Foraging difference, potential competition, and resource overlap between Western honey bees
(*Apis mellifera* L.) and Asian honey bees (*Apis cerana* F.)

By
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Abstract

The anthropogenic introduction of *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) to the endemic range of Asian honey bees (*Apis cerana* Fabricius) began in the late 19th century. This human-induced movement of the non-native species to Asia has caused major repercussions. The number of managed colonies of *A. mellifera* in Asia has continued to increase. It is certainly a problem for the conservation of its Asian counterparts. The key concerns include abrupt displacement of *A. cerana* by *A. mellifera* in the apicultural industry, competition between *A. mellifera* and *A. cerana* for floral resources, and the introduction and exchange of pests and novel diseases. The field work for this research was conducted in Nepal, where four species of honey bees are native and where *A. mellifera* has been introduced for apicultural practices less than 30 years ago. This dissertation compares the foraging behavior of *A. mellifera* and *A. cerana* in relation to environmental variables, potential competition, and resource overlap. The last chapter presents for the first-time data on nesting biology, distribution, and traditional uses of the stingless bees (Apidae: Meliponini) in Nepal.

Extensive literature review in the first chapter warns that steep replacement of Asian honey bees by managed colonies of *A. mellifera* might cause local or small regional extinction of *A. cerana* from many regions of Asia. Moreover, there is continuous risk of exchange of diseases from one species to the other.

In the second chapter, foraging differences between *A. cerana* and *A. mellifera* in relation with time of the day, ambient temperatures, light intensity, and relative humidity are presented. I found that *A. cerana* colonies have longer foraging hours than *A. mellifera* colonies, and start foraging earlier in the morning and keep working later in the evening, at lower temperatures and with less ambient light. *A. mellifera*'s foraging peaked only once per day, at local noon, when temperatures and ambient light are high. In contrast, *A. cerana* foraging peaked twice once earlier in the morning and again later in the afternoon, before and after *A. mellifera*'s peak foraging. *A. cerana* foraged at lower temperatures and light

intensities than *A. mellifera*. *A. cerana* colonies also maintained their brood nest at temperatures significantly lower than did *A. mellifera*.

The third chapter compares floral resources —pollen and nectar—used by these two honey bees species to explore resource overlap and competition. Results show that *A. cerana* collects significantly greater pollen loads when they are in a single-species apiary than in mixed-species apiary with *A. mellifera*. Nectar volume, nectar concentration, and mg of sugar in nectar collected by *A. cerana* are also significantly higher in single-species apiary than mixed-species apiary. In contrast, *A. mellifera* does not show significant differences in the volume and concentration of nectar or weight of pollen loads collected when in single-species and mixed-species apiaries. *Pianka's index of niche overlap* between *A. mellifera* and *A. cerana* from this study is 0.73. This indicates that there is extensive overlap in the resources used by the two species.

Nesting biology, local indigenous nomenclature, and traditional practices of stingless bees management in Nepal are documented for the first time. Eighteen specific uses of stingless bees' products in food, medicine, crafts, and religious beliefs are documented. Ecological niche models suggest *Tetragonula iridipennis* Smith (Hymenoptera: Apidae, Meliponini) is broadly distributed in southern Nepal. In combination with my field data, niche model suggests areas where it may be locally extinct and areas where it may have been introduced by humans. Finally, the conservation status and future directions for the sustainable use of this bee species in Nepal are discussed.

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Chapter 1

Consequences of introduced Western honey bees (*Apis mellifera* L.) on the conservation status of Asian

honey bees (*Apis cerana* F.) and its ecological system

Abstract

The anthropogenic introduction of Western honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) to the endemic range of Asian honey bees (*Apis cerana* Fabricius) began in the late 19th century. This human-induced movement of the non-native species to Asia has caused major repercussions. When *A. mellifera* in Asia acquires parasites and pathogens indigenous to the Asian *A. cerana* or other *Apis* species, transportation of infected *A. mellifera* can spread the novel pathogens to *A. mellifera* outside of Asia. For example*, A. mellifera* in far northeastern Asia or Siberia were successfully colonized by two strains of the brood-parasitic mite, *Varroa destructor* Anderson & Trueman (Acari: Varroidae).

The number of managed colonies of *A. mellifera* in Asia has continued to increase. It is certainly a problem for the conservation of its Asian counterparts. Here, I review the current literature on the present-day distribution of Western honey bees in Asia and the known and potential consequences to the survival of Asian honey bees. These include: the abrupt displacement of the apiculture industry from *A. cerana* to *A. mellifera*, competition for floral resources, mating interference by *A. mellifera* drones to *A. cerana* queens, and the introduction and exchange of pests and diseases. This review highlights the cumulative effects of *A. mellifera'*s introductions to many Asian countries which might have caused local or small regional extinction of *A. cerana*. It also warns the continuous risk of novel disease transfer between them.

Introduction

The majorities of invasive insect introductions are accidental and may lead to major ecological and agricultural disruptions (Oldroyd 1999, Gray et al. 2009, Koetz 2013a, Lovett et al. 2016). However, some insects have been intentionally introduced outside of their native range, often without careful thought about potential negative impacts on the ecological system they are introduced to (Russo 2016). There are many examples of insects spreading beyond the areas of deliberate introduction and becoming invasive (Morales et al. 2013).

