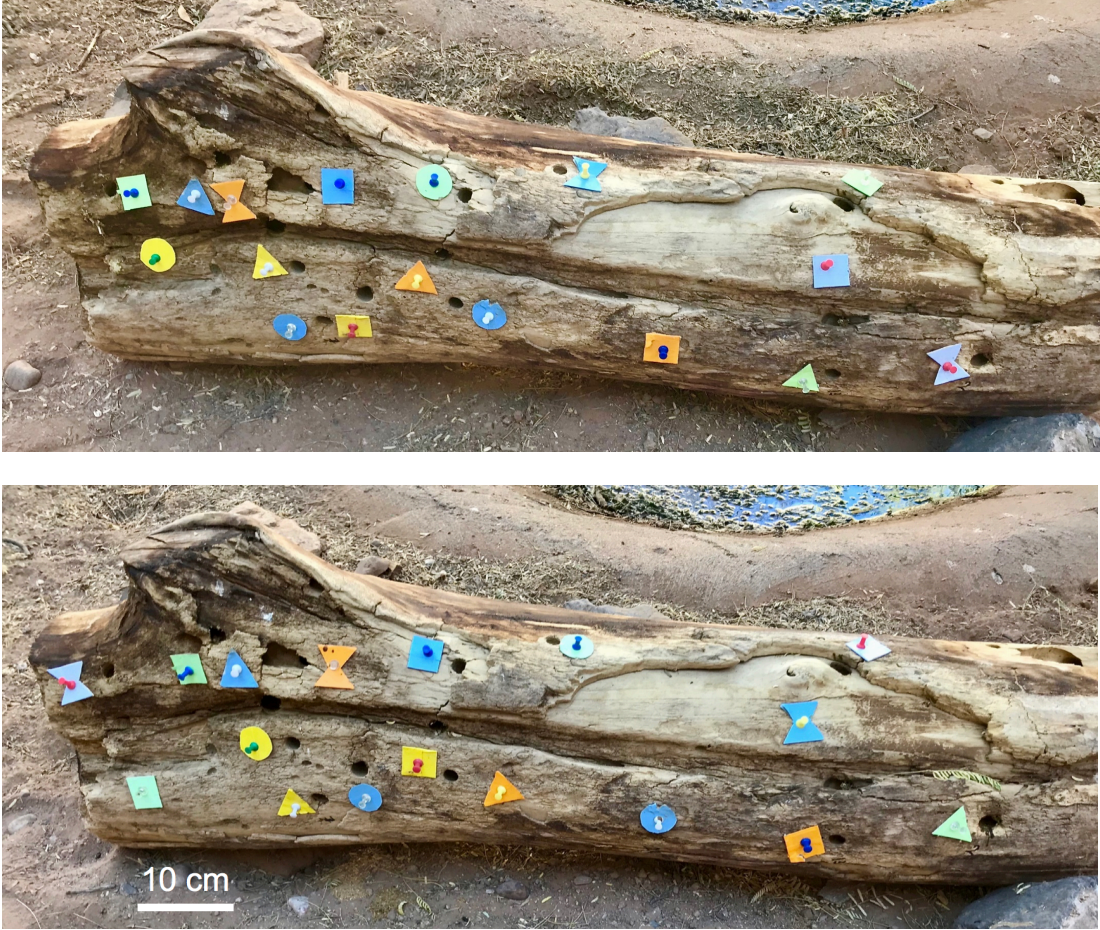


Figure 6.2. Nesting log with a symbol configuration to which bees had been trained for one week (top), and shifted symbol configuration (bottom) used to determine the effect of proximal visual landmarks on nest localization. Circular holes are nest entrances.

Results

I performed four treatments that assessed the effects on nest localization of (1) distal visual cues, (2) proximal visual cues, (3) the removal of olfactory cues, and (4) the addition of unfamiliar conspecific odors. I found a significant effect of treatment on search time (Kruskal-Wallis test: $H_5=43.72$, $P=2.6 \times 10^{-8}$), with both visual treatments

increasing search time, the removal of odors having no significant effect, and the addition of foreign odors decreasing search time (Fig. 3).

Log rotation: Foragers returning to an unmanipulated log found their nests quickly and accurately, searching for an average of 8.7 ± 1.3 s, with no errors observed ($n=22$). Search time was significantly increased following the 180° log rotation, with foragers searching for approximately eight times as long as they had during baseline, where no errors were seen (70.5 ± 14.3 s, $n=16$, $P=2.5 \times 10^{-5}$); (Fig. 3). Bees were significantly more likely to make nest recognition errors in this treatment when compared to baseline (Fisher's exact, $P=0.02$); (Table 1). In this treatment, four of sixteen bees inaccurately identified their nests, with one bee entering four other nests before locating her own.

Movement of visual symbols peripheral to the nest entrance: Shifting the geometric symbols near nest entrances significantly increased search time relative to baseline, more than doubling it on average (20.4 ± 3.4 s, $n=28$, $P=0.05$); (Fig. 3). However, there was no difference in the number of bees making nest recognition errors in this treatment versus baseline (3 errors, Fisher's exact, $P=0.25$); (Table 1).

Removal of chemical cues: Treating the outside of the nest with hexane to control for intrinsic deterrent effects of the olfactory treatments produced a mean search time of 12.7 ± 2.5 s ($n=18$). Compared to this control, removing chemical cues from the inside of the nest entrance through application of hexane did not significantly change search time (7.9 ± 1.2 , $n=18$, $P=0.18$); (Fig. 3). Likewise, the olfactory removal treatment had no effect on search time when compared to baseline values ($P=0.92$). There was no difference in the number of bees making nest recognition errors in the olfactory removal treatment (2 errors) versus the baseline (Fisher's exact, $P=0.23$), or in the olfactory removal treatment versus the olfactory control (1 error, Fisher's exact, $P=1$); (Table 1).

Addition of foreign-bee chemical cues: The addition of cuticular hydrocarbon extract from foreign bees significantly *decreased* search time relative to the olfactory control (5.0 ± 0.6 , $n=16$, $P=0.006$) and to the baseline ($P=0.05$). No bee made a nest recognition error in this treatment.

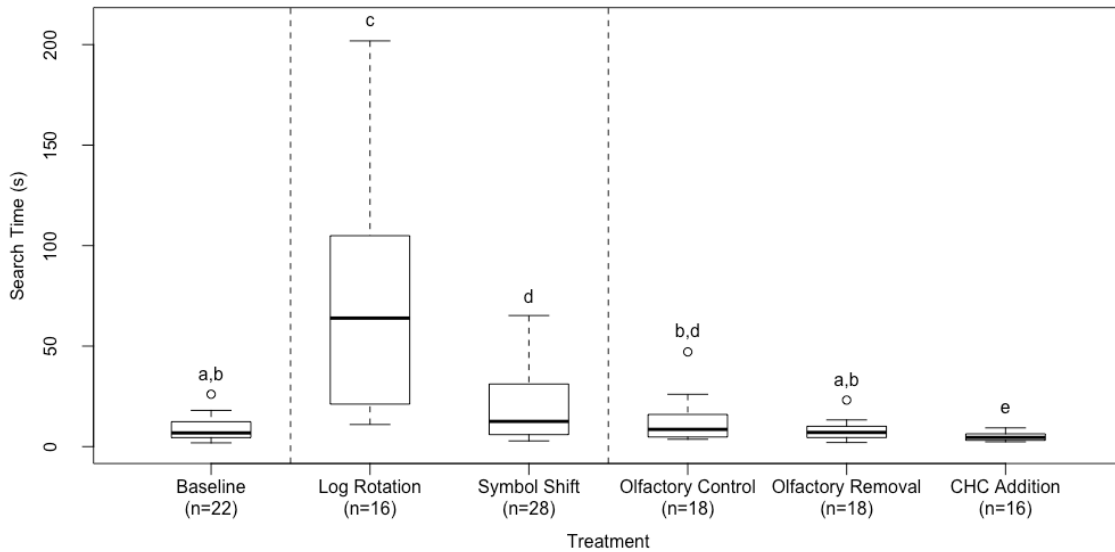


Figure 6.3. Time spent searching for the home nest in the baseline condition (left), visual treatments (center), and olfactory treatments and control (right). Both the symbol shift and log rotation treatments significantly increased search times relative to baseline ($P=2.5 \times 10^{-5}$ and $P=0.04$, respectively). The CHC addition significantly decreased search time relative to the olfactory control ($P=0.006$), and when compared to the baseline ($P=0.05$). Letters indicate significant differences and open circles represent outliers.

Table 6.1. Counts of returning foragers that made one or more nest recognition errors versus those that made no errors. Bees made significantly more errors in the log rotation treatment (in bold) than in the baseline ($P=0.02$). No other treatment comparison was significant.

	Baseline	Log Rotation	Symbol Shift	Olfactory Control	Olfactory Removal	Olfactory Addition
Made Errors	0	4	3	1	2	0
No Error	22	12	25	17	16	16

Discussion

The evolution of group living is likely facilitated by adaptive cognitive processes that maintain social boundaries. These mechanisms are particularly relevant for aggregative or colonial species, in which individuals maintain separate nests within the group, and for which the social group presents a spatially complex environment to navigate. Many bees, particularly ground-nesting species, are known to aggregate in large groups sometimes exceeding 100,000 nesting females (Hanson & Ascher, 2018). Some carpenter bees also form dense aggregations, likely because they have strong preferences for a nesting substrate that can be limiting in their environment (Gerling et al., 1989). In these aggregations, several hundred individuals can occupy a single log, creating a complex three-dimensional nesting landscape. The ability to distinguish among nests at close range allows *X. sonora* to capitalize efficiently on a rare but valuable nesting substrate while minimizing conflict with neighbors. Furthermore, nest recognition ability functions in predator avoidance. For bees, bird predation is a significant cost of prolonged hovering outside of nests (Alcock, 1995, 1996), and in the study treatments that delayed nest entry resulted in attempts at prey capture by birds. This study demonstrates that nest recognition in this species may be facilitated by information from multiple sensory modalities.

The utility of multimodality in nest recognition

Interest in the evolution of multimodal information processing is increasing, both in terms of animal communication and cognition, particularly navigation (Buehlmann et al., 2013; Dovey et al., 2013; Higham & Hebets, 2013; Partan & Marler, 2005). Nest recognition provides a suitable context for the evolution of multimodal cue processing due to its importance in maintaining social boundaries (von Frisch, 1956). I manipulated visual and olfactory cues independently and observed significant delays in nest localization following visual manipulation. However, the fact that foragers ultimately did locate their nests under each of the experimental conditions suggests that their nest recognition system is generally robust to disruptions of individual cues.

In animal communication, multimodality increases signal detectability, provides redundancy, and conveys complex messages (Johnstone, 1996; Rowe, 1999). Multimodal communication strategies can also facilitate spatial localization. Female túngara frogs (*Physalaemus pustulosus*), for example, are better able to locate calling males when presented visually with vocal sac inflation, along with acoustic signals (Rosenthal et al., 2004). Multimodality in nest localization may confer similar benefits. For homing bees, chemical cues may have a shorter range of detection, greater specificity, and less persistence than visual cues. As discussed above, chemical cues provide important individual- or colony-specific information when nests have only subtle visual distinctions, as in ground-nesting bee aggregations. However, chemical cues can sometimes be unreliable if they are not a suitable temporal match to behavior patterns. For example, chemical cues may not always keep pace with frequent emigration or usurpation, which is common among *Xylocopa* throughout the reproductive season (Hogendoorn & Leys, 1993; Peso & Richards, 2011). In *X. sonora*, general conspecific odors appear to play a minor role in supplementing more temporally reliable visual cues.

It appears that unimodal (visual) processing is sufficient for nest localization in this species, but that multimodal processing can be advantageous, potentially in assessing logs as nesting sites. A potentially weighted organization of cue use may allow for prioritization of cues in order of stability and reliability.

The use of visual cues in nest recognition

The importance of vision in nest localization by group living and social Hymenoptera is well established (Butler et al., 1970; T. Collett et al., 1992; von Frisch, 1967; Wehner et al., 1996). Tinbergen (1972) demonstrated the importance of proximal visual landmarks for digger wasps, which nest in soil aggregations. Use of more distant landscape cues can precede this local landmark recognition, as in bumble bees, which orient first to distal cues as they approach the nest, and then to proximal cues around the nest entrance (Plowright et al., 1995; Robert et al., 2018). Proximal and distal cues may also be integrated continuously but at different weights at different distances from the nest in order to dynamically optimize the utility of multiple information sources (Hoinville & Wehner, 2018). This navigation scheme could conceivably account for the differences in the magnitude of search time between the two visual treatments, with rotation of the log delaying nest entry significantly more than the manipulation of proximal cues. The rotation of nesting logs disrupted long-range cues potentially including panorama cues, celestial cues, or magnetic cues, all of which may be heavily weighted at relatively greater distances from the log. Simultaneously, proximal cues are likely more important at short distances from the log, and their manipulation consequently has a smaller effect on search time. Together, these results suggest that carpenter bees may integrate visual information related to panorama and positional cues associated with the nest as well as

fine-scale cues in the immediate proximity of the nest entrance, and that the relative weight afforded to these cues may depend on distance from the nest.

The use of olfactory cues in nest recognition

Hölldobler and Michener (1980) hypothesized that olfactory nest recognition is an important pre-adaptation for olfactory nestmate recognition in the social insects; as such, it should occur frequently in solitary and subsocial bees (W. Wcislo, 1992). Indeed, the importance of olfaction in nest localization has been demonstrated widely across the Apoidea (R. L. Foster & Gamboa, 1989; Guédot et al., 2006; W. Wcislo, 1992). The results of the olfactory manipulations suggest that olfactory cues can influence a bee's ability to find her nest, but that the absence of these cues can be overcome by other cues, likely visual. The removal of olfactory cues at nest entrances impeded nest search efforts in the ground-nesting solitary sweat bee *Lasioglossum figueresi* (W. Wcislo, 1992). In this study, by contrast, removal of intrinsic olfactory cues by the same methods did not present any significant obstacle to carpenter bee nest localization. It may be that wood nesting substrate provides more visual heterogeneity than does soil, making visual navigation more reliable for wood-nesters like carpenter bees. Conversely, *Lasioglossum* may be more likely to rely on supplemental olfactory cues to navigate their visually homogenous soil habitat. The variation in cue use by species with different nesting ecologies highlights that nesting substrate presents particular recognition challenges, and that these challenges may drive interspecific variation in hierarchical cue use.

Interestingly, I found that the addition of scent cues from foreign bees to the nest entrance helped bees find their nests more quickly, rather than hindering their search. Conversely, a similar treatment significantly delayed nest entry for *L. figueresi* (W. Wcislo, 1992). This result suggests that carpenter bees may not respond to nest scent

cues on the level of an individual bee, but rather that a generic “bee odor” can be sufficient to indicate nest occupation and aid nest localization. As nesting logs age, *X. sonora* preferentially inhabit newer nests, leaving older nests unoccupied. To navigate logs riddled with nesting holes, only a fraction of which are occupied, *X. sonora* may use species- but not individual-specific odors to filter out empty nests and find their occupied nest faster. Similarly, house-hunting wild honey bees probably use general odor cues to locate tree cavities that have been previously occupied by bees, which they prefer (Visscher et al., 1985). Other insects, especially beetles, use non-specific odors as aggregation pheromones to orient to a single nesting site (Francke & Dettner, 2004), suggesting that species odor cues can provide information that facilitates nest selection for group living species. Alternatively, the common threat of nest invasion may make carpenter bees sensitive to foreign odors at the home nest, potentially speeding their flight to defend the nest.

In contrast to *X. sonora*, Hefetz (1992) found that returning foragers of *X. pubescens* delayed entry when their nest entrance was swapped with a neighbor’s, which was identical visually but presumably not chemically. In this case, unfamiliar odor cues acted as a deterrent. It is not clear why these two species respond so differently to unfamiliar nest entrance odors. *X. pubescens* appears to localize to an individual nest-specific odor, whereas *X. sonora* uses non-specific odor information. This study examined the role of CHCs in nest recognition, but it is likely that other compounds, especially glandular secretions, are also relevant for creating a characteristic nest odor (Gerling et al., 1989).

Conclusions

These results support the roles of both vision and olfaction in nest recognition, but suggest that visual cues may be sufficient in the absence of olfactory information. General scent cues may play a role in facilitating quick nest recognition, however. Furthermore, accuracy in nest localization likely preserves important boundaries between social groups. The generally low recognition error rates observed in this study suggest that bees avoid entering a nest until they have processed sufficient information to confirm they have identified it correctly, likely to avoid aggressive interactions with guards. At the same time, these aggressive interactions are not often fatal. As such, there may not be a significant cost to inaccurate nest entry nor a strong selective pressure for identification of individual-specific odors. The overall resilience of bees' homing behavior to the manipulations in this study likely reflects the adaptive value of a robust nest recognition system in this social context.

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APPENDIX A

FLUID NEST MEMBERSHIP DRIVES VARIABLE RELATEDNESS IN GROUPS OF A
FACULTATIVELY SOCIAL BEE, *FRONTIERS IN ECOLOGY AND EVOLUTION*



Fluid Nest Membership Drives Variable Relatedness in Groups of a Facultatively Social Bee

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Kin selection theory has dominated our understanding of the evolution of group living. However, many animal groups form among non-relatives, which gain no indirect fitness benefits from cooperating with nestmates. In this study, we characterized the relatedness and inter-nest migration behavior of the facultatively social carpenter bee, *Xylocopa sonorina*. Nesting constraints due to costly nest construction in this species give rise to intense intraspecific competition over access to existing nests. We used mark-recapture techniques to characterize patterns of dispersal and nest relocation within a nesting aggregation of spatially clustered nests. Two-thirds of bees relocated at least once during the reproductive season, likely to seek reproductive opportunities in another nest. This fluid nest membership creates opportunities for association among non-relatives. To assess the effects of this dynamic nesting behavior on group relatedness, we used microsatellite analysis to estimate relative relatedness within and between nests in the aggregation. We found that relatedness was variable across sampling years, but that in many cases nest-mates were no more related to one another than they were to non-nestmate bees in the population. Together, these results suggest that group composition in *X. sonorina* may result from strategies to maximize direct fitness. This study supports the hypothesis that factors beyond kinship, such as ecological constraints, are likely to drive group formation in this species.

Keywords: carpenter bees, *Xylocopa*, microsatellite, dispersal, social evolution, non-kin, relocation, drifting

INTRODUCTION

For many animal groups, kin selection theory has served as the central paradigm for understanding the evolution of social behavior (Hamilton, 1964; West-Eberhard, 1975; Eivers and Hare, 1976). Nevertheless, many animals form social groups with non-kin, and gain little to no indirect fitness benefits from cooperation (Bernasconi and Strassmann, 1999; Clutton-Brock, 2009; Riehl, 2013). Particularly within the Hymenoptera, these groups tend to be understudied relative to kin groups, but offer valuable opportunities to test hypotheses about drivers of social evolution while controlling for indirect fitness benefits (Ostwald et al., in review, this issue). However, the extent to which animals form alliances with non-relatives and the mechanisms by which these groups arise remain unknown for many social taxa.

Non-kin groups may arise through shared exploitation of limiting resources, especially nesting sites. These conditions may prompt individuals to disperse and seek reproductive opportunities by joining established groups or constructed nests. Nest joining by non-relatives is common within

the cooperatively breeding birds, which may gain direct fitness benefits of cooperation even when relatedness is low (Piper et al., 1995; Young, 1998; Baglione et al., 2002; Richl. 2011). Likewise, among the communal and polygynous wasps and bees, females may join nests established by non-relatives, where they may benefit from reduced costs of guarding, provisioning, and/or nest construction (Danforth et al., 1996; Johnson, 2004; Weislo and Tierney, 2009; Mora Kepfer, 2014; Ostwald et al., in review, this issue).

The large carpenter bees (genus *Xylocopa*) represent useful candidates for testing hypotheses about social evolution, particularly in the context of non-kin sociality. Carpenter bees may be solitary or may form small, fluid societies in which a single dominant female performs all or most of the egg laying, provisioning, and nest construction/maintenance (Gerling et al., 1989; Richards and Course, 2015; Buchmann and Minckley, 2019). Subordinate females may perform guarding duties but otherwise contribute little to the productivity of the nest (Hogendoorn and Velthuis, 1993; Richards, 2011; Prager, 2014). Instead, subordinates are likely waiting for opportunities to inherit existing nests (Velthuis and Gerling, 1983; Richards, 2011; Schwarz et al., 2011; Vickruck and Richards, 2018), which can be less costly than new nest construction (Ostwald et al., 2021).

Because nests are costly and valuable resources, most females will breed in existing nests rather than undertaking new nest construction (Peso and Richards, 2011), which is energetically expensive (Ostwald et al., 2021). This limitation creates a shortage of available breeding space that can give rise to intense intraspecific competition for reproductive opportunities (Gerling et al., 1989; Buchmann and Minckley, 2019). Following emergence, adult *Xylocopa* often (but not always—see Gerling, 1982; Velthuis, 1987) overwinter with siblings in the natal nest in mutually tolerant pre-reproductive assemblages that become aggressive and break up at the onset of the reproductive season (Michener, 1990). These family groups become aggressive at the onset of the reproductive season, prompting dispersal and the formation of dominance hierarchies (Velthuis, 1987; Michener, 1990; Richards and Course, 2015). To secure reproductive opportunities, females may compete for dominance in their natal nests or may attempt to usurp reproductives in nearby nests (Hogendoorn and Leys, 1993; Hogendoorn, 1996; Richards, 2011). Alternatively, females may disperse from their natal nests to join neighboring nests, perhaps seeking to advance their position in a reproductive queue or to minimize competition with close kin (Vickruck and Richards, 2018, 2021).

Nest joining behavior has been observed in several *Xylocopa* species and is expected to create opportunities for association among non-relatives (Gerling, 1982; Velthuis, 1987; Camilo and Garofalo, 1989; Peso and Richards, 2011). Peso and Richards (2010) used mark-recapture techniques to examine the extent of nest joining in the eastern carpenter bee, *Xylocopa virginica*, and found that roughly half of recaptured females were found at a different nest from the one at which they were originally marked. The high rate of relocation can explain low within group relatedness in social groups of this species (Vickruck and Richards, 2021). Aside from this study, genetic relatedness of nesting groups is unknown for

any other species of carpenter bee, despite ample behavioral observations indicating that carpenter bees tolerate non-relatives in their nests.

We examined nest joining behavior and relatedness in the facultatively social valley carpenter bee, *Xylocopa sonorina*. Like most carpenter bees, this species is characterized by high reproductive skew and intense nest-site competition (Gerling, 1982). Gerling (1982) observed adult females joining active nests during the reproductive season, and also found that some recently emerged offspring dispersed from their natal nests soon after emergence. We predicted that dispersal and nest relocation may dilute relatedness within nests, leading to mixed associations of kin and non-kin. Using complementary behavioral and genetic approaches, we characterized the dynamic group membership of *X. sonorina* and the consequences of these behaviors for relatedness within and among nesting groups. In doing so, we aim to highlight mechanisms of group formation that can evolve in the absence of helping behavior and indirect fitness returns.

MATERIALS AND METHODS

Study Design and *X. sonorina* Seasonal Activity

To characterize nest relocation and relatedness patterns in *X. sonorina*, we collected genetic and behavioral data from a single nesting aggregation (an occupied log of Goodding's willow, *Salix gooddingii*, 206 cm length × 23 cm diameter) sourced from a riparian area in Phoenix, AZ, United States (33.41988 N, -112.07062 W). In central/southern Arizona, winter quiescence for *X. sonorina* typically ceases in March (Minckley, 1987). Mating activity occurs in March and April (Minckley and Buchmann, 1990), and female reproductive activities, including nest construction/renovation, egg laying, and offspring provisioning, occur primarily in April and May (Minckley, 1987; Ostwald et al., 2020), and offspring emerge in late May to June (Minckley, 1987; Ostwald et al., 2020). This species is univoltine and produces an average of 11.5 brood per nest (Ostwald et al., 2020), laid by a single reproductive female. Nests may be solitary or may contain as many as 9 adults during the spring (Ostwald et al., 2020), though the distribution of group sizes is expected to depend strongly on local factors such as nesting density.

To capture dynamic nest movement behavior across the reproductive season but prior to offspring emergence, we conducted behavioral observations from mid-March to early May of 2021. Likewise, to capture group relatedness at the onset of dispersal and reproductive activity we collected genetic samples in late March to early April of 2019 and 2020.

Behavioral Observations of Dispersal and Nest Joining

We used mark-recapture techniques to examine dispersal and nest joining by female bees over the course of the reproductive season. During spring 2021, we caught and/or observed bees

entering and departing nest entrances in our focal log (as in Pesó and Richards, 2010; Pesó and Richards, 2011). Upon first capture of an individual, we recorded the nest of origin and marked each bee with a unique two-color paint marking on the thorax and abdomen using Testors enamel paint (Testors, Vernon Hills, IL). For all subsequent observations we recorded the identity of the bee and the nest of departure or arrival. These nests may have been natal nests or non natal nests to which they had dispersed. To estimate total population size, we extrapolated from counts of the number of marked and unmarked female bees entering and exiting the log over the course of 1 h at the end of the spring, after all focal bees had been marked.

We observed nest entry and departure activity for 17 days between March 18 and May 2, 2021. On each sampling day we observed bees for 1–4 h within the daily window of peak flight activity, for a total observation period of 30 h 25 min over the course of the spring. We observed all entries and exits during these observation periods, and recorded the nests visited. Ambient temperatures at the time of observation ranged from approximately 20–34°C.

Genetic Analysis

We collected genetic samples by capturing bees upon departure from their nests. We anesthetized all females on ice then removed the most distal tarsal segment from one metathoracic leg using a sterile razor blade. Removal of this tarsal segment is not known or expected to significantly impair mobility (Vickruck and Richards, 2017). Tarsal samples were stored in ethanol at –20°C for later genetic analysis.

We extracted DNA from all tarsal samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, United States). We amplified DNA at 6 microsatellite regions characterized for the congener *X. virginica* (Vickruck, 2015: XV7, XV9, XV27, XV28, XV30, XV42), having previously confirmed the presence of these loci in *X. sonorina* using gel electrophoresis of amplified PCR products. Loci were amplified in three 12.5 µl PCR multiplex reactions of two or three primers per multiplex. Forward primers were tagged with a fluorescent probe (6FAM, TET, PET, HEX, or VIC) for fragment identification (Supplementary Table 1). Genotypes were analyzed by fragment analysis and scored by visual inspection of the tracefiles using Geneious R8 (Kearse et al., 2012).

Relatedness Calculation and Statistical Analysis

We estimated relative relatedness of sampled individuals using methods developed by Queller and Goodnight (1989), using the R package *related* (Pew et al., 2015). Data are presented as pairwise comparisons of the relative relatedness of all possible pairs of individual female bees in the sample. We used Wilcoxon tests to compare relative relatedness of nestmates (within nest comparison) vs. non-nestmates (between nest comparison) within each year of collection. We excluded from analysis any individuals that were missing genotype information at two or more loci ($N = 6$). In addition, we tested for adherence to Hardy Weinberg equilibrium at all loci, and estimated F_{ST} and

F_{IS} between collection years, using the *genepop* package (Rousset, 2008). All statistical analyses were conducted in R 4.1.9 using the *base* and *stats* packages (R Core Team, 2021).

RESULTS

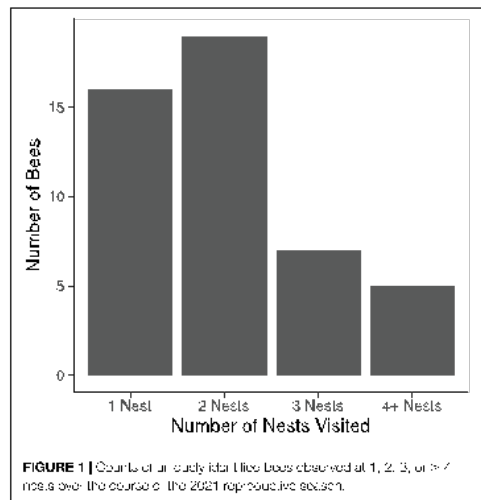
Fluid Group Membership

Over the course of spring 2021, we marked a total of 75 unique female bees at 25 focal nests. We estimate that there were approximately 147 female bees residing in the log at this time based on the estimated ratio of marked to unmarked bees. Further, we estimate that there were approximately 40 active nests over the spring observation period. Of the 75 marked female bees, we observed 47 bees on more than one occasion, with an average of 2.57 ± 0.22 (range: 1–12) observations per individual. The bees that were only observed once may have dispersed to nests other than our 25 focal nests (including nests in other, distant aggregations) or may not have left the nest during our chosen sampling times. Of the 47 bees observed more than once, 16 (34.04%) were observed only at a single nest. The remaining 31 bees (66.96%) were observed at multiple nests: 19 bees (40.43%) were observed at 2 different nests, 7 bees (14.89%) were observed at 3 different nests, 3 were bees (6.38%) observed at 4 different nests, and 2 bees (4.26%) were observed at 5 different nests (Figure 1). No bee was observed re-visiting a nest she had previously occupied.

Importantly, the number of nests visited by each bee is likely to be greater than what we were able to observe during this limited observation period. Many of the 28 bees we marked but did not recapture may have relocated to non-focal nests. For all bees observed more than once, we observed a significant correlation between number of observations of each bee and the observed number of nests visited ($r = 0.49$, $df = 44$, $P < 0.001$). This correlation suggests that more intensive sampling would reveal even lower nest fidelity.

Relatedness Within and Between Groups

We sampled genetic material from 68 adult females in spring 2019 and 2020. In 2019 we sampled 29 females from 12 nests and in 2020 we sampled 39 females from 18 nests. We sampled between 1 and 7 females per nest (mean = 2.27, S.E. = 0.28). Population genetic analyses showed little genetic differentiation between years (across loci $F_{ST} = 0.0488$; Supplementary Table 2). Hardy-Weinberg equilibrium was verified for all but two loci (XS7 and XS30; Supplementary Table 2). In XS7 F_{IS} analysis showed an extreme overabundance of heterozygotes ($F_{IS} = 0.97$). We calculated the relative relatedness of all pairwise comparisons of individuals sampled (Queller and Goodnight, 1989). In this metric of relatedness, a value of 0 refers to the average relatedness of all individuals sampled. Positive values refer to above-average relatedness and negative values refer to below-average relatedness. A relative relatedness value of 1 indicates that the two individuals share alleles at all six loci tested. In 2019, the estimated relatedness (r) of nestmates (mean = –0.09, S.E. = 0.15, median = 0.10) was indistinguishable from the relatedness of non nestmates (mean = 0.10, S.E. = 0.03,



median = -0.023 ; Wilcoxon test: $P = 0.500$; **Figure 2**). In 2020, the relatedness of nestmates (mean = 0.35 , S.E. = 0.07 , median = 0.336) was significantly higher than the relatedness of non-nestmates (mean = -0.05 , S.E. = 0.02 , median = -0.01 ; Wilcoxon test: $P < 0.001$; **Figure 2**).