The Western honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini), the most commonly managed bee species in the world, is one such species. It is native to Europe, Africa and the Middle East (see, for example, Ruttner 1988) and has been introduced and naturalized in North and South America, Asia and Australia (Huryn 1997, Goulson 2003, Yang 2005, Russo 2016). The introduction of *A. mellifera* to North America by European colonists for honey and wax production began in the early 17th century (Sheppard and Smith 2000).

Regardless of the importance of *A. mellifera* as pollinators of agricultural plants, its role in non-managed landscapes is still unclear. Research on the effects of *A. mellifera* on native bees and plant communities has yielded inconsistent results. However, there have been noted impacts due to *A. mellifera's* potential to exploit resources (Hatfield 2016), relatively high level of pathogen loads (Goulson and Hughes 2015), generalists foraging habits (Menzel et al. 1993), and their interactions with native bee fauna (Sugden and Pyke 1991, Goulson 2003, Paini and Roberts 2005, Yang 2005). A standard colony of *A. mellifera* collects 20-30 lbs. pollen and 120-900 lbs. honey per year (Goulson, 2003). Depending upon the floral abundance, density of *A. mellifera* and other native bees, and other climatic conditions, the effects of this resource depletion by *A. mellifera* to native bees in the given area is variable. However, it still represents a substantial strain on resources (Anderson and Anderson 1989, Wills et al. 1990, Horskins and Turner 1999) and many studies found that *A. mellifera* generally exploits the most abundant

resources of a given area (Mallick and Driessen 2009, Shavit et al. 2009) and can have negative impacts on fecundity of native bees by displacing them from flowers and altering the suite of flowers they had the potential to visit (Hudewenz and Klein 2015).

Additional research is needed to address the effects of *A. mellifera* on native bee species. In controlled experiments, researchers have observed some indication of competitive exclusion of native bees by *A. mellifera*, where the honey bees force the native bees to switch to the less abundant and rewarding resources (Tepedino et al. 2007, Goulson and Sparrow 2009, Roubik 2009, Hudewenz and Klein 2013). There are many studies on competitive effects of *A. mellifera* on bumble bees showing lower reproductive success and smaller body size (Walther-Hellwig et al. 2006, Goulson and Sparrow 2009, Elbgami et al. 2014).

Another problem caused by domesticated livestock like *A. mellifera,* which is often used in migratory beekeeping, is spillover of infectious disease from mass-flowering crops into wildlife populations and natural habitats and vice versa (Fürst et al. 2014, González-Varo and Vilà 2017). There are many instances where *A. mellifera* has transmitted diseases to species of native bees including bumble bees upon sharing the same flower patches (Singh et al. 2010, Fürst et al. 2014).

Disease and pathogen transmission amongst honey bees species is being a growing concern. For instance, *Tropilaelaps clareae* Delfinado & E.W. Baker, a parasite of the *Apis dorsata* Fabricius found a breeding ground in the colonies of *A. mellifera* (Joshi et al. 2002). Similarly, *Melisococcus pluton* Bailey & Collins, a causative agent of European Foul Brood disease of *A. mellifera* detected in the colonies of *Apis laboriosa* Smith (Allen et al. 1990). In the same manner, *Nosema ceranae* Fries, a common disease of *Apis cerana* Fabricius*,* was detected in *A. mellifera* about two decades ago (Fries 2010, Martín-Hernández et al. 2018). It has been implicated as an important factor in colony collapse disorder because of its high virulence in *A. mellifera* (Paxton 2010, Graystock et al. 2013). *N. ceranae* has already been detected in *A. mellifera* of Canada and the United States (Williams et al. 2008) as well as bumble bees of

South America (Plischuk et al. 2009). There is a strong probability of *N. ceranae* detection soon in bumble bees of North America. One suspected culprit of disease transmission between managed and wild bees is the commercial use of pollinators, primarily *A. mellifera* (Manley et al. 2015). Spillover of the pathogens, pathogen spillback to *A. mellifera* with strong virulence capacity, and facilitation are three mechanisms by which managed bees negatively impact wild bees (Graystock et al. 2015, Graystock et al. 2016, González-Varo and Vilà 2017).

The first introduction of *A. mellifera* in the endemic distribution area of the *A. cerana* resulted in a host shift of *Varroa destructor* Anderson & Trueman from the native Asian honey bees to the Western honey bees (Akratanakul and Burgett 1975). *V. destructor*, a deadly parasite of *A. mellifera* has been globally introduced in many different episodes (Pirk et al. 2017). Gradually, *Varroa* has spread all over the world (Crane 1978, Jong et al. 1982) except the recorded presence in Australia (Rosenkranz et al. 2010, Koetz 2013b, Iwasaki et al. 2015). It is primarily due to the subsequent movement of this host-parasite interaction. In most cases of *Varroa* infestations, the area with pre-dominant numbers of human managed colonies lacking wild or feral colonies are easy targets for the mite due to the lack of the resistance and resilience (Jaffe et al. 2010). In contrast, feral populations have been shown to mitigate the effects of *Varroa* at population level by maintaining genetic diversity in different places of North America and Africa (Sgro et al. 2011).

It is evident that anthropogenic introduction of *A. mellifera* into Asia has resulted in the complex interactions between managed and unmanaged honey bees species (Dietemann et al. 2013, Spleen et al. 2013, Steinhauer et al. 2014). It contributed to the cosmopolitan distribution of *V. destructor* which otherwise is a parasite of *A. cerana* to whom it is not as problematic. Promoting beekeeping with *A. mellifera* in Asia provides a continuous introduction into to new habitats, thereby allowing another potential novel disease spillover to *A. mellifera* and spillback of highly virulent strain of *Varroa* to *A. cerana* (Beaurepaire et al. 2015, Pirk et al. 2017). A worrisome consequence of this pathogen exchange is another era of global colony collapse disorder leading to severe economic and ecological losses.

The potential risks posed by *A. mellifera* are not only to native bees but also to native plant communities. *A. mellifera* has shown preference on visiting invasive plants (Huryn and Moller 1995, Morales and Aizen 2002, Hanley and Goulson 2003) thereby increasing seed sets and initiating mutualism among invasive species (Barthell et al. 2005, Goulson 2005). Healthy pollinator populations are essential to maintain healthy plant communities given that more than 85% of all flowering plants depend on an animal pollination for reproduction (Ollerton et al. 2011). Therefore, this invasive-invasive mutualism has the potential to cause significant damage to native plant communities (Brown and Paxton 2009). It might cause a considerable amount of economic and ecological damage to the ecosystem (Goulson 2005). Also, several studies indicate that honey bees only collect pollen from 25-42% of the plant species available in a given natural area (Wills et al. 1990, Thorp et al. 1994, Buchmann 1996). In addition, there are many thousands of plant species which are not effectively pollinated by honey bees because of the required "buzz pollination" behavior to free pollen grains from the plant (De Luca and Vallejo-Marín 2013). As such, natural systems dominated by exotic *A. mellifera* are unlikely to be effectively pollinated thereby posing threats to the native bees and native plants. While *A. mellifera* is an effective pollinator of many mass-flowering crops (González-Varo and Vilà 2017), it should not be considered as an effective pollinator of all native plants (De Luca and Vallejo-Marín 2013). More thorough surveys and research is needed prior to future introductions of this species into new habitats to avoid possible negative impacts.

Precautionary measures are especially important prior to placing *A. mellifera* or any other managed pollinators in habitats with vulnerable and declining native bee populations. Recently, many ecologists and entomologists have warned about the potential negative impacts of *A. mellifera*, with some efforts to eradicate them locally (Wenner et al. 2009). While keeping introduced honey bees on public lands and natural areas has been a matter of debate (Pyke 1999); there is a pressure for the beekeeping industries to find pesticide free forage-areas and evidence-based technical knowledge to decide whether the presence of *A. mellifera* is appropriate for that landscape (Hatfield 2016). It is therefore important to

consider how *A. mellifera* will impact native honey bees species in Asian landscapes before placing them for commercial beekeeping and pollination services. Herein, I am presenting the current status of *A. mellifera* in Asia focusing on its introduction and timeline, the current trends of apicultural shift from *A. cerana* to *A. mellifera*, and the potential consequences of this shift on the conservation status of Asian honey bees and its ecological system.

Introduction of western honey bees in Asia: a timeline and history

Ruttner (1988) recognized 24 subspecies of the Western honey bees through multivariate analysis of morphological characters. *A. mellifera* lives in colonies ranging from 15,000 to 60,000 individuals (Southwick and Heldmaier 1987). Asiatic hive bees are similar in appearance to the Western honey bees but have a smaller average body size and colonies ranging from 2,000 to 20,000 individuals (Koetz 2013b). Asiatic hive bees have adapted to highly diverse geographical ranges from tropical low land to high Himalayan regions. Four subspecies: *A. c. cerana*, *A. c. indica*, *A. c. japonica*, and *A. c. himalaya* were previously recognized (Ruttner 1988); however, recent work classifies the Asiatic hive bee into six different morpho-clusters based on twelve morphological features (Radloff et al. 2010). More intensive taxonomic and molecular research are still lacking when comparing Asian honey bees to its European counterpart.

The native home range of *A. cerana* includes parts of Asia ranging from tropical and moist rainforests to dry grasslands and taiga (Radloff et al. 2010). In the 1970s, they were deliberately introduced to Papua New Guinea and have since gone feral and spread over the Solomon Islands and northeastern Australia (Koetz 2013b). More than 450 colonies of *A. cerana* have been detected in Queensland, Australia from 2007 to 2011 (Hyatt 2012). Due to the multiple episodes of incursion in the northern Cairns region, eradication attempts by Australian authorities shifted to mitigating the negative effects of *A. cerana* (Commerford 2013, Koetz 2013b, Pirk et al. 2017). In practice, Australia has been

destroying *A. cerana* colonies aggressively to avoid *Varroa* introduction (Les Anderson 2012). Despite this, it seems *Varroa* has already found its new home in Australia (Iwasaki et al. 2015).