DISCUSSION

Choosing whom to live with is one of the most consequential social decisions animals make. Kinship can factor strongly into this decision when individuals receive indirect fitness benefits from helping relatives reproduce. When helping behavior is limited, as for many carpenter bees (Gerling et al., 1989; Richards, 2011; Prager, 2014), incentives for nesting with kin may be likewise minimal. We explored nesting decisions in the valley carpenter bee (*X. sonorina*), which face severe intraspecific competition over nesting opportunities (Gerling, 1982; Ostwald et al., 2021). We demonstrated that nest joining is common throughout the reproductive season in this species. Joining behavior may prompt associations between non-relatives. Indeed, we present genetic evidence suggesting a mix of kin and non-kin in nesting groups, with many close relatives nesting apart, and many unrelated individuals nesting together. These highly dynamic social groups raise important questions about the costs and benefits of group living in different social contexts.

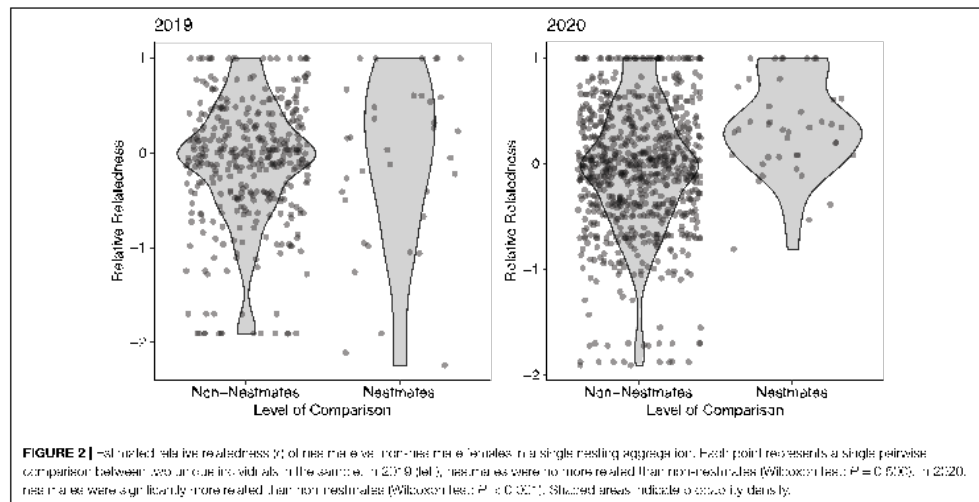
Inter-nest migration creates opportunities for individuals to associate with non-relatives. Often, these movements reflect adaptive strategies to access limited reproductive opportunities through resource sharing or cooperation, as in many communal birds and insects (Abrams and Fickwort, 1981; Weislo, 1993; Vehrencamp, 2000; Weislo and Tierney, 2009; Riehl, 2011). In

other cases, relocation may reflect usurpation (Klahn, 1988; Hogendoorn and Leys, 1993). In our study, a majority of bees (67%) relocated from the nests at which they were originally captured (similar to rates observed in other Hymenoptera species; *Megachile rotundata*, Goerzen et al., 1995; *X. virginica*, Pesó and Richards, 2011; *Polistes canadensis*, Sumner et al., 2007), and 27% relocated more than once. These moves may be temporary or permanent, reflecting both relocation and perhaps inspection of possible nesting sites. Notably, however, we never observed a bee relocate and then return to her previous nest, suggesting that relocations are often long-term.

The high rates of nest relocation in our study likely represent attempts to seek out reproductive opportunities within saturated nesting space. Bees that relocate may be attempting to usurp dominant reproductives in nearby nests (Hogendoorn and Leys, 1993; Hogendoorn, 1996; Richards, 2011). Alternatively, they may join existing groups as subordinates, but perhaps with a greater chance of nest inheritance than they had in their natal nests (Richards and Course, 2015). Notably, nest relocation was common despite the fact that intruders are usually treated aggressively by resident bees (Velthuis and Gerling, 1983; Hogendoorn and Velthuis, 1995), suggesting that the potential benefits of relocation can outweigh the costs of physical conflict. The fitness outcomes of the relocation strategy compared with remaining in the natal nest remain to be investigated.

Often termed “drifting,” nest relocation behavior should not be conflated with navigational errors. For example, navigational errors are a well-documented apicultural phenomenon in which honey bees enter unfamiliar hives in crowded apiaries (Free, 1958; Pfeiffer and Crailsheim, 1998; Oliveira et al., 2021). Studies of inter-nest movements in the paper wasp *Polistes canadensis* and the eastern carpenter bee *X. virginica* found evidence that nest relocation in these species was not merely the result of navigational errors (Sumner et al., 2007; Pesó and Richards, 2011). In a study of the navigational abilities of *X. sonorina*, females made very few navigational errors, even in treatments designed to disrupt homing cues (Ostwald et al., 2019). These observations, coupled with the high incidence (67%) of nest relocation in our study, suggest that nest relocation here represents an active strategy rather than simply a consequence of navigational errors.

Our behavioral data support the results of our genetic analysis, which suggests that nestmates are not always close relatives. In 2019, we found that nestmates were no more related to one another than they were to non-nestmates. Frequent inter-nest migration, as observed in our mark-recapture data, was likely to be the mechanism diluting relatedness in these nestmate groups. In 2020, however, we found nestmates to be significantly more related than non-nestmates, despite sampling at the same time of year across sampling years. This suggests that levels of relatedness vary, across years and likely seasonally. If females overwinter with siblings, then we would expect relatedness to progressively decrease over the reproductive season, as bees disperse and are driven from the nest by dominant bees (Velthuis, 1987; Richards and Course, 2015; Vickruck and Richards, 2021). Also, annual variation in the timing of environmental cues regulating carpenter bee social phenology (Minckley, 1987;



Ostwald et al., 2020) could account for observed differences across years if, for example, bees began foraging and dispersing later in 2020 than in 2019.

Alternatively, the extent of dispersal and nest relocation across years may depend on factors such as population density and the degree of intraspecific competition. Further sampling throughout the year and across years would usefully clarify the extent to which relatedness changes over time and how these patterns are shaped by environmental factors. Our observed differences in relatedness may be, in part, an issue of limitations on genetic markers. We examined genetic loci characterized for another species, *X. virginica* (Vickruck, 2015), which we demonstrated to be present and variable in *X. sonorina*. However, developing species-specific genetic markers will enable greater resolution of relatedness estimates in future studies.

Why might bees leave their natal nests to join individuals to which they are not closely related? Many non-kin groups benefit from task sharing that improves survival or fitness by reducing the individual labor burden (Bernasconi and Strassmann, 1999; Tibbets and Reeve, 2003; Cahan and Fewell, 2004; Wilkinson et al., 2016). Carpenter bees, however, do not share the labor costs of reproduction, with only the reproductively active female contributing meaningfully to foraging and nest construction (Richards, 2011; Richards and Course, 2015). As such, additional group members may not improve the productivity of the nest (Prager, 2014; Buchmann and Minckley, 2019). In the absence of helping behavior, the indirect fitness benefits of remaining in the natal nest with relatives are likely to be low or absent. Instead, females may prioritize seeking direct fitness opportunities wherever they may be available, with kin or non-kin.

At the same time, social decisions may not necessarily be made irrespective of kinship. Temporary matrifilial societies may

arise from generation overlap between mothers and recently emerged offspring, in which offspring may guard the nest and receive food from their mother (Gerling, 1982; Gerling et al., 1983; Velthuis and Gerling, 1983). Conversely, non-kin nesting may actually represent a strategy to maximize reproductive opportunities among kin. Data from *X. virginica* even suggest that females may actively avoid nesting with relatives during the reproductive season to reduce kin competition (Vickruck and Richards, 2021). Indeed, our data show many instances of closely related non-nestmates. Further study is needed to determine whether females can benefit from associating with relatives, and if so, what conditions and life history stages favor these associations.

In conclusion, we found evidence for variable relatedness within carpenter bee nesting groups, suggesting that groups can consist of a dynamic mix of kin and non-kin nestmates. This study represents one of only two to quantify genetic relatedness in *Xylotopa* groups (Vickruck and Richards, 2021). Observations of nest relocation in an additional two *Xylotopa* species suggests that low relatedness may be common among the social species in the genus (*X. pubescens*, Gerling et al., 1983; *X. salicetipes*, Velthuis, 1987). Nest membership in our study was highly fluid, with most females spending time in multiple nests over the course of the reproductive season. This nest relocation strategy likely reflects attempts to secure reproductive opportunities among strongly limited nest sites. Changes in nest membership demonstrate that relatedness is not a fixed condition, but rather may shift with seasonal and social variables. Instances of low relatedness among our sampled bees suggest limited indirect fitness benefits for nestmates, and instead emphasize the importance of ecological factors, especially nesting constraints, in facilitating sociality in *X. sonorina*.

(Ostwald et al., 2020, 2021). Though sociality is often interpreted through the lens of kin selection, systems such as these with low and dynamic relatedness highlight the complexity of social decisions beyond the role of kinship.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MO collected the data and wrote the manuscript. MO and RD analyzed the data. All authors contributed to study design and manuscript editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/feco.2021.767380/full#supplementary-material>

Supplementary Table 1 | Amplified microsatellite with sequence, dye, and multiplex information. Loci were characterized for *X. variegata* in Vičrusk, 2019.

Supplementary Table 2 | Summaries population genetic information across microsatellite loci, including F_{ST} , F_{IS} , X^2 test for Hardy-Weinberg Equilibrium of associated DF and P-values.

Supplementary Data Sheet 1 | Queen-Queen relatedness values for all comparisons within and between colonies of the focal population presented in the paper, as well as for non-focal populations (“Outgroup” comparisons).

Supplementary Data Sheet 2 | Mark-recapture raw data including date of capture and nest identity.

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APPENDIX B

TEMPORAL AND SPATIAL DYNAMICS OF CARPENTER BEE SOCIALITY REVEALED

BY CT IMAGING, *INSECTES SOCIAUX*



Temporal and spatial dynamics of carpenter bee sociality revealed by CT imaging

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Abstract

Facultatively social animals adaptively match social strategy to environmental context; as such, they offer unique insights into the ecological factors facilitating social evolution. We investigated temporal (seasonal) and spatial (nest architectural) factors governing flexible social behavior in the carpenter bee *Xylocopa varipuncta* Patton using repeated, non-destructive computerized tomography scans of nesting logs. We tested the hypothesis that group living is mediated by environmental factors, specifically ecological constraints and phenological parameters. These imaging data support a facultative social organization strongly influenced by seasonal shifts in life-history strategy. Our results also illuminate patterns of structural change associated with nest inheritance and eventual nest abandonment. This dynamic use of space mediates the within-nest interactions that determine social organization. Furthermore, constraints on the usefulness of inherited nest structures compound an existing limitation on nest sites that may underlie the origins of this flexible social strategy. These findings emphasize the importance of including spatial dynamics in considerations of the ecological contexts in which sociality evolved.

Keywords Carpenter bees · Nest architecture · Phenology · Social polymorphism

Introduction

The initial transitions from solitary to group living are likely facultative, with some individuals in a solitary population adopting a social lifestyle (West-Eberhard 1987; Weislo and Fewell 2017). As such, facultatively social species found among diverse taxa including birds, mammals, and insects, provide valuable insights into the conditions promoting the evolution of social behavior. Studies of the drivers of flexible sociality have variously highlighted ecological factors (Field et al. 2010; Haney and Fewell 2018), social factors (Kapheim et al. 2015; Lawson et al. 2016), and indirect fitness effects (Reyer 1984; Yagi and Hasegawa 2012). Within these broader contexts, individuals must weigh the

fundamental considerations of when and where it is adaptive to be social. In this study, we use CT imaging to characterize a facultative social organization in the carpenter bee *Xylocopa varipuncta* Patton, for which both seasonal and spatial selective pressures likely play roles in the formation of social groups.

Complex social behavior often has its evolutionary origins in ecological and spatial constraints that compel organisms to forego independent reproductive opportunities. In particular, environmental factors that change seasonally can drive cyclical variation in social behavior. For example, temporary grouping behavior can maximize seasonal opportunities for resource exploitation (Bos et al. 2004; Watanuki et al. 2004; Smith et al. 2019). Additionally, climatic stressors can drive periods of mutual tolerance between conspecifics seeking shared refugia. This is especially the case for temperate species with limited ability to withstand winter conditions outside of hibernacula or aggregations (Arnold 1988; Dapporto and Palagi 2006).

Habitat saturation and costly nest founding can also encourage social nesting (Emlen 1982; Hatchwell and Komdeur 2000; Barve et al. 2019). Inheritance of nesting structures by descendants is a common adaptation to environments lacking in opportunity for dispersers (Harris and

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Muric 1984; Myles 1988; Leadbeater et al. 2011). Extended multi-generational use of inherited nests may have implicit limitations, however, when nest structures decline in quality over time (Holmes et al. 2003; Møller and Fritzke 2006). Furthermore, these changes in nest architecture can shape interactions within societies. Effects of the built environment on social behavior are well established in human societies (Baum and Valins 1977; Allen 2000; Williams 2005) and increasingly in the eusocial insects (Pinter-Wollman 2015; Pinter-Wollman et al. 2017), but spatial determinants of social complexity are underexplored in discussions of social evolution (He et al. 2019).

Here we explore the nest as the interface at which organisms shape their environment and which in turn shapes the social interactions of its inhabitants. To examine interactions among sociality, ecological factors, and the built environment, we observed seasonal changes in the social behavior of the carpenter bee *X. varipuncta* and correlated impacts on nest architecture. Carpenter bees are known for their diversity of social behaviors (Michener 1990; Rehan et al. 2012), with a number of species expressing intra-population social polymorphism (Gerling and Hermann 1978; Gerling et al. 1981). Nest site limitation has often been invoked as a primary ecological driver of carpenter bee social nesting (Gerling and Hermann 1978; Gerling et al. 1989). Likely due to this limitation, carpenter bees are strongly philopatric, inheriting and renovating nests from older generations (Michener 1990). The ultimate abandonment of these nesting tunnels, however, suggests an eventual functional expiration of inherited nests. One possible mechanism by which nest quality may decline over time is through tunnel widening that accompanies brood-rearing activities (Gerling et al. 1981). Wider tunnels require the construction of larger-diameter nest partitions, which may increase the cost of constructing these partitions. More importantly, however, the diameter of nest tunnels has important implications for within-nest behaviors, particularly dominance interactions, which often involve physically blocking a rival's passage through the nest (Gerling et al. 1981; Hogendoorn and Velthuis 1993; Richards and Course 2015). As such, important behaviors mediating social organization would be compromised by tunnels that are wide enough to permit non-consensual passing.

We conducted a year-long series of CT scans of carpenter bee (*X. varipuncta*) nesting logs to test the hypothesis that social organization responds dynamically to seasonal and architectural changes. The destructive nest surveys that are typically used to characterize insect social structure fail to capture important structural changes caused by long-term interactions between the social group and its extended phenotype, the nest. For many taxa, the nest is the site of interactions that are essential for understanding group dynamics; however, it is often inaccessible to non-destructive observation. Recent work has made use of imaging technology to

visualize ant and termite nests (Fuchs et al. 2004; Halley et al. 2005; Perna et al. 2008; Varoudis et al. 2018), but these studies have focused on characterizing nest construction by large eusocial colonies rather than examining the ways in which the use of space may shape sociality at its evolutionary origins. Some of the foundational work on carpenter bee sociality has made use of two-dimensional, field X-ray views of nest structures in thin wooden boards (Gerling and Hermann 1978; Gerling et al. 1981; Hogendoorn and Velthuis 1993). Our study builds on this foundation by using CT techniques to produce a three-dimensional view of nest architecture and social strategies in natural nesting logs. This approach allows visualization of social structure over time and demonstrates flexible matching of social strategy with seasonal and spatial conditions.

Methods

Population, nesting logs, and *X. varipuncta* life history

To track the social dynamics of naturally nesting carpenter bees, we established a population of *X. varipuncta* in a desert riparian research area at Arizona State University (33°42' N, -111°93' W). We collected and relocated two logs (Log A: *Prosopis* sp. wood, 51 cm long, and Log B: *Salix gooddingi* wood, 107 cm long) containing multiple *X. varipuncta* nests from local residential and park areas in December 2017. A third log, Log C (*Prosopis* sp. wood, 135 cm long) was used for dissection in February 2018 immediately after it was removed from its original location in a nearby residential area. Each log contained over a dozen previously constructed nest structures in varying stages of decay, only a fraction of which were occupied at a given time. Logs in the courtyard experienced ambient temperature and humidity, and received morning shade.

In the desert Southwest, *X. varipuncta* spend the winter inactive in their nests, before emerging in early March (Minckley 1987). Mating activity generally occurs between March and May (Alcock and Johnson 1990). Beginning in March, females provision brood and perform any necessary excavation of nesting tunnels (Minckley 1987). Emergence of adult offspring generally begins in June, after which activity levels taper through the fall into November and December, by which time adults enter winter-quiescence (Minckley 1987; personal obs.).

CT scanning

To estimate frequencies of social and solitary nesting strategies, nesting logs were scanned once every two months between January 2018 and March 2019, for a total of eight

scans per log, using a CT machine (General Electric, Light-Speed VCT) at St. Joseph's Hospital in Phoenix, AZ. In January 2018 only Log B was scanned, and in March 2018 only Log A was scanned; for all subsequent scan dates, both logs were scanned together. Scans took place between 1.00 AM and 6.00 AM to ensure that all bees were inactive inside their nests during scanning. To prevent escape, nest entrances were plugged with cotton prior to transport to the hospital. Scan image slices were 2.5 mm thick and provided in sagittal and coronal views relative to the long axis of the log. Following scanning, logs were placed in their original locations at the research site.

3D reconstructions: social organization and nest architecture analysis

To assign bees in scan images to their particular nests, we manually reconstructed the 3D structure of nests and visually identified all bees, brood, and pollen inside the nest tunnels (Fig. 1). 3D images were constructed from scans using Avizo™ version 9.0 (Thermo Scientific™), (Fig. 2). For each 3D nest structure, we counted the number of unique tunnel branches per nest.

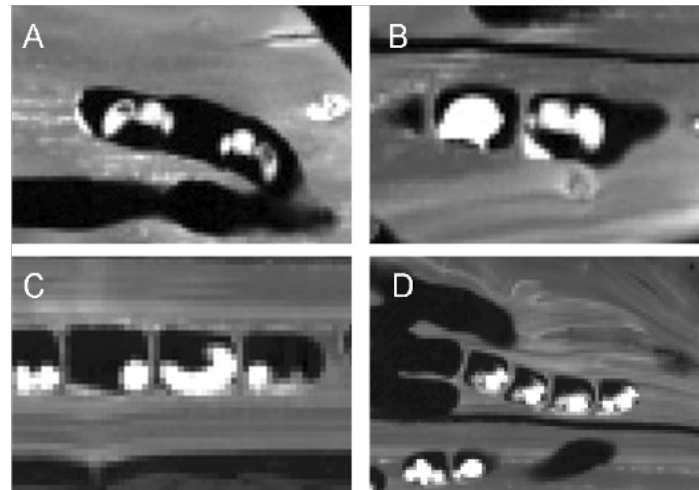
To determine the effect of brood cell construction on tunnel diameter, we measured the diameter of tunnels at their widest point within three brood-cell lengths of the terminal end, in spaces where brood cells had been or would be constructed. We measured diameters of tunnels occupied by brood in the May 2018 scans (for both logs) and compared them to the diameter of these same tunnels before the brood cells were constructed (Log A: March 2018 scan; Log B:

January 2018 scan). We also measured tunnel diameter of abandoned nests in both logs in the May 2018 scans. Tunnel diameter was measured using ImageJ version 2.0.0 (National Institutes of Health).

The 3D reconstructions of nests and bees in scan images allowed us to estimate the prevalence of social (multi-female) and solitary (single female) nesting at different times throughout the year. It is important to note that we were unable to distinguish the female from male bees on the CT images. Due to this limitation, any nests that may have been comprised of a single female and one or more males would have been counted as a possible multi-female nest, causing us to underestimate the number of solitary nests. Conversely, any nests consisting of a single male would have been counted as a possible solitary female nest, causing us to overestimate the number of solitary nests. However, we do not expect these counterbalancing potentials for over- and underestimation to affect observed ratios of solitary to social nests throughout the year. Males are active in the spring through May when defending mating territories (Alenck and Johnson 1990, Minkley and Buchmann 1990), before dying and being replaced by the new generation of males in June (personal obs.). Due to this relative continuity of male presence in nests, we do not expect significant seasonal shifts in sex ratio that would impact our social nest frequency estimates.

To additionally overcome the limitation associated with not distinguishing males and females in scan images, we dissected nesting logs and censused occupied nests near the beginning and the end of the 14-month study. In February 2018, a month after the first scan, we dissected Log C, which

Fig. 1 Scan images showing nesting tunnels with a adult bees **b** a full pollen provision and a “pollen slant” or in-progress pollen provision tended by an adult bee, **c** brood cells containing larvae, and **d** brood cells containing pupae



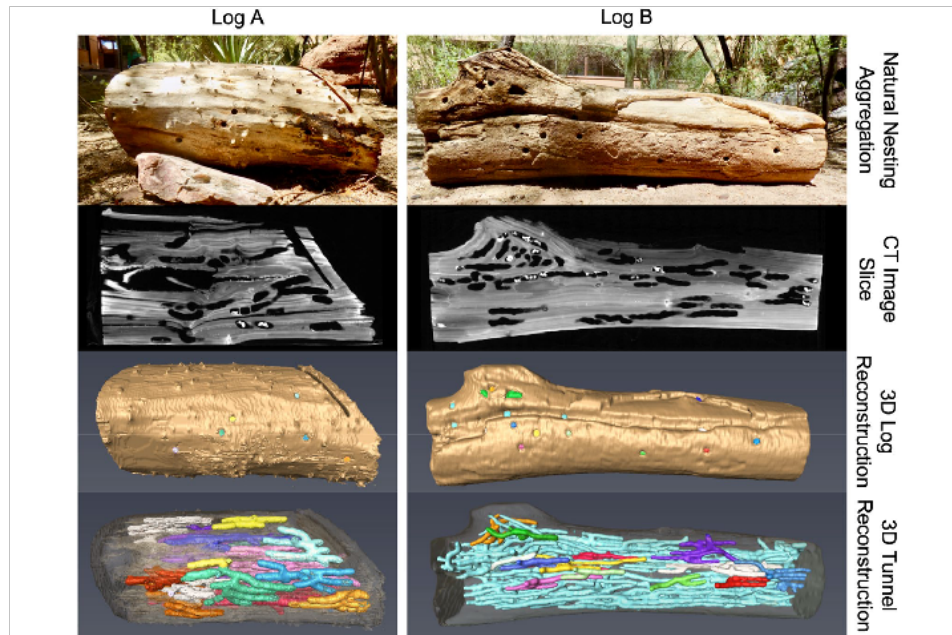


Fig. 2 Images showing nesting logs, CT scan image slices (July 2018), and 3D reconstructions of logs and nest tunnels. Tunnels and nest entrances shown in different colors represent separate uncore-

ected nests. The blue tunnel network in Log B consists most likely of a series of passively merged nests

was not used for any scans. In March 2019, after all scans were completed, we dissected Logs A and B, the subjects of our series of eight scans. During log dissection, we exposed nesting tunnels with a hammer and chisel, and censused bees in each nest to categorize it as social or solitary. We also measured head widths of females using digital calipers to contextualize our measurements of tunnel diameter.

Statistical analysis

We evaluated the effect of season on social strategy (solitary or social) with a Fisher's exact test. We used Pearson's correlation analysis to test for a relationship between the number of tunnel branches in a nest and the number of adults per nest in July 2018. For our analysis of nest tunnel diameter, we confirmed normality and homoscedasticity of data with Shapiro tests and Levene's tests, respectively. To determine the effect of brood cell construction on tunnel diameter, we compared tunnel diameter measurements before and after brood cells had been constructed, using a paired *t* test with a Bonferroni correction for multiple pairwise comparisons.

To compare these data to the diameters of tunnels in abandoned nests as well as to the doubled head width of females (a measure of the ease of passing other adults in the nest), we conducted *t* tests as appropriate and Mann-Whitney *U* tests when assumptions were violated. *P* values are reported with Bonferroni corrections for multiple pairwise comparisons. Results are presented as mean \pm standard error. All statistical analyses were performed in R version 3.4.2 (R Development Core Team 2017).

Results

Phenology of social organization

The total population (Logs A and B) numbered 29 bees when logs were CT scanned in May 2018, during early brood provisioning and before the emergence of the first brood (Fig. 3a). The adult population more than quadrupled to 129 bees after the emergence of brood in the July 2018 CT scans. However, this increase was still less than half the

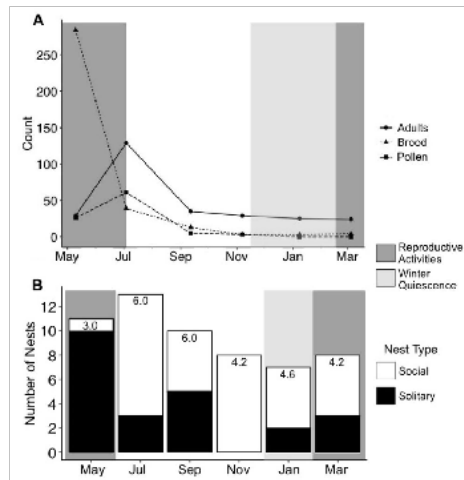


Fig. 3 Phenology of carpenter bee sociality represented as a total census across both logs of brood cells including eggs, larvae or pupae, and “pollen slants” representing the early stage of provisioning future brood. Times shaded orange represent the general period of activity devoted to reproductive activities such as mating, nest construction, and brood provisioning. Times shaded blue represent the period of winter quiescence. **b** Proportions of solitary and social nests in Logs A and B combined, between May 2018 and March 2019. Numbers in white bars represent mean numbers of adult bees in social nests. Total nest number changed throughout the year as nests were variously occupied and abandoned. Time of year had a significant effect on the frequency of social nesting ($P < 0.001$)

potential population increase suggested by the emergence of 245 brood between May and July. By September 2018, the within-log population had fallen to below 40 adults, probably due to a combination of mortality and dispersal where it remained until the following year. Overwintering mortality, estimated as the decrease in the number of adults present in scans between November and January, was relatively minimal at 15% (4 bees).

Social and solitary nests co-occurred throughout the year, demonstrating facultative sociality. The number of active nests in the two nests fluctuated throughout the year between a minimum of 7 and a maximum of 13, due to migration, new nest construction, and nest abandonment. There was a significant effect of time of year on the frequency of social nesting in this population (Fisher’s exact $P < 0.001$); (Fig. 3b). Specifically, social nesting was common (50% of nests or more) throughout the year, except when solitary nesting predominated in May (10 of 11 nests) when females were provisioning their first brood. In July 10 of 13 nests were social, likely because young adults remained in the nest after emergence. Social nests at this time contained

6.50 ± 1.12 adults. In September, nests were split evenly between solitary and social strategies, and social nesting predominated through the winter (Fig. 3b).

Nest censuses from our log dissections indicated that our estimates of social and solitary nesting frequencies were roughly consistent with actual proportions of social and solitary nests, and that error resulting from ambiguous social states was minimal. Of 18 total nests dissected, four contained a solitary female and one or more males (which would have caused an underestimate of the number of solitary nests) and two contained a single male (which would have caused an overestimate of the number of solitary nests). The counterbalancing effects of these two types of potential error help mitigate the effects of ambiguity in social strategy assignment using CT scans.

Nest architecture

Our 3D reconstructions of nests revealed a diversity of nest structures (Fig. 4) ranging from a single linear tunnel to large tunnel networks with more than fifteen branches. Additionally, the reconstructions suggested that multiple nests had become connected such that a continuous tunnel structure contained multiple adjacent entrances, probably from formerly independent nests (see blue tunnel network, Log B, Fig. 2). For those nests occupied when the population reached its peak size in July, we found no relationship between the number of adults in a nest and the number of branches ($r < 0.01$, $n = 12$, $P = 0.98$).

The construction of brood cells significantly increased tunnel diameter, from 15.06 ± 0.16 mm before brood to 16.80 ± 0.24 after brood cell construction (Paired t test, $t = -7.43$, $n = 24$, Bonferroni-adjusted $P < 10^{-6}$); (Fig. 5). Abandoned tunnels (16.87 ± 0.23 mm, $n = 24$) were also significantly wider than tunnels before brood cell construction (Welch’s t -test, Bonferroni-adjusted $P < 10^{-6}$), and similar to those after brood cell construction (Welch’s t test, Bonferroni-adjusted $P = 1$); (Fig. 5). Because measurements of tunnel diameter before brood laying were taken before nest excavation activity had begun, we assume that these tunnels had been constructed the prior season (spring 2017) or earlier. Therefore, it is likely that the tunnels measured in this study had housed brood for at least two consecutive seasons.

The doubled head width of females (a measure of ease of passing in tunnels) (15.27 ± 0.03 mm; $n = 144$) was significantly less than the diameter of abandoned tunnels (Mann–Whitney U test, Bonferroni-adjusted $P < 10^{-7}$) and the diameter of tunnels after brood cell construction (Mann–Whitney U test, Bonferroni-adjusted $P < 10^{-7}$), but no different from the diameter of tunnels before brood cell construction (Mann–Whitney U test, Bonferroni-adjusted $P = 0.93$); (Fig. 5).

Fig. 4 Sample of 3D nest reconstructions with a number of branches per nest (top left of boxes). Yellow dots indicate the position of nest entrances

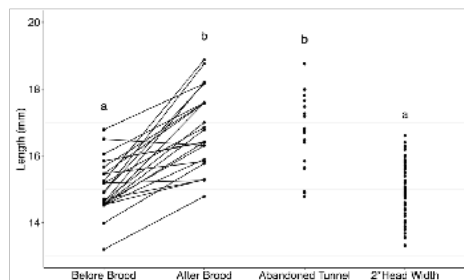
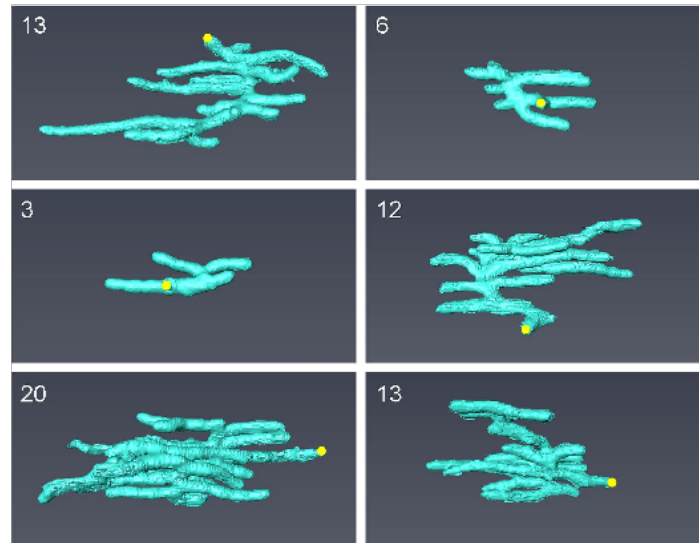


Fig. 5 Comparison of tunnel diameters before and after brood cell construction ($N=24$), diameters of abandoned tunnels ($N=24$), and the doubled head width of females ($N=14$), from Log e^2 as a contextualizing measure of ease of passing in tunnels

Discussion

Plasticity in social behavior allows animals to adaptively match life history strategy to changing environmental conditions. In this study, we used non-invasive, repeated CT imaging of carpenter bee nesting logs to demonstrate flexible sociality strongly influenced by seasonal effects. We also examined the interplay between nest use and nest architecture and found that reused nests change in a way that has implications for within-nest social interactions. Our results

suggest important effects of phenology and nest-site limitation on the incidence of social nesting and capture the fluidity of social behavior in a facultatively social species.