Indigenous cultures have managed Asian honey bees across Asia for up to two thousand years (Crane 1978). However, starting in late the 19th century, beekeepers across Asia began to import *A. mellifera* for the economic benefits of honey and wax production (Abrol 2013). European honey bees are considered to be more productive because of their significantly larger colonies, so they are preferred by commercial beekeepers in Asia (Atwal 2000, Abrol 2013) and have been regularly introduced to Asian countries (Table 1-1). The first introduction of *A. mellifera* to Asia was likely in 1877 to Japan from United States (Sakai and Okada 1973, Watanabe 1981, Yoshida et al. 1994, Solignac et al. 2005, Theisen-Jones and Bienefeld 2016). India unsuccessfully introduced them in 1880, but it took more than 80 years to firmly establish the species (Abrol 2013). China, on the other hand introduced them a little more than 100 years ago (Yang 2005, Tan et al. 2012) and *A. mellifera* is now extant throughout the country surpassing more than 9 million colonies (FAO 2016). Subsequently, Vietnam got them from China around 1960 (Tan and Binh 1994). Thailand had multiple unsuccessful attempts in the 1940s (Wongsiri 1988, Suppasat et al. 2007) and 1950s (Akratanakul 2000, Suppasat et al. 2007) for research at Chulalongkorn and Kasetsart Universities in Bangkok. Later, it has successfully imported them from Taiwan to Lampoon and Chiang Mai in northern Thailand for commercial purposes in 1970 (Wongsiri et al. 2000). Pakistan introduced them in the late 1970s from Australia (Sivaram 2012). Nepal had its first unsuccessful introduction in 1978 but later successfully established them in 1990 from Northern India and Kashmir (Allen 1995). Bangladesh recently introduced them from India in 1995 (Sivaram 2012). More interestingly, beekeepers and bee enthusiasts are still trying to introduce them to high altitude habitats in countries like Nepal (Personal observation by Chet Bhatta in Nepal, 2017).

Current status of *A. mellifera* beekeeping in Asia

Beekeeping with indigenous *A. cerana* is a well-known and ancient tradition across the Asian countries. It is evident by the mysterious poetic expressions of the *Rig-Veda*, 1500-2000 B. C. (Kapil 1971). Even today, local people still keep bees in traditional ways, e.g., in hollowed logs, house walls, and varieties of boxes made of locally available materials (Kapil 1971, Verma and Rinderer 1993, Verma and Attri 2008, Hisashi 2010, Kohsaka et al. 2017). In the late nineteenth century, a small prototype of a modern hive with movable frames was introduced in south India by a visiting priest. This was later modified to form the Newton A and Newton B types hives to accommodate two varieties of *A. cerana* found in India: the yellow plains bees, which inhabit warmer, low altitude habitats and black hill bees, which occupy higher, cooler areas (Kapil 1971, Verma 1998, Sivaram 2012). Today, beekeeping enterprises in Asia range from small traditional family-owned apiaries to large-scale, intensive businesses that practice migratory beekeeping (Akratanakul 1990, Tan and Binh 1994, Verma 1998, Sivaram 2012). Hive boxes and equipment for *A. cerana* beekeeping are still not standardized across the Asian bee-keeping landscape, as they have been done in *A. mellifera*.

Many beekeepers have started small and large-scale, commercial *A. cerana* beekeeping throughout Asia (Sivaram 2012), otherwise beekeeping is generally considered as a zero investment and zero management practice (Joshi et al. 2002). Local people catch swarms and keep them in locally designed log or wall hives and harvest small amounts of honey annually. Based on their indigenous knowledge, farmers multiply the colonies and share among the neighbors spreading this profitable enterprise throughout the communities with no initial investment (Verma and Attri 2008). *A. cerana* beekeeping is not only profitable for marginalized communities but also well-acclimated for foraging under extreme weather conditions thereby pollinating a great diversity of native flora in these mountain ecosystems. *A. mellifera* beekeeping in Asia on the other hand involves substantial capital, large foraging areas, and potential risk of failure along with a host of ecological consequences (Koetz 2013b, Pirk et al. 2017).

The recent questionnaire survey among apiculturists of Asia on the population status of Asiatic hive bee by Theisen-Jones and Bienefeld (2016) confirmed the decline of *A. cerana* populations in Afghanistan, Bhutan, China, India, Japan, South Korea, Myanmar, Pakistan, the Philippines, Taiwan, and Vietnam. The decline rate reported is roughly 5% in Bhutan to 95% or higher in Thailand, South Korea, Afghanistan, and Pakistan. This result is consistent with the findings of other honey bees researchers from those regions (Abrol 2013, Koetz 2013b). Even though *A. cerana* is more tolerant to pest and disease than *A. mellifera*, low productivity and absconding uncertainties are the main drivers of species switch by beekeepers in Asia (Koetz 2013b).

Beekeeping with *A. mellifera* seems to be a clear winner over *A. cerana* in instances where there are no problems in environmental settings or of high startup costs for sophisticated systems (Verma and Attri 2008, Oldroyd and Nanork 2009), yet ecological consequences and biodiversity issues are far more important than short-term economic returns (Pirk et al. 2017). Despite the considerable attention of introducing *A. mellifera* to ecological systems in Australia and the Americas, it is very surprising that colonies of *A. mellifera* has continued to increase in many of the Asian countries (Figure 1-1). The honey exports and imports data posted by FAO (2016) clearly indicates how increases in numbers of managed colonies of *A. mellifera* in selected Asian countries and losses in Western Europe and the United States are highly correlated with the global honey exports and imports (Figure 1-2 & 1-3). This state of promotion and attraction of alien species for short-term benefits from hive products might have long term ecological significance. Promoting alien species instead of inventing new possibilities on breed management of native species leaves *A. cerana* more susceptible than ever.