Facultative sociality and social phenology

Alternative social strategies within populations often arise from complex interactions between organisms and their environment. Plastic responses to environmental variables are likely to underlie the evolutionary transition from solitary to group living (Michener 1974; West-Eberhard 1987; Weislo and Fewell 2017). Social organization may shift according to seasonal changes in ecological and behavioral variables, particularly those affecting resource availability, social competition, and climatic challenges. Our results reveal a dynamic social organization likely driven by seasonally fluctuating costs and benefits of group nesting.

Seasonal periods of increased competition can constrain sociality, as in red deer stags which form bachelor groups for 10 months of the year that break up during the mating season (*Cervus elaphus*; Lincoln et al. 1972), or in female wild sheep, which group when food resources are plentiful and disperse in summer when meadows are impoverished (*Ovis orientalis*; Bon et al. 1990). Social groups of carpenter bees likewise disband when within-group competition increases, as mutually tolerant overwintering groups become competitive and produce dispersers in the spring (Gerling et al. 1989; Hogendoorn and Velthuis 1993). Our results are consistent with this typical behavioral pattern, with a high

incidence of social nests found in winter and predominantly solitary nests found in spring. Seasonal factors are likely to impact important differences in the nature of group living, whether as family-based pre-reproductive assemblages or as female-based reproductive social groups. Because subordinate females in *Xylocopa* nests contribute only minimally, if at all, to brood provisioning (Gerling et al. 1989; Richards and Course 2015), females may prefer to nest solitarily in the spring to minimize reproductive competition and resource sharing.

Social groups may also form according to the seasonality of certain adaptive benefits of sociality. Adaptive group thermoregulation, for example, explains temporary social behavior in winter groups of taiga voles (*Microtus xanthognathus*; Wolff and Lüdicker 1981), sea snails (*Nerita ornamentalis*; Chappéron and Scuront 2012), and night lizards (*Xantusia vigilis*; Rabosky et al. 2012). For the facultatively eusocial sweat bee, *Megalopta genalis*, tropical seasonal variation drives fitness effects of alternative social strategies: social nests reap productivity benefits in the dry season when resources are abundant, and survival benefits in the wet season, when the risk of nest failure is high (Smith et al. 2019). Likewise, it may be that seasonal physiological demands encourage group nesting by carpenter bees. In our study, socially nesting bees in the January CT scans appeared in densely packed groups at the terminal ends of tunnels, suggesting potential thermoregulatory grouping. Future studies should examine the effect of social strategy on potential adaptive winter behaviors such as thermoregulation and water conservation. Additionally, in some species, subordinate females play an important role in guarding the nest against pollen robbers when floral resources are limiting (Hogendoorn and Velthuis 1993). As such, the benefits of tolerating potential rivals in the nest vary with season.

Importantly, a persistent minority of individuals nested socially in the spring and solitarily in the winter. This evidence of persistently facultative sociality indicates that season alone does not govern social behavior. Within-population variation in social behavior has been broadly interpreted as a plastic behavioral response to environmental and social cues (Field et al. 2010; Schradin et al. 2012; Shell and Rehan 2017). Flexible social behavior in carpenter bees could allow for matching of life history strategy with variable ecological and social conditions. Furthermore, the mechanisms underlying this plasticity may be central to the evolution of more complex forms of sociality.

Nest architecture and implications for social behavior

Across taxa, passive architectural features of nesting structures are known to influence behavior (Dawkins 1982; Collias and Collias 1984; Turner 2000; Pinter-Wollman et al.

2017). Human social behavior is often shaped by architecture: connectivity in workspace layouts can influence levels of innovation (Wineiman and Davis 2009) and scientific collaboration (Kabo et al. 2014). More fundamentally, dwelling structural complexity may have been an important precursor to the development of complex hominid societies (Jaubert et al. 2016). Likewise, the relationship between structural and social complexity in insects is becoming increasingly clear (Theraulaz et al. 1998; Pinter-Wollman 2015; Pinter-Wollman et al. 2017). Our study situates this discussion at the evolutionary origins of sociality.

Our use of non-destructive CT imaging techniques allowed us to track dynamic architectural features and their associated social effects. Like many animals, carpenter bees construct nests that can be inherited and modified by subsequent generations (Rau 1933; Laland et al. 2003; Prager and Hunter 2011). As such, we did not observe a relationship between the number of adults in a nest and the number or tunnel branches per nest, since only a fraction of tunnels in large nests is actively used for provisioning brood at a given time. Conversely, Prager and Hunter (2011) found a positive association between founder number and number of tunnel branches in *X. virginica*, but this species generally constructs smaller, less branched nests than *X. varipuncta* (Gerling and Hermann 1978). Our results suggest that tunnels, and even whole nests, may become abandoned when their over-use leads to tunnel widening. Carpenter bees lay eggs in linear sequence along tunnels, with each egg separated by partitions made of wood pulp. Females gather raw materials for this pulp by chewing wood from the sides of the tunnel (Gerling et al. 1981), such that the annual need for new partition material may cause tunnels to progressively widen over years of reuse (Gerling et al. 1983). The preference for newer, narrower tunnels likely motivates nest renovation and produces larger nest structures with many branches.

The width of tunnels shapes social interactions because it determines bees' ability to effectively defend valuable food provisions and vulnerable offspring, both from invaders and from rival nestmates. When a pair of bees meet in a nest tunnel and one attempts to pass by, the other has the option to either block her, back up or maneuver her body such that both can move past one another, ventral side to ventral side (Brothers and Michener 1974). As such, successful passing is usually interpreted as a tolerant behavior because it entails mutual maneuvering in a position that exposes each bee to the other's mandibles and stinger (Breed et al. 1978; Arneson and Weislo 2003; Pego and Richards 2010). Spatial effects on the ease of passing nestmates thus can influence within- and between-group social dynamics (Jeanson et al. 2005).

Prevention of passing is associated with social dominance in *Xylocopine* bees (Michener 1990). Dominant *X. pubescens* females prevent subordinates from entering nest

tunnels used for provisioning brood, both because adults may consume pollen from growing “pollen slants” intended for larvae and because usurpation events involve the destruction of the dominant’s brood (Hoogendoorn and Velthuis 1993). Females are likely reluctant to lay brood in wide tunnels that they are unable to defend. Furthermore, tunnel blocking is an important behavior mediating food sharing. Mother bees of *X. pubescens* are accosted by hungry progeny upon return from foraging trips and are prevented from passing until they offer a food concession to their offspring, who jockey for positions nearest the nest entrance in order to receive the largest share of food (Gerling et al. 1981, 1989). Wider than average tunnels would permit non-consensual passing that would neutralize the effectiveness of behaviors maintaining dominance hierarchies and nest defense. Thus, the architecture of nest tunnels and their progressive restructuring via repeated use may impact nest utility over time, intensifying the competition over already scarce nesting resources.

Conclusions

Environmental factors are known to shape social behavior (Emlen 1982; Lion and Gandon 2009), and, conversely, social interactions can dictate the spatial use of the environment (Theraulaz et al. 1998; Smith et al. 2015; Pinter-Wollman et al. 2017). Our study provides evidence for the reciprocity of these effects by characterizing a seasonal effect on social behavior and an effect of nest reuse that may influence social interactions. We found that group nesting is common throughout the year, but disfavored during brood provisioning when reproductive competition encourages dispersal from the natal nest. Furthermore, the functional deterioration of nests caused by multi-generational reuse leads to nest abandonment and exacerbates nest site competition. These interactions between spatial constraints and social competition are likely to underlie plasticity in social behavior more broadly.

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APPENDIX C

SOCIAL CONSEQUENCES OF ENERGETICALLY COSTLY NEST CONSTRUCTION IN

A FACULTATIVELY SOCIAL BEE, *PROCEEDINGS OF THE ROYAL SOCIETY: B*

Research



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Social consequences of energetically costly nest construction in a facultatively social bee

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Social groups form when the costs of breeding independently exceed fitness costs imposed by group living. The costs of independent breeding can often be energetic, especially for animals performing expensive behaviours, such as nest construction. To test the hypothesis that nesting costs can drive sociality by disincentivizing independent nest founding, we measured the energetics of nest construction and inheritance in a facultatively social carpenter bee (*Xylocopa sonorina* Smith), which bores tunnel nests in wood. We measured metabolic rates of bees excavating wood and used computerized tomography images of nesting logs to measure excavation volumes. From these data, we demonstrate costly energetic investments in nest excavation of a minimum 4.3 kJ per offspring provisioned, an expense equivalent to nearly 7 h of flight. This high, potentially prohibitive cost of nest founding may explain why females compete for existing nests rather than constructing new ones, often leading to the formation of social groups. Further, we found that nest inheritors varied considerably in their investment in nest renovation, with costs ranging more than 12-fold (from 7.08 to 89.1 kJ energy), probably reflecting differences in inherited nest quality. On average, renovation costs were lower than estimated new nest construction costs, with some nests providing major savings. These results suggest that females may join social groups to avoid steep energetic costs, but that the benefits of this strategy are not experienced equally.

1. Introduction

Sociality can arise as an adaptive strategy to mitigate the costs of independent breeding [1,2]. Especially in risky, unpredictable or harsh environments, individuals may have little chance of success without the buffering advantages supplied by the social group [3,4]. In particular, group living can provide shared access to limiting resources, especially habitat space [5,6]. As such, environments lacking in opportunities for dispersers can provide fitness incentives for offspring to remain at the natal nest [7,8], or for individuals to form cooperative alliances [9,10]. Thus, evolutionary transitions from solitary to group living may be facilitated by adaptive avoidance of costly life histories.

These costs of independent breeding may be energetic in nature. Relative to group-living animals, independent breeders may make greater personal investments in energetically costly behaviours such as dispersal, foraging and nest founding [11–13]. Nest-building behaviour in particular has been implicated as a context favouring the evolution of sociality [14]. Animals living and breeding in complex nests often need to expend considerable energy gathering building materials and then constructing and maintaining these structures. The substantial energetic expense associated with nest construction has given rise to various strategies that circumvent or minimize such costs. Nest inheritance is one such tactic common across diverse cooperative taxa [8,15,16]. Inheritance strategies can result in group living if offspring delay dispersal or fail to disperse from the natal nest [5,17,18]. Alternatively, social groups may form when individuals seek access to non-natal nests via usurpation or nest

joining, often delaying reproduction until inheriting the nest [19–21]. High nest-building costs thus favour sociality as competitors saturate low-cost nesting opportunities.

The social implications of founding costs are particularly observable in facultatively social animals, which plastically express social behaviour in response to ecological and social context. Because initial transitions to group living are likely to be facultative [22], these systems provide important insights into conditions shaping sociality at its evolutionary origins. Studies of facultatively social animals have emphasized the roles of genetic, ecological and social factors in shaping sociality [23–27]. However, the evolution of sociality in these groups has rarely been explored from an energetic perspective. Energetic considerations are likely to factor strongly into the context-dependent behavioural decisions underlying facultative sociality.

With their flexible sociality and impressive nest construction behaviours [28–31], large carpenter bees (genus *Xylocopa*) represent ideal candidates for examining the influence of energetic costs on social evolution. Unlike bees that nest in hollow cavities or soft piths [32], many carpenter bees construct their nests by boring tunnels into wood with their mandibles, a process that is predicted to entail significant time and energetic costs [29,33]. Perhaps due to these costs, some carpenter bees seek reproductive opportunities in already-constructed nests rather than undertaking the construction of a new nest [28,34,35]. This nest inheritance strategy leads to the formation of fluid, often ephemeral nesting groups comprising kin and/or non-kin, often with overlapping generations [29,34]. The carpenter bee *Xylocopa sonorina* nests either solitary or in small groups, in which only a single female dominates egg laying, foraging and excavating behaviours at a given time [35,36] (note that *X. varipuncta* has been synonymized with *X. sonorina* [37]). As such, additional group members do not strongly reduce the individual labour burden for dominant bees, aside from potential shared nest guarding [36] (but see [34,38]). In the absence of strong helping behaviour, sociality may be influenced more strongly by nesting constraints [29,39]. While nesting substrate is not expected to be limiting in our study population, costs of exploiting available substrate may constrain founding behaviour. Nest inheritors could reap substantial energetic savings if metabolic costs of construction are high. These savings are not guaranteed, however, and benefits could vary considerably across nests when inheritance entails addition or expansion of tunnels to replace over-used structures [40].

In this study, we investigate the energetic costs underlying social and life-history strategies in *X. sonorina*. As with many nest-building species, sociality in carpenter bees has been widely attributed to the costs of new nest construction [29,33,39]; however, the energetic costs of constructing a nest have been difficult to quantify empirically. Using respirometric techniques coupled with three-dimensional (3D) structural analysis enabled by computerized tomography (CT) imaging, we directly measured metabolic costs during nest construction. From these data, we estimate the metabolic cost of nest excavation per offspring provisioned, providing a fitness-relevant estimate of this neglected component of offspring production costs. In doing so, we provide quantitative support for the claim that high excavation costs underlie nest limitation. Further, we quantify costs of nest renovation behaviour to understand the range

of costs incurred by nest inheritors. Our results demonstrate that avoidance of costly nest building may provide an important fitness benefit of sociality.

2. Methods

To assess metabolic costs of various behaviours associated with reproduction, we measured the metabolic rates of female *X. sonorina* during excavation, flight and resting behaviours. Experiments were conducted between March and July 2019 (the season of peak nest excavation activity) using free-living bees nesting at Arizona State University in Tempe, Arizona (33°25'12" N, 111°35'48" W). Bees were captured at dawn upon their first departure from the nest to ensure that none had fed that day, because feeding status influences metabolic rate [41]. Bees were weighed upon capture (A&D GR-200; repeatability 0.0001 g).

(a) Respirometry

To measure metabolic rates of excavating bees (*X. sonorina*, female mass = 0.95 ± 0.02 g), we passed dry, CO₂-free air through a 15 ml syringe containing a bee and a small (8 cm³) cube of wood. Dry, CO₂-free air was supplied at 500 ml min⁻¹ by a FLUK Purge Gas Generator (Purge Gas, Palmer, MD, USA) connected to 11 columns of cobalt-doped copper (II) sulfate (Drierite, W.A. Hammond Drierite Co Ltd, Xenia, OH, USA) and NaOH (Ascarite II, Sigma-Aldrich, St Louis, MO, USA). Each bee was given either balsam (*Orientalis pyramidalis*), a soft wood (density = 0.389 g cm⁻³, *n* = 18) or Goodding's willow (*Salix gooddingii*), a hard wood species (density = 0.539 g cm⁻³, *n* = 15) that we sampled from a log occupied by nesting *X. sonorina*. These wood species are both known to be used by *X. sonorina*, and span much of the range of densities of known nesting substrates for this species [37,42]. The respirometry traces were closely matched to behaviours; we only report CO₂ emission rates for bouts of digging behaviour that were longer than 5 s, which is greater than the 95% washout time for our system (time constant 0.03 min, 2.5 s for 95% washout).

For comparison to excavation metabolic rate, we measured metabolic rates of resting bees and bees in flight, using flow-through respirometry as above. For quantification of resting metabolic rate, we only used bees that exhibited no locomotion or activity during measurement (*n* = 10). For flight metabolic measurements, we only included bees that achieved sustained hovering behaviour for a minimum of 10 s and recorded steady-state CO₂ emission during this flight period (*n* = 17). Bees were motivated to fly by agitation of the chamber prior to the 10 s hovering period and orientation of the chamber towards a lit window in a darkened room. Resting bees were measured in 15 ml syringes with air supplied at 500 ml min⁻¹, and flying bees were measured in a 465 ml glass chamber with air supplied at 1850 ml min⁻¹.

Flow rate (SIP) was regulated using a Flowbar-8 Mass Flow Meter System (Sable Systems International, Las Vegas, NV, USA). Excurrent CO₂ was measured using a Li-Cor 6252 CO₂ infrared gas analyzer (Li-Cor, Lincoln, NE, USA). Measurements were digitized (UI-2, 8S) then recorded and analysed using the Expedata data acquisition software, v. 1.9.13 (8S). The mean VCO₂ (ml min⁻¹) was calculated using the equation

$$VCO_2 = FCO_2 \times SIP / \text{Flow rate}_{\text{respirator}}$$

where FCO₂ represents the fractional content of CO₂ in the excurrent stream [43]. Conversions from VCO₂ to watts assumed a respiratory quotient (RQ) of one for metabolism of simple carbohydrates [44]. All measurements were taken at 25 ± 1°C to control for temperature effects on metabolic rate; further, we observed that bees initiated daily flight activity at ambient temperatures roughly near this temperature range.

(b) Behavioural analysis

We continuously observed each bee in the respirometry chamber for 30 min and recorded the time periods spent excavating. To calculate the average excavation rate, we weighed wood cubes before and after the sampling period to measure total mass of wood excavated, and divided by the total time spent excavating. Sample videos of bees excavating the two wood types are available in the electronic supplementary material.

(c) Computerized tomography scans and nest volume reconstructions

To measure nest structure volumes and assess the amount of nest excavation that occurs within a single breeding season, we CT scanned a nesting log occupied by *X. sororiva* at three different time points: (i) during winter quiescence, prior to the breeding season (January 2018); (ii) during peak brood production activity (May 2018); and (iii) after the end of the breeding season, by which time nest excavation activity has ceased (September 2018). The scanned log also provided the hard willow wood used in the respirometry trials, to ensure equivalent wood density in our estimations. Scans were conducted using a CT machine (General Electric, LightSpeed VCT) at St Joseph's Hospital in Phoenix, AZ, USA, with 2.5 mm thick scan image slices provided in sagittal and coronal views relative to the long axis of the log. We manually reconstructed the 3D structure of nests in scans and calculated their volumes using the imaging software Avizo v. 9.0 (Hermo Scientific); [40]. Additionally, we measured brood production rates by counting the number of occupied brood cells per nest on the May scans. This population of *X. sororiva* is primarily univoltine, with most brood emerging between late May and early June [40].

(d) Estimations of energetic costs of nest construction and renovation

We used nest volume measurements and metabolic data to calculate (i) costs of nest excavation per offspring and (ii) costs of nest renovation. We chose eight focal nests that were occupied during the period of active brood production (May), and measured their volumes prior to (January), during (May) and after (September) the breeding season. The change in nest volume over the course of the entire breeding season (January–September) provided us with an estimate of the potential renovations necessary for a female breeding in an existing nest. Additionally, we measured the volume of the tunnel space used to house a single developing offspring (a 'brood cell'; $n = 30$). The majority of the occupied nest volumes consist of brood cells, but additional space is constructed at entrances, to join adjacent nest tunnels, and to house adult bees. As such, brood cells represent only a portion of the total excavation costs, and a conservative estimate of per-offspring excavation costs.

These measured volumes, the average metabolic rate of hard wood excavation and the rate of hard wood excavation were used to calculate the total energetic expenditure associated with excavation of different nest volumes, according to the following equation: energetic cost (J) = metabolic rate ($W = J s^{-1}$) \times volume excavated (cm^3) \div rate of excavation ($cm^3 s^{-1}$). We used hard rather than soft wood metabolic rate in this calculation because the wood excavated in hard wood trials was sourced from the same log we CT scanned for nest volume analysis. In this way, we controlled for potential interactions between wood hardness and nest volume. Finally, to contextualize our measurements of metabolic costs, we calculated a flight-time equivalent of these energy costs using our measurements of flight metabolic rate. Flight provides relevant energetic context as it is a necessary provisioning behaviour for reproductive

Xylocopa [28,29,36], and because insect flight is among the costliest animal behaviours [45,46].

(e) Statistical analysis

To assess differences in mass-specific metabolic rate among resting, flying and excavating bees, we used a Kruskal–Wallis test. We ruled out analysis of variance due to departures from normality and homoscedasticity revealed by Shapiro's tests and Levene's tests, respectively. We performed pairwise Wilcoxon tests on treatment pairs for *post hoc* analysis, because individual bees were measured in all three behavioural activities: excavating, flying and resting. Similarly, we used Mann–Whitney *U*-tests to assess differences in excavation rate and excavation rate-adjusted metabolic rates. We used linear regression to examine the relationship between the metabolic rate and excavation rate, and analysis of covariance (ANCOVA) to assess the nature of this relationship with respect to wood hardness. Samples for this analysis were independent; individual bees were either measured excavating hard wood or excavating soft wood, but not both. Results are presented as mean \pm s.e. All statistical analyses were performed in R v. 3.6.2 (R Development Core Team, 2017).

3. Results

(a) Metabolic rate during nest excavation, resting and flight

The metabolic rate during nest excavation ($0.016 \pm 0.002 W$, $n = 33$) was significantly higher than the metabolic rate of resting bees ($0.005 \pm 0.001 W$, $n = 10$, Wilcoxon rank-sum test; $p < 0.001$), but significantly lower than the metabolic rate of bees in flight ($0.179 \pm 0.008 W$, $n = 17$, Wilcoxon rank-sum test; $p < 0.001$) (figure 1a). Our flight metabolic rate was lower than previous estimates for *Xylocopa* [34,47–49], such that our estimates of flight-time equivalents for excavation behaviour may be conservative. The resting metabolic rate was similar to that recorded for *X. capitata* [44]. Our results indicated a significant positive linear relationship between the metabolic rate and excavation rate both for the soft wood ($p = 0.037$, $n = 18$, adjusted $R^2 = 0.196$) and the hard wood ($p = 0.008$, adjusted $R^2 = 0.391$, $n = 15$). Regression lines did not differ in slope (ANCOVA: $p = 0.750$) or intercept (ANCOVA: $p = 0.245$). Wood hardness did not significantly affect metabolic rates of excavators (Mann–Whitney *U*-test, $p = 0.34$), but bees excavating soft wood excavated at a significantly faster rate (soft wood: $1.62 \pm 0.12 cm^3 h^{-1}$, hard wood: $0.12 \pm 0.02 cm^3 h^{-1}$, Mann–Whitney *U*-test; $p < 0.001$). As such, when mass-specific metabolic rate is adjusted for the excavation rate, the excavation of hard wood ($621 = 181 J cm^{-3}$) is significantly more energetically costly than the excavation of soft wood ($42.1 \pm 3.96 J cm^{-3}$; Mann–Whitney *U*-test; $p < 0.001$) (figure 1b).

(b) Energetic costs of nest construction and renovation

To estimate the energetic costs of nest construction and renovation, we repeatedly measured the volumes of nest structures across a single breeding season (figure 2). Whole nest structures measured prior to the breeding season (January) averaged $257 \pm 41.2 cm^4$. By the time brood production was under way (May), these nests had increased in volume by $47.7 \pm 23.8\%$ to a total volume of $353 \pm 37.3 cm^4$. Between May and the end of the season (September), nests were further expanded by $10.2 \pm 4.52\%$ to a final average volume

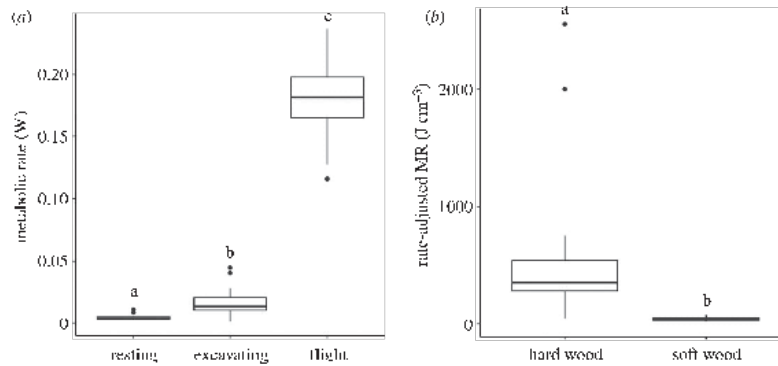


Figure 1. (a) Metabolic rates (W) of bees during resting, excavation and flight. The metabolic rate of bees during wood excavation was significantly higher than that of resting bees ($p < 0.001$), but lower than that of flying bees ($p < 0.001$). (b) Cost of excavation (J cm^{-3}) is significantly higher for excavation of hard wood than for soft wood ($p < 0.001$).

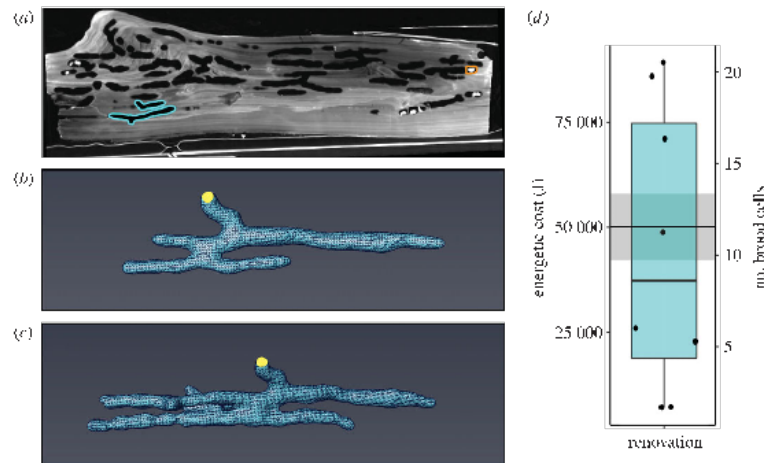


Figure 2. (a) CT scan image slice of an occupied nesting log, showing one focal nest, highlighted blue, and one brood cell, highlighted orange. (b) Three-dimensional reconstruction of focal nest prior to the breeding season (January). (c) Three-dimensional reconstruction of the same focal nest after the breeding season (September). The change in tunnel volume from (b) to (c) represents the renovation volume excavated by the female who inherited this nest. Yellow dots indicate the position of nest entrances. (d) Estimates of energy costs (J) to renovate a nest (left axis; $n = 8$ nests). The axis on the right corresponds to estimated energy costs associated with excavating brood cells (i.e. one brood cell – space to provision a single offspring). The horizontal line and grey region represent the observed mean \pm s.e. number of offspring provisioned per nest in this population. (Online version in colour.)

of $380 \pm 53.5 \text{ cm}^3$, for a total expansion of $63.1 \pm 28.2\%$ or $122 \pm 32.8 \text{ cm}^3$ across the entire breeding season. The age and full history of these nests prior to this year of observation is unknown. As such, nest measurements taken in January may include older nests that had undergone some expansion in previous years.

We used these volume measurements to estimate energy investment associated with brood production and nest renovation (figure 2d). Spring CT scans indicated that bees in this population provisioned 11.5 ± 1.81 offspring per nest (therefore, per reproductive female) over the reproductive season. To excavate the space necessary to rear a single offspring (a brood cell; $11.9 \pm 0.21 \text{ cm}^3$) within a nest would require

$4.3 \pm 0.1 \text{ kJ}$ of energy. A female bee using an existing nest and undertaking nest renovations of the average volume observed in this study would require $44.8 \pm 1.2 \text{ kJ}$ of energy (range: 7.08–89.1 kJ).

4. Discussion

Group living can provide immediate opportunities for individuals to increase fitness by minimizing energetic costs. For animals that build costly nests, group living can arise when individuals compete for access to existing nest space [8,14,16]. Our findings support the assertion that high energetic savings of nest inheritance can favour group living

even in the absence of strong cooperative behaviour among group members. For the carpenter bee *X. smaragdina*, females that opt out of nest excavation activity by joining an existing nest, often with other females [36], may bypass significant energetic costs if they inherit the nest. We used metabolic rate measurements in combination with CT imaging to demonstrate high costs of nest excavation by the carpenter bee *X. smaragdina*. These costs favour the reuse of existing nests, perhaps even at the expense of delayed or uncertain reproduction. The effects of this energetic investment represent a significant but neglected component of reproductive fitness, and one with important implications for the formation of social groups.

(a) Nest construction as a context for the evolution of sociality

Life histories involving costly building behaviours may facilitate social evolution by placing a premium on constructed nest habitats [14]. In these cases, the risks and/or costs associated with nest founding can outweigh the potential benefits of independent breeding, encouraging offspring to remain in the natal nest. Nest inheritance has been implicated as an important selective pressure in the evolution of sociality in the Hymenoptera [7,50], colonial rodents [18,51,52] and cooperatively breeding birds [15,16,53]. Fewer studies, however, have directly quantified the energetic costs of nest building [54,55]. This evidence can provide essential empirical support for hypotheses that seek to explain the origins of social nesting strategies.

In *X. smaragdina*, as in many *Xylocopa*, only a single female per nest reproduces at a given time, though females may join established, occupied nests throughout the reproductive season [26] (M.M.O. 2019, personal observation). In addition to monopolizing reproduction, dominant bees perform most or all of the foraging and nest excavation, such that non-reproductive subordinates contribute little, if at all, to the productivity of the nest, aside from possible nest guarding duties [29,36]. Why, then, do females join or invade nests rather than constructing their own? Nest limitation has been widely proposed as the major driving force in the evolution of carpenter bee sociality, but this hypothesis rests on the assumption that nest construction is challenging, costly or otherwise prohibitive [29,39,56].

Our results validate the assertion that nest construction entails high metabolic costs. Per offspring produced, we conservatively equate this expense to that of approximately 7 h of bee flight, one of the most energetically demanding behaviours that animals perform [45,46]. For context, the carpenter bee *X. capitata* spends 3.5 h flying among flowers to provision a single offspring [33] (note that this estimate includes flower handling time and excludes commuting time), suggesting that nest building ranks among the principal energetic investments associated with offspring production. A female excavating the volume necessary to rear the average brood size (11.5) in this population would expend just under 50 kJ of energy, which is equivalent to nearly 80 h of flight. Further, foundresses must expend additional energy to excavate non-brood rearing space in the nest. Together, this amounts to an expense exceeding the average cost of nest renovation (about 45 kJ). Importantly, the large variation in renovation costs observed in our study, ranging more than 12-fold from about 7 to 90 kJ, suggests that

nests inherited in good condition (requiring little renovation) provide even greater savings. In other scenarios, inheriting females may invest as much in renovation as they could have in new nest construction. As such, the advantages of nest inheritance can vary widely, likely depending strongly on inherited nest quality. On average, however, the high energetic cost of excavation in this species should incentivize social strategies that rely on nest inheritance.