Impacts on Asian honey bees and its ecological system

Regardless of profitability of *A. mellifera* in beekeeping enterprises, its introduction into the native range of *A. cerana* might have been problematic for several reasons such as: competition for floral resources, interference with *A. cerana* queen mating by *A. mellifera* drones, and introduction and

exchange of pests and diseases (Theisen-Jones and Bienefeld 2016). These factors in combination might have negatively impacted *A. cerana* in their ecology and population health. Indigenous honey bees, especially *A. cerana*, *A. laboriosa*, *A. dorsata*, and *A. florea* have co-existed together for centuries in a harmonized way until the introduction of *A. mellifera* (Koeniger et al. 1999, Joshi et al. 2002). Thai Sac Brood Virus (TSBV), European Foul Brood (EFB), and many acarine diseases were introduced to *A. cerana* that led to diminished populations of *A. cerana* in south Asian countries (Theisen-Jones and Bienefeld 2016). Later, resistant wild colonies of *A. cerana* slowly emerged in different regions retreating their populations after two decades of *A. mellifera* introduction (Reddy 1999, Ahmad and Partap 2000, ICIMOD 2001). On the other hand, introduction of *A. mellifera* to the Hindu Kush Himalayan (HKH) regions and other countries of Asia without knowing the fact that they require migratory beekeeping, monoculture based large foraging grounds, and intensive management practices made themselves prone to new pests and diseases (Ahmad and Partap 2000). It resulted in failure to adapt to the temperature regimes in the mountain ecosystem while impacting indigenous bee populations and its ecological system (Joshi et al. 2002).

In this section of the dissertation, I collated the information reviewed from over 138 papers on recorded consequences of *A. mellifera* to the native bees and their ecological system. 66 papers presented negative impacts of *A. mellifera* on Asiatic hive bee causing apicultural shift, local extinction, floral resource competition, exchange of pathogens and parasites, introgression, and drones/queen interference. 46 papers reported negative impacts of *A. mellifera* on ecological system *via* pollination of exotic plants and invasive weeds, decreasing plant fitness and altering pollination webs, and facilitating spillover from migratory beekeeping around mass flowering crops. Regardless of the negative impacts on native bees and their ecological system, 36 papers provided positive impacts of *A. mellifera* introduction due to its role in economics of agricultural pollination, bee hive products, and its adaptability on varied climates (Table 1-2).

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Discussion

Suitability and economic success of beekeeping with *A. mellifera* is still uncertain in many regions of Asia primarily due to the varied local climates, intensive labor, money, and technicalities involved in migratory beekeeping. For example, commercial beekeeping with *A. mellifera* has fully overtaken *A. cerana* beekeeping in several temperate regions of Asia such as northern India and several regions of China and Japan (Theisen-Jones and Bienefeld 2016). On the other hand, the practicality of beekeeping with *A. mellifera* doesn't make sense in tropical and humid countries like Cambodia and Philippines where all *A. mellifera* beekeeping activities were diminished immediately upon the termination of funding from European and American NGOs (Oštir 2014). Even in the cases where *A. mellifera* is critical for commercial agricultural pollination and large-scale honey production, it is very important to have the best understanding of the local flora and fauna, local conditions, local land management goals, and the sensitivity of habitat before making a decision on whether to allow access of the natural areas to this introduced species (Hatfield 2016).

More research is needed to understand how native honey bees and other bee species will be impacted by the further intrusion of western honey bees in Asia. Further consideration of potential impacts and relevance raised by the researchers when deciding whether to place *A. mellifera* apiaries in new habitats is needed until we have additional promising evidence documenting some neutral effects of it to native bees and their habitats. More importantly, considering research that highlight several potential risks posed by *A. mellifera* on native pollinators and their ecology, it would be very logical to conserve and promote locally available honey bees and other native bees rather than promoting introduction of this alien species to the landscapes with less disturbed native ecological system.

Apiculturists and native bee activists in Asia are struggling to encourage beekeepers for a beekeeping enterprise with less profitable native honey bees in several regions of Asia despite there has been clear evidence that *A. mellifera* is a perfect winner if beekeepers have sufficient fund to invest

(Oldroyd and Nanork 2009). It is suggested that low productivity of *A. cerana* colonies could be compensated by increasing colonies, as one can easily catch and take care of swarms from wild colonies (Verma and Rinderer 1993, Tan and Binh 1994). However, it may be more profitable to keep a single high-performing colony than keeping many low performing colonies from a purely economic stand point (Theisen-Jones and Bienefeld 2016). It is noteworthy to mention that *A. mellifera* colonies historically produced lower yields of honey (2-5 kg per colony) before the introduction of the selective breeding (Crane 1978). Therefore, these approaches could also be successful if applied to *A. cerana*. Controlled mating used in the *A. mellifera* population should be practiced improving the regional stock of *A. cerana*. Moreover, intensive taxonomic and molecular studies should be carried out with in *A. cerana* populations to encourage native beekeeping enterprises while maintaining ecological integrity.