With finite energy budgets, nest inheritors could feasibly reinvest energy savings into other reproductive efforts, such as egg production and foraging, to produce more or higher-quality offspring than they might following new nest excavation. For *Xylocopa*, which lay massive eggs relative to their body size [57,58], egg production is a high but unavoidable cost of reproduction; potentially avoidable reproductive behaviours such as nest construction may, therefore, provide important opportunities for reproductive energy savings. Nest inheritance may also provide important time savings. Gerling [36] observed one *X. smaragdina* female spend 14 days excavating a single tunnel, of which there are usually several per nest. Avoidance of this considerable time investment could therefore have important fitness implications by increasing available time for provisioning. The average time spent waiting for nest inheritance in this species is unknown, but is likely to vary widely based on local conditions, the degree of competition and individual competitive ability among other factors. Females may acquire nests immediately through usurpation, or may delay reproduction until a nest becomes available; subordinates of the carpenter bee *X. virginica* may even postpone reproduction for as long as a year [35]. Future studies should aim to clarify fitness consequences of nest inheritance, quantifying both time and energetic costs of all reproductive behaviours to more thoroughly contextualize the energetic trade-offs underlying patterns of social nesting.

(b) Interactions between nest properties and social strategies

Social strategy decisions involving nest inheritance are likely made in consideration of the quality of existing nest structures. For most animals, nest sites are a finitely renewable resource. Many birds, for example, experience a trade-off between the advantages of nest inheritance and the potential for exposure to parasites in older nests [59,60]. Carpenter bees, likewise, tend to cease reuse of nest tunnels once they have grown past a certain threshold diameter [40]. With each successive season of nest reuse, the reconstruction of brood cell partitions progressively widens tunnels to the point where they may be indefensible to usurpers [40]. As nest tunnels decline in quality, bees may construct additional, new tunnels off of existing nest structures. In this way, even bees that acquire nests through inheritance may be forced to undertake some degree of nest excavation, depending on the state of the nest at the time of inheritance. The extent of necessary renovation can vary substantially, ranging in this study by a factor of more than 10. This wide variation implies that some inheritors may experience significant energetic savings relative to foundresses, while others invest substantially in renovation, perhaps losing this advantage.

The properties of available nesting substrate likewise may influence nesting decisions. We measured excavation costs for bees using two wood species spanning much of the range of wood hardness available to *X. smaragdina* [42], and measured

lower metabolic rates for bees excavating softer wood, when adjusted for excavation rate. However, these immediate energy savings may be counter-balanced by long-term disadvantages of nesting in soft wood, such as reduced durability and vulnerability to weathering and predators. Several species are known to be vulnerable to predation by woodpeckers [29,31,61], and may benefit from nesting in more protective, harder wood, despite initial time costs associated with nest excavation. Furthermore, nesting in hard wood could increase the longevity of the nesting log, which may be continuously occupied for as long as 15 years (S. L. Burhmann 2020, personal communication). If the aggregation occupying the log is closely related, long-term success of descendants may be important. *Xylocopa sumatrana* may invest more in nest excavation than carpenter bee species that nest principally in stalks or culms, taking advantage of the plant material's soft pith or hollow interior to minimize construction costs [29,42]. Indeed, of the eight social *Xylocopa* species noted in Corling *et al.*'s review of the genus [29], seven species nest in solid wood [36,62–66] and just one nests in stems [67]. Variation in nesting ecologies may, therefore, represent an important determinant of social organization within and across species.

5. Conclusion

Energetic considerations underlie life-history trade-offs across taxa [68,69], yet have remained underexplored in the context of social evolution. Efforts to describe comprehensive energy budgets encompassing a range of relevant behaviours could provide important insights into the fitness outcomes of

alternative social strategies. Our findings support a critical role for energetically costly nest excavation as a driver of carpenter bee sociality, providing quantitative support for a widely cited hypothesis [34,56]. These results form an important mechanistic link between behavioural decisions and ecological conditions, and emphasize the significance of energetic trade-offs at the origins of group living.

Ethics. This study was conducted in accordance with guidelines for animal research established by Arizona State University. No bees were killed or harmed for this experiment. No permits or licences were required for this study.

Data accessibility. The dataset associated with this study is available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.2280y65r0> [70].

Authors' contributions. All authors contributed to experimental design and manuscript drafting. M.M.O. and T.P.F. collected the data. V.M.O. wrote the manuscript and analysed the data.

Competing interests. We declare no competing interests.

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APPENDIX D

MULTIMODAL CUES FACILITATE NEST RECOGNITION IN CARPENTER BEE
AGGREGATIONS, *ANIMAL BEHAVIOUR*



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Multimodal cues facilitate nest recognition in carpenter bee aggregations

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The advantages of group living are partially offset by the cognitive challenges associated with maintaining social boundaries. These challenges can give rise to recognition mechanisms that adaptively integrate information across multiple sensory modalities. The valley carpenter bee, *Xylocopa varipuncta*, nests in dead wood in large aggregations of up to several dozen nests. This study investigates the proximate mechanisms by which returning foragers quickly and reliably identify their own nest entrance within a high density nesting site. We manipulated long- and short-range visual cues associated with nest entrances, removed chemical cues on the inside of nest entrances and added chemical cues from foreign conspecific bees. By measuring the effect of these manipulations on nest search time and search accuracy, we assessed the importance of visual and olfactory sensory modalities in allowing carpenter bees to locate their nests within aggregations. Our results support the hypothesis that both visual and olfactory cues can facilitate nest localization. Removal of nest olfactory cues did not significantly disrupt homing, suggesting that olfactory information may not be necessary for nest localization when visual information is available. However, the addition of olfactory cues from unfamiliar conspecific bees actually aided nest localization rather than disrupting it, suggesting that bees may use generalized species odour cues for homing. Due to intense nest site competition within aggregations, nest localization may have important social implications for maintenance of high-density nesting.

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Social organisms experience a trade-off between benefits of group living and the potential fitness costs generated by spatial crowding. To mitigate these costs, individuals must adaptively respond to the cognitive challenges imposed by frequent social contacts. Group-living organisms must navigate densely occupied social landscapes and minimize within-group conflict by maintaining social boundaries. Densely nesting individuals also face the particular challenge of reliably recognizing their nest and distinguishing it from those in the immediate vicinity. The potential consequences of inaccurate nest identification can be significant. These repercussions include robbery or aggressive interactions for the resident individual (Hogendoorn & Velthuis, 1995), loss of contact with vulnerable offspring for the drifting individual (Hogendoorn & Velthuis, 1993) and potential pathogen spread for both individuals (Forrest et al., 2015). Given these costs, aggregative

nesting is likely maintained by effective nest recognition mechanisms.

The sensory cues on which animals rely for nest localization vary with environmental conditions and with the sensory capabilities of the animal. Acoustic communication, for example, is particularly important for nest recognition in birds and can facilitate breeding site localization when the use of olfactory and visual cues is impractical, as in colonial penguins (Juventin, 1982). Similarly, nocturnal activity of bats precludes meaningful use of visual cues, so Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, use acoustic cues to locate their pups within colonies that can contain millions of individuals (Balcombe, 1990). Most insects, however, do not possess hearing, and therefore rely on alternative cues for nest localization (Cöpler & Hennig, 2016). Niko Tinbergen's landmark study of the homing behaviour of the digger wasp *Philanthus triangulum* elegantly demonstrated the importance of visual cues for nest localization (Tinbergen, 1972). Other studies have likewise emphasized the significance of visual cues for insect navigation, particularly landmark cues and cues associated with the panoramic view of the nest (Collett, Graham, Harris, & de Ibarra, 2006; Collett, Chittka, & Collett, 2013; Harris, Graham, & Collett, 2007; Hoinville & Wehner, 2018; Mandal, 2018; von Frisch, 1967; Wehner, Michel,

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& Antonsen, 1996; Wehner & Rüber, 1979). Beyond the use of visual cues, insects are known to recognize nests at close range using olfactory cues (Buehlmann et al., 2012; Butler, Fletcher, & Watler, 1969; Huber & Knaden, 2018; Steck, Hansson, & Knaden, 2009). Bees, in particular, have been shown to orient to specific chemical cues associated with nest entrances (Foster & Gamboa, 1989; Guédot, Bosch, & Kemp, 2007; Weislo, 1992).

To provide redundancy and aid recognition under a variety of conditions, nest recognition mechanisms may integrate cues across multiple sensory modalities. Several hypotheses have emerged to explain the complexity of signals and cues, which may vary in their intensity or persistence in the environment, in their ease of detection or in the information contained. First, the use of multiple modalities may provide 'back-ups' that either reinforce or conflict with the original message (Johnstone, 1996). This redundancy can improve accuracy in decision making. Mosquitofish, *Gambusia affinis*, for example, are better able to locate conspecifics and avoid predators when provided with visual and chemical cues in tandem, rather than either cue separately (Ward & Mehner, 2010). Second, different modalities can convey different types of information or have modulatory effects on the primary message, as in human speech perception, which is modulated by visual cues (McGurk & MacDonald, 1976; Partan & Marler, 2005). Furthermore, the use of multiple cues or signals can specify context. Female red-winged blackbirds, *Agelaius phoeniceus*, and Cuban grassquits, *Tiaris canora*, sing different songs to clarify the meaning of a single visual display that can signal either aggression or courtship (Baplista, 1978; Belitsky, 1983). Due to this functional variation, the weights accorded to different cues may vary depending on their information content in a given context.

We investigated the importance of visual and olfactory cues in the nest recognition ability of an aggregating carpenter bee, *Xylocopa varipuncta*. This species nests primarily in decaying stumps and logs, forming aggregations of up to several dozen nests. To maximize use of this nesting substrate, which is rare in their desert habitat, *X. varipuncta* nest densely, with nest entrances often only centimetres apart. As such, *X. varipuncta* probably process multiple cues at long- and short-range to avoid entering a neighbour's nest. Previous studies found evidence for visual and olfactory nest localization in African *Xylocopa* species, but they differed in their interpretation of the relative importance of these cues. Anzenberger (1985) suggested that olfactory but not visual cues are important for homing, while Hefetz (1992) found that both play a role, with olfactory cues useful at very close range. Weislo (1992) demonstrated the importance of olfactory cues for nest localization in the tropical sweat bee *Lasiglossona figueresi* by removing chemical cues at nest entrances as well as supplementing with odour cues from foreign conspecific bees, both of which delayed nest entry by returning foragers. In the present study, we distinguish between the effects of proximal and distal visual landmarks in visual homing, and between the presence and absence of scent cues in olfactory homing. This experimental design allows us to explore the relevant sensory cues that *X. varipuncta* uses to function in a high-density nest site.

METHODS

Experiments were performed from June 2018 through August 2018 at Arizona State University in Tempe, Arizona, U.S.A. (33°25'12"N, 111°55'48"W). Four logs, each containing approximately 5–20 active nests of *X. varipuncta*, were transferred to the university campus from a nearby park and residential areas in the spring of 2018. Over the course of the experiment, bees abandoned certain nests and colonized others. The number of active nests—and therefore the number of replicates per

treatment—varied over the course of the season as bees emerged, dispersed and experienced mortality and usurpation.

We performed four treatments that manipulated visual or olfactory cues at nest entrances: (1) rotating the log 180° about its vertical axis; (2) moving coloured shapes placed next to nest entrances; (3) rinsing entrances with hexane to remove olfactory cues; (4) applying cuticular hydrocarbon (CHC) extracts from nonresident bees to nest entrances. We compared the results of these treatments to an unmanipulated baseline condition. A period of at least 1 week was allowed between each treatment to allow bees to adjust to the changing conditions.

To determine the effect of each treatment on a returning forager's ability to find her nest, we measured search time and search error rate during peak foraging time (05:30–08:30 hours). To standardize measurements of search time, logs were surrounded by three to six curved rebar poles, 85 cm in height, that were placed 90 cm from the central axis of the log (see Fig. 1). The population was given 5 days to adjust to the presence of the rebar poles before measurement began, and it did not appear to be disturbed by their introduction.

Measurement of search time began when a returning bee passed into the region defined by the poles and it ended when the returning bee entered her home nest. We quantified nest recognition errors as the number of nests that a bee entered and exited before returning to her home nest. Bees were unmarked, so we did not determine nest ownership through monitoring of individually marked bees. Rather, we defined search success as entering a nest and remaining there for at least 30 s without leaving to search nearby nests. Any time spent in the wrong nest was not included in search time. Orientation flights, characterized by slow, nestward-facing circling of the logs (Zeil, Kelber, & Voss, 1996), were observed across treatments, but rarely. We collected data from one foraging female per nest and used each nest a maximum of once per treatment. Because male *Xylocopa* have lower nest fidelity than females (Pezo & Richards, 2011), we included only female bees in this study. Females were distinguished by their coloration (black integument; whereas males have yellow integument).

For each log, we chose focal nests that were present on the same face of the log, considering that these nests could reasonably be confused with neighbouring nests in that same region. We excluded nests on the ends of the logs or on opposite faces of the log because we expected that bees orient to these major features of the log.

Log Rotation

To determine whether bees use distal visual cues to orient to their nests, we rotated logs 180° around their vertical axes and measured search time and the number of errors made. This manipulation altered in tandem various distal cues known to be used in insect navigation, including both celestial and terrestrial cues, notably the position of the sun relative to the nest and the panoramic view associated with it, respectively (Maudsl, 2018; Wystrach, Beugnon, & Cheng, 2011). These measurements were compared to baseline search times and error counts for unmanipulated logs. If bees make use of distal cues, we reasoned that rotation would increase search times and errors, by altering the apparent relationship between visual cues and nest location. Logs were rotated a single time on the morning of 14 August. Observations took place that morning and the following morning during periods of peak foraging activity in an attempt to observe each bee's first return trip following the manipulation. It is possible that some bees observed on the second day had foraged the previous day after the end of the observation period. In this case, our measurement of search time would be conservative, given the additional opportunity



Figure 1. Example of experimental set-up including rebar poles surrounding a nesting log to create a standard space in which to measure search time. Circular holes are nest entrances.

for the observed bee to learn the new orientation of the log. However, foraging activity past early morning is extremely low at this time of year, and it is likely that most, if not all, bees spent the hottest part of the day inside the nest. Additionally, some bees in this treatment had an opportunity to learn the new orientation of the log when they departed from their nest, potentially through orientation flights. As such, our measurement of search time is a conservative estimate of the effect of the disturbance on homing.

Movement of Visual Symbols Peripheral to the Nest Entrance

We also assessed the importance for nest localization of close-range visual features in the periphery of nest entrances. One week before data collection, we placed artificial visual symbols (coloured geometric shapes) approximately 1–2 cm from each nest entrance on the focal side of the log. There is strong evidence for colour and shape learning in honey bees (Gould, 1984, 1987; Leonard & Masek, 2014). Colours chosen for the shapes were within the visual spectrum for bees (Chittka & Waser, 1997; von Frisch, 1956). Immediately before recording search times and error rate, we laterally shifted the symbols, moving each marker to the periphery of a neighbouring nest entrance (see Fig. 2). Because data collection occurred over multiple days during peak foraging time, we returned the symbols to their original position after each observation period, then shifted them identically before the next data collection bout. In this way, any bees that we had not observed that day could not have left the nest during the observation period and thus remained naïve to the new symbol configuration.

Removal of Chemical Cues

To determine whether chemical cues on the inside of the nest entrance influence a forager's ability to find her nest, we washed the inside of the nest entrance with hexane immediately after a bee left, and recorded search time and accuracy upon her return. We did this by applying approximately 2 ml of hexane with a cotton

swab on the walls of the nest gallery, a short 1–2 cm entrance tunnel that leads into the main nesting tunnels. Our protocols for the chemical removal treatment and the chemical addition treatment (below) followed those of Weislo (1992), which produced positive behavioural results for nest localization in the ground-nesting solitary sweat bee *Lasioglossum figueresi*.