Figures and figure legends

Figure 1-1. Status of *Apis mellifera* colonies in selected Asian countries between 1961-2016 (FAO 2016)
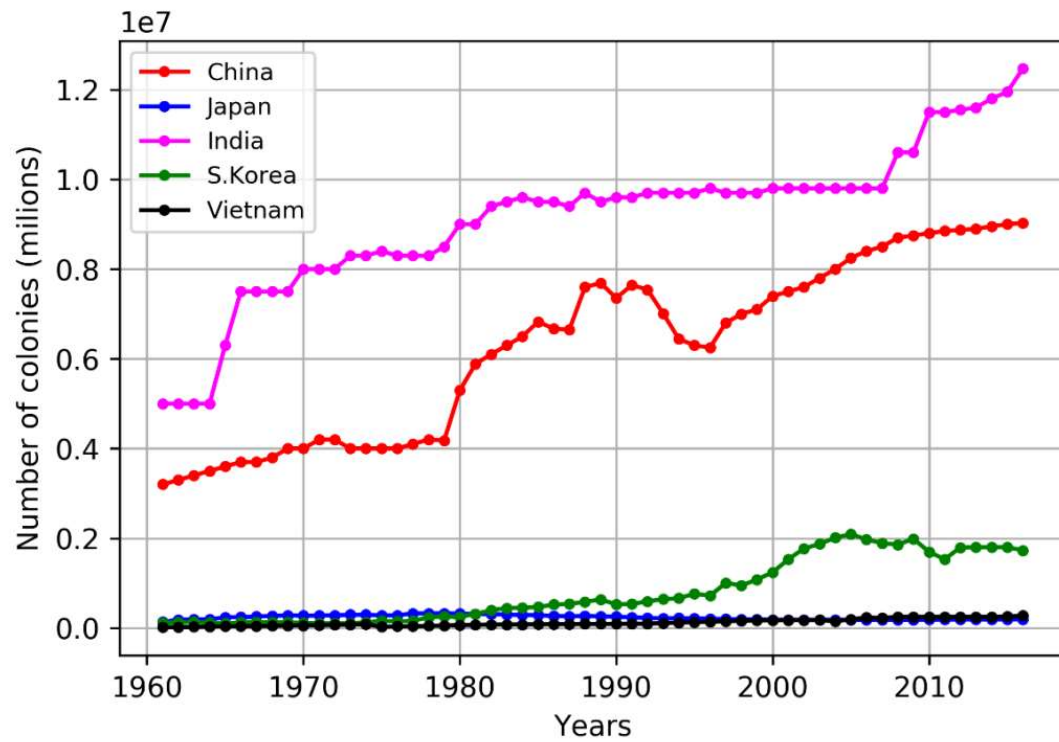
Figure 1-1

Figure 1-2. Honey export trend of selected Asian countries between 1961-2016 (FAO 2016).
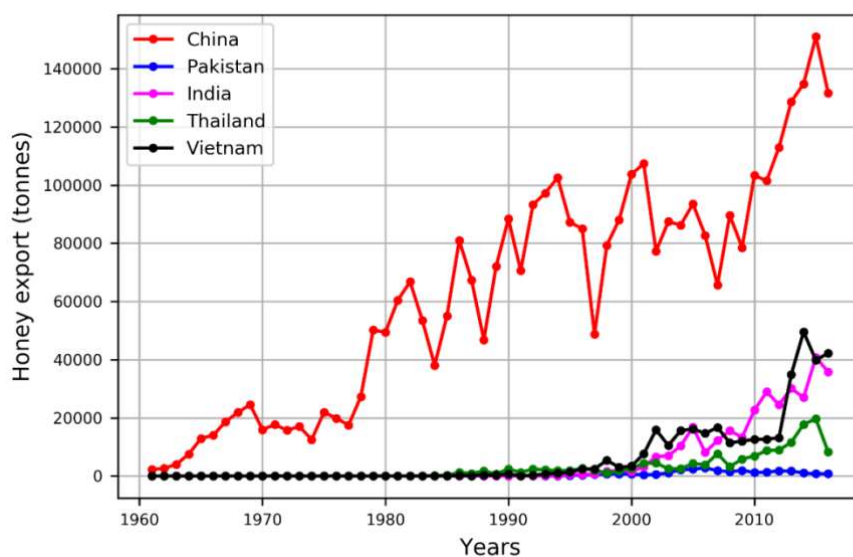
Figure 1-2

Figure 1-3. Honey import trend of selected European and American countries between 1961-2016 (FAO 2016)
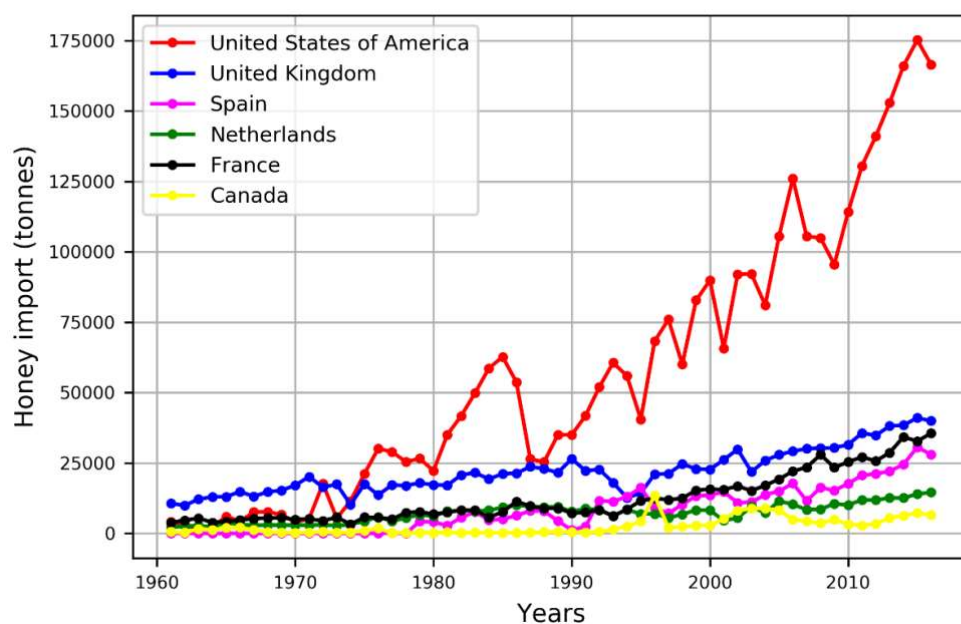
Figure 1-3

Tables and table legends

Table 1-1. Selected countries in Asia and their timeline of *A. mellifera* introductions and establishments.

Country	Timeline of *A. mellifera* introductions and establishments in Asian countries	
	Introductions	Establishments
Japan	1877 (Sakai and Okada 1973, Watanabe 1981, Yoshida et al. 1994, Solignac et al. 2005)	1915 (Theisen-Jones and Bienefeld 2016, Kohsaka et al. 2017)
India	1880s (Abrol 2013)	1960 (Abrol 2013)
Vietnam	1887 (Theisen-Jones and Bienefeld 2016)	1960s (Tan and Binh 1994)
China	1896-1913(Theisen-Jones and Bienefeld 2016)	Early 1900 (Yang 2005, Tan et al. 2012)
Taiwan	1896-1912 (Theisen-Jones and Bienefeld 2016)	---
South Korea	---	Early 1900 (Choi 2001, Kohsaka et al. 2017)
Thailand	1940s and 1950s (Wongsiri 1988, Akratanakul 2000, Suppasat et al. 2007)	1970s (Wongsiri et al. 2000)
Pakistan	---	1977-1978 (Sivaram 2012)
Nepal	1978 (Allen 1995)	1992 (Allen 1995)
Bangladesh	---	1995 (Sivaram 2012)

Table 1-1

Table 1-2. Selected references of potential positive and negative impacts of *Apis mellifera* L. on native bees of Asia and their ecological system. References on italics and bolded refer citations with an empirical component and non-italicized and unbolded references refer citations based on opinions.

Impacts	References
Negative consequences on native bees	
1. Apicultural Shift	*(Verma and Rinderer 1993, Atwal 2000, Yang 2005, Verma and Attri 2008, Aizen and Harder 2009, Oldroyd and Nanork 2009, Sivaram 2012, Abrol 2013, Moritz and Erler 2016, Theisen-Jones and Bienefeld 2016, Pirk et al. 2017)*
2. Local extinction of Asiatic hive bees	(Sakagami 1959, Atwal and Sharma 1971, Yang et al. 2011, Pirk et al. 2017)
3. Floral resource competition	*(Roubik 1980, Ruttner 1988, Sugden and Pyke 1991, Nagamitsu and Inoue 1999, Goulson 2003, Paini 2004, Paini and Roberts 2005, Yang 2005, Greenleaf and Kremen 2006, Semida and Elbanna 2006, Magnacca 2007, Michener 2007, Goulson and Sparrow 2009, Badano and Vergara 2011, Carvalheiro et al. 2011, Hudewenz and Klein 2013, Koetz 2013b, Herbertsson et al. 2016, Torné-Noguera et al. 2016, Cane and Tepedino 2017)*
4. Exchange of pathogens and parasites 22	*(Akratanakul and Burgett 1975, Crane 1978, Jong et al. 1982, Thomas et al. 2002, Goulson 2003, Hoffmann et al. 2008, Dietemann et al. 2009, Mayack and Naug 2009, Oldroyd and Nanork 2009, Fries 2010, Rosenkranz et al. 2010, Singh et al. 2010, Kojima et al. 2011, Bailey 2013, Spleen et al. 2013, Van Dooremalen et al. 2013, Fürst et al. 2014, Graystock et al. 2014, Steinhauer et al. 2014, Beaurepaire et al. 2015, Goulson and Hughes 2015)*
5. Introgression and drones/queen interference	*(Ruttner and Volprecht 1983, Ruttner 1988, Barthell et al. 2001, Ji et al. 2003, Wang et al. 2003, Pirk et al. 2011, Byatt et al. 2016)*
Negative consequences on ecological system	
1. Pollination of exotic plants and invasive weeds	*(Stimec et al. 1997, Barthell et al. 2001, Morales and Aizen 2002, Stout et al. 2002, Goulson and Derwent 2004, Simpson et al. 2005, McIver et al. 2009, Groom et al. 2015, Miller et al. 2015)*
1. Decreasing plant fitness and altering pollination webs (Impacts on native flora and fauna)	*(Roubik 1978, Gross and Mackay 1998, England et al. 2001, Morales and Aizen 2002, Goulson 2003, Dupont et al. 2004, Paini 2004, Moritz et al. 2005, Rymer et al. 2005, Traveset and Richardson 2006, Aizen et al. 2008, González-Varo et al. 2010, Polatto and Chaud-Netto 2013, Aizen et al. 2014, Fürst et al. 2014, Valido et al. 2014, Carman and Jenkins 2016, Montero-Castaño et al. 2016, Torné-Noguera et al. 2016, Cane and Tepedino 2017)*
2. Spillover from migratory beekeeping 18	*(Rand et al. 2006, Hoffmann et al. 2008, Blitzer et al. 2012, Tscharntke et al. 2012, Driscoll et al. 2013, Vasseur et al. 2013, Couvillon et al. 2014, Fürst et al. 2014, Härtel and Steffan-Dewenter 2014, Macfadyen et al. 2015, Manley et al. 2015, Requier et al. 2015, Cavigli et al. 2016, Danner et al. 2016, González et al.*