Addition of Foreign Bee Chemical Cues

To determine whether the presence of chemical cues from a foreign bee influences a bee's homing ability, we collected bees from a distant (2.54 km) aggregation of *X. varipuncta* and extracted cuticular hydrocarbons by rinsing chilled bees in 10 ml of hexane for 60 s each. After the departure of each forager, we applied these extracts to the inner wall of nest galleries in the same manner as in the chemical removal treatment, recording search time and accuracy upon each forager's return.

Olfactory Control

Hexane is highly volatile, with 2 ml spread over the area of the nest gallery fully evaporating in under 7 min (Braun & Caplan, 1989). Average summer foraging durations are highly variable but have been recorded as 47.2 min in *Xylocopa virginica* (Richards & Course, 2015), and in our study were no shorter than approximately 15 min. As such, the hexane probably had sufficient time to evaporate fully by the time foragers returned to the nest. Nevertheless, to additionally control for any potential deterrent effects of the hexane itself, we applied hexane in a ring around the outside of the nest entrance, where we did not expect any chemical cues to be deposited (as in Weislo, 1992). We then recorded search time and error rate and compared these to our results from both olfactory treatments.

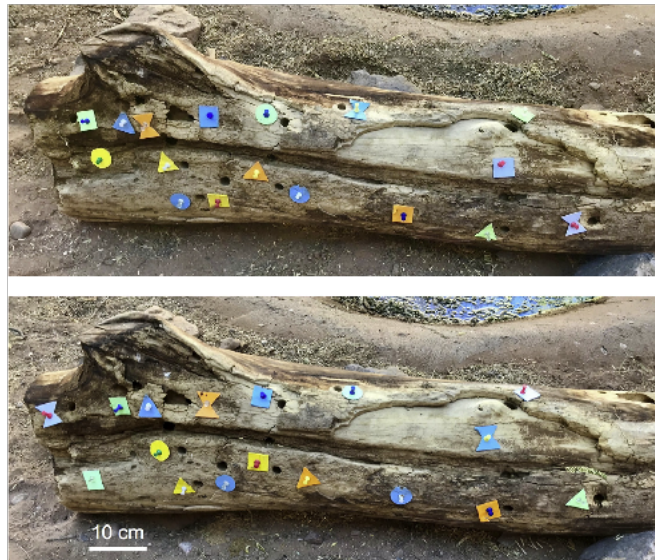


Figure 2. Nesting log with a symbol configuration to which bees had been trained for 1 week (top), and shuffled symbol configuration (bottom) used to determine the effect of proximal visual landmarks on nest localization. Circular holes are nest entrances.

Statistical Analysis

We used a Kruskal–Wallis test to detect differences in search time among the four treatments, the baseline and the olfactory control. We ruled out analysis of variance due to departures from normality and equal variance shown by normal Q–Q plots and Levene’s tests for untransformed data as well as log-transformed data. For post hoc tests, we performed Wilcoxon tests on treatment pairs. Results are reported as means \pm SE. To assess differences across treatments in the tendency of bees to make nest recognition errors, we performed Fisher’s exact tests on the number of bees making one or more errors across treatment pairs. All statistical analyses were performed in R v.3.4.2 (R Development Core Team, 2017).

Ethical Note

This study was primarily observational and conducted in accordance with guidelines for animal research established by Arizona State University. No permits or licenses were required for this study.

RESULTS

We performed four treatments that assessed the effects on nest localization of (1) distal visual cues, (2) proximal visual cues, (3) the removal of olfactory cues and (4) the addition of unfamiliar conspecific odours. We found a significant effect of treatment on search time (Kruskal–Wallis test; $H_3 = 43.72$, $P = 2.6 \times 10^{-6}$), with both visual treatments increasing search time, the removal of odours having no significant effect, and the addition of foreign odours decreasing search time (Fig. 3).

Log Rotation

Foragers returning to an unmanipulated log found their nests quickly and accurately, searching for an average of 8.7 ± 1.3 s, with no errors observed ($N = 22$). Search time was significantly increased following the 180° log rotation, with foragers searching for approximately eight times as long as they had during baseline, where no errors were seen (70.5 ± 14.3 s, $N = 16$, $P = 2.5 \times 10^{-5}$; Fig. 3). Bees were significantly more likely to make nest recognition errors in this treatment when compared to baseline (Fisher’s exact test; $P = 0.02$; Table 1). In this treatment, 4 of 16 bees inaccurately identified their nests, with one bee entering four other nests before locating her own.

Movement of Visual Symbols Peripheral to the Nest Entrance

Shifting the geometric symbols near nest entrances significantly increased search time relative to baseline, more than doubling it on average (20.4 ± 3.4 s, $N = 28$, $P = 0.05$; Fig. 3). However, there was no difference in the number of bees making nest recognition errors in this treatment versus baseline (3 errors; Fisher’s exact test; $P = 0.25$; Table 1).

Removal of Chemical Cues

Treating the outside of the nest with hexane to control for intrinsic deterrent effects of the olfactory treatments produced a mean search time of 12.7 ± 2.5 s ($N = 18$). Compared to this control, removing chemical cues from the inside of the nest entrance through application of hexane did not significantly change search time (7.9 ± 1.2 , $N = 18$, $P = 0.18$; Fig. 3). Likewise, the olfactory removal treatment had no effect on search time when compared to baseline values ($P = 0.92$). There was no difference in the number of

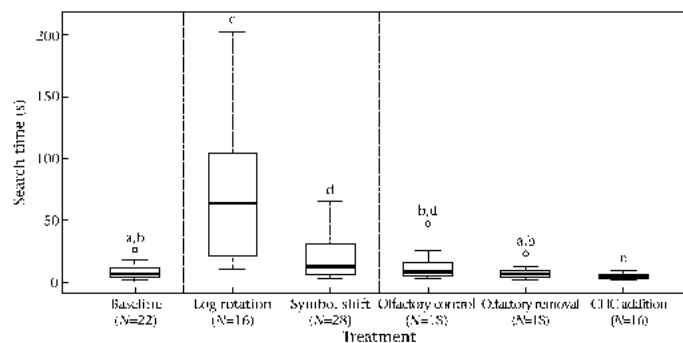


Figure 3. Time spent searching for the home nest during baseline, visual treatments (log rotation; symbol shift) and olfactory treatments (control; removal; cuticular hydrocarbon (CHC) extract). Letters indicate significant differences ($P < 0.05$); open circles represent outliers.

bees making nest recognition errors in the olfactory removal treatment (2 errors) versus the baseline (Fisher's exact test: $P = 0.23$), or in the olfactory removal treatment versus the olfactory control (1 error; Fisher's exact test: $P = 1$; Table 1).

Addition of Foreign Bee Chemical Cues

The addition of cuticular hydrocarbon extract from foreign bees significantly decreased search time relative to the olfactory control (5.0 ± 0.6 , $N = 16$, $P = 0.006$) and to the baseline ($P = 0.05$). No bee made a nest recognition error in this treatment.

DISCUSSION

The evolution of group living is probably facilitated by adaptive cognitive processes that maintain social boundaries. These mechanisms are particularly relevant for aggregative or colonial species, in which individuals maintain separate nests within the group, and for which the social group presents a spatially complex environment to navigate. Many bees, particularly ground-nesting species, are known to aggregate in large groups sometimes exceeding 100 000 nesting females (Hanson & Ascher, 2018). Some carpenter bees also form dense aggregations, probably because they have strong preferences for a nesting substrate that can be limiting in their environment (Gerling, Velthuis & Hieftz, 1989). In these aggregations, several hundred individuals can occupy a single log, creating a complex three-dimensional nesting landscape. The ability to distinguish among nests at close range allows *X. varipuncta* to capitalize efficiently on a rare but valuable nesting substrate while minimizing conflict with neighbours. Furthermore, nest recognition ability functions in predator avoidance. For bees, bird predation is a significant cost of prolonged hovering outside of nests (Alcock, 1995, 1996), and in our study treatments that delayed nest entry resulted in attempts at prey capture by birds. Our study demonstrates that nest recognition in this species may be facilitated by information from multiple sensory modalities.

The Utility of Multimodality in Nest Recognition

Interest in the evolution of multimodal information processing is increasing, both in terms of animal communication and cognition, particularly navigation (Buchmann, Hansson, & Knaden, 2013; Dovey, Keniford, & Towne, 2013; Higham & Hebets, 2013; Partan & Marler, 2005). Nest recognition provides a suitable

context for the evolution of multimodal cue processing due to its importance in maintaining social boundaries (von Frisch, 1956). We manipulated visual and olfactory cues independently and observed significant delays in nest localization following visual manipulation. However, the fact that foragers ultimately did locate their nests under each of the experimental conditions suggests that their nest recognition system is generally robust to disruptions of individual cues.

In animal communication, multimodality increases signal detectability, provides redundancy and conveys complex messages (Johnstone, 1996; Rowe, 1999). Multimodal communication strategies can also facilitate spatial localization. Female túngara frogs, *Physalaemus pustulosus*, for example, are better able to locate calling males when presented visually with vocal sac inflation, along with acoustic signals (Rosenthal, Rand, & Ryan, 2004). Multimodality in nest localization may confer similar benefits. For homing bees, chemical cues may have a shorter range of detection, greater specificity and less persistence than visual cues. As discussed above, chemical cues provide important individual or colony-specific information when nests have only subtle visual distinctions, as in ground-nesting bee aggregations. However, chemical cues can sometimes be unreliable if they are not a suitable temporal match to behaviour patterns. For example, chemical cues may not always keep pace with frequent emigration or usurpation, which is common among *Xylocopa* throughout the reproductive season (Hogendoorn & Leys, 1993; Peso & Richards, 2011). In *X. varipuncta*, general conspecific odours appear to play a minor role in supplementing more temporally reliable visual cues. It appears that unimodal (visual) processing is sufficient for nest localization in this species, but that multimodal processing can be advantageous, potentially in assessing logs as nesting sites. A potentially weighted organization of cue use may allow for prioritization of cues in order of stability and reliability.

The Use of Visual Cues in Nest Recognition

The importance of vision in nest localization by group-living and social Hymenoptera is well established (Butler, Fletcher, & Watter, 1970; Collett, Dillmann, Giger, & Wehner, 1992; von Frisch, 1967; Wehner et al., 1996). Tinbergen (1972) demonstrated the importance of proximal visual landmarks for digger wasps, which nest in soil aggregations. Use of more distant landscape cues can precede this local landmark recognition, as in bumble bees, which orient first to distal cues as they approach the nest, and then to proximal

Table 1
Counts of returning foragers that made one or more nest recognition errors versus those that made no errors

	Baseline	Log rotation	Symbol shift	Olfactory control	Olfactory removal	Olfactory addition
Made errors	0	4	3	1	2	0
No errors	22	12	25	17	16	16

Bees made significantly more errors in the log rotation treatment (i.e. hold) than in the baseline ($P < 0.02$). No other treatment comparison was significant.

cues around the nest entrance (Plowright, O'Connell, Roberts, & Reid, 1995; Robert, Frasnelli, de Ibarra, & Collett, 2018). Proximal and distal cues may also be integrated continuously but at different weights at different distances from the nest in order to dynamically optimize the utility of multiple information sources (Hoinville & Wehner, 2018). This navigation scheme could conceivably account for the differences in the magnitude of search time between our two visual treatments, with rotation of the log delaying nest entry significantly more than the manipulation of proximal cues. Our rotation of nesting logs disrupted long-range cues, potentially including panorama cues, celestial cues or magnetic cues, all of which may be heavily weighted at relatively greater distances from the log. Simultaneously, proximal cues are likely to be more important at short distances from the log, and their manipulation consequently has a smaller effect on search time. Together, these results suggest that carpenter bees may integrate visual information related to panorama and positional cues associated with the nest as well as fine-scale cues in the immediate proximity of the nest entrance, and that the relative weight afforded to these cues may depend on distance from the nest.

The Use of Olfactory Cues in Nest Recognition

Hollnagel and Michener (1980) hypothesized that olfactory nestmate recognition is an important pre-adaptation for olfactory nestmate recognition in the social insects; as such, it should occur frequently in solitary and subsocial bees (Wcislo, 1992). Indeed, the importance of olfaction in nest localization has been demonstrated widely across the Apoidea (Foster & Gamboa, 1989; Guédot, Piltz-Singer, Buckner, Bosch, & Kemp, 2006; Wcislo, 1992). The results of our olfactory manipulations suggest that olfactory cues can influence a bee's ability to find her nest, but that the absence of these cues can be overcome by other cues, likely visual. The removal of olfactory cues at nest entrances impeded nest search efforts in the ground-nesting solitary sweat bee *L. figueroesi* (Wcislo, 1992). In our study, by contrast, removal of intrinsic olfactory cues by the same methods did not present any significant obstacle to carpenter bee nest localization. It may be that wood-nesting substrate provides more visual heterogeneity than does soil, making visual navigation more reliable for wood-nesters like carpenter bees. Conversely, *Lasiosiglossa* may be more likely to rely on supplemental olfactory cues to navigate their visually homogenous soil habitat. The variation in cue use by species with different nesting ecologies highlights that nesting substrate presents particular recognition challenges, and that these challenges may drive interspecific variation in hierarchical cue use.

Interestingly, we found that the addition of scent cues from foreign bees to the nest entrance helped bees find their nests more quickly, rather than hindering their search. Conversely, a similar treatment significantly delayed nest entry for *L. figueroesi* (Wcislo, 1992). Our result suggests that carpenter bees may not respond to nest scent cues on the level of an individual bee, but rather that a generic 'bee odour' can be sufficient to indicate nest occupation and aid nest localization. As nesting logs age, *X. varipuncta* preferentially inhabit newer nests, leaving older nests unoccupied. To navigate logs riddled with nesting holes, only a small fraction of

which are occupied, *X. varipuncta* may use species-specific but not individual specific odours to filter out empty nests and find their occupied nest faster. Similarly, house-hunting wild honey bees probably use general odour cues to locate tree cavities that have been previously occupied by bees, which they prefer (Visccher, Morse, & Seeley, 1985). Other insects, especially beetles, use nonspecific odours as aggregation pheromones to orient to a single nesting site (Francke & Detuner, 2005), suggesting that species' odour cues can provide information that facilitates nest selection for group-living species. Alternatively, the common threat of nest invasion may make carpenter bees sensitive to foreign odours at the home nest, potentially speeding their flight to defend the nest.

In contrast to *X. varipuncta*, Hefetz (1992) found that returning foragers of *Xylocopa pubescens* delayed entry when their nest entrance was swapped with a neighbour's, which was identical visually but presumably not chemically. In this case, unfamiliar odour cues most likely acted as a deterrent. It is not clear why these two species respond so differently to unfamiliar nest entrance odours. *Xylocopa pubescens* appears to localize to an individual nest-specific odour, whereas *X. varipuncta* uses nonspecific odour information. Our study examined the role of CHCs in nest recognition, but it is likely that other compounds, especially glandular secretions, are also relevant for creating a characteristic nest odour (Gerling, Velthuis, & Hefetz, 1989).

Conclusions

Our results support the roles of both vision and olfaction in nest recognition but suggest that visual cues may be sufficient in the absence of olfactory information. General scent cues may play a role in facilitating quick nest recognition, however, Furthermore, accuracy in nest localization likely preserves important boundaries between social groups. The generally low recognition error rates observed in our study suggest that bees avoid entering a nest until they have processed sufficient information to confirm they have identified it correctly, most likely to avoid aggressive interactions with guards. At the same time, these aggressive interactions are not often fatal. As such, there may not be a significant cost to inaccurate nest entry nor a strong selective pressure for identification of individual-specific odours. The overall resilience of bees' homing behaviour to the manipulations in this study probably reflects the adaptive value of a robust nest recognition system in this social context.

Competing Interests

We declare no competing or financial interests.

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