	2016, Montero-Castaño et al. 2016, González-Varo and Vilà 2017, Pirk et al. 2017)
Positive consequences on ecological system	
1. Economics of Agricultural pollination and bee hive products	(Davis et al. 1988, Greenleaf and Kremen 2006, Cook et al. 2007, Klein et al. 2007, Aizen et al. 2008, Ricketts et al. 2008, Aizen and Harder 2009, Brown and Paxton 2009, Freitas et al. 2009, Zayed 2009, Potts et al. 2010, Carvalheiro et al. 2011, Aebi et al. 2012, Calderone 2012, Klein et al. 2012, Rucker et al. 2012, Garibaldi et al. 2013, Breeze et al. 2014, Melin et al. 2014, Pirk et al. 2014, Glatz 2015, Cunningham et al. 2016, Gaines-Day and Gratton 2016, Pirk et al. 2017)
2. Alternative to native species and adoption in the varied climates	*(Huryn 1997, Goulson 2003, Junker et al. 2010, Sun et al. 2013, Chamberlain and Schlising 2014, Lowenstein et al. 2014, Sanguinetti and Singer 2014, Giannini et al. 2015)*
3. Organisms for lab reared studies	*(Williams 1998, Page Jr and Peng 2001, Grozinger et al. 2003, Mullin et al. 2015)*

Table 1-2

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Chapter 2

Foraging differences between Western honey bees (*Apis mellifera* L.) and Asian honey bees (*Apis cerana* F.) in relation to environmental variables

Abstract

The foraging differences between *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) and *Apis cerana* Fabricius in relation to environmental variables were observed. Observation experiments were conducted at four apiaries in Kathmandu valley, Nepal. Colonies of both species were kept together in two mixed-species apiaries and colonies of only *A. cerana* or only *A. mellifera* were kept in each of two single-species apiaries. Ambient temperature, relative humidity, and light intensity were recorded hourly after the commencement of foraging activities for six months. Brood nest temperatures and thoracic temperatures of brood nest workers and departing foragers were also measured at specific ambient temperatures. We found *A. cerana* colonies have longer foraging hours than *A. mellifera* colonies. *A. cerana* started foraging earlier in the morning and later in the evening, at lower temperatures and lower light intensity than *A. mellifera*. *A. mellifera* foraging (measured as departures per minute of foraging workers) peaked only once at local noon while *A. cerana* foraging peaked twice, earlier in the morning and later in the afternoon, at lower temperatures and moderate light intensities. *A. cerana* colonies maintained their brood nest at temperatures significantly lower than did *A. mellifera and* were able to forage in the morning and on colder days. Neither *A. cerana* nor *A. mellifera* showed significant differences in these foraging parameters, when they were kept in mixed-species *versus* single-species apiaries. Both *A. cerana* and *A. mellifera* foraging were best predicted by the time of the day rather than with specific environmental variables: light intensity, ambient temperature or relative humidity. This study suggests that *A. mellifera* foragers stay inside the hive at low ambient temperatures to maintain a higher brood nest temperature but *A. cerana* foragers are still able to forage at the colder temperatures because of their lower brood nest temperature.

Keywords

Introduction

Honey bees carry out activities both inside and outside of the hive which help them maintain their ecological success (Winston 1991). Activities inside the nest include homeostatic maintenance of internal colony temperature, brood rearing and food storing (Southwick and Moritz 1987). Worker foraging for pollen, nectar, and water, mating flights of queens and drones, and scouting for new nest sites are primary activities outside the nest (Dyer and Seeley 1987). The fluctuations of ecological abiotic factors, i.e., relative humidity, ambient temperature, and light intensity play vital roles on the foraging activities of honey bees (Lundie 1925).

According to Dyer and Seeley (1987), every flying insect has a species specific minimum thoracic temperature to initiate their flight activities, maintaining which must be detrimental to reach out to the available floral resources before the competing species. Foraging bees have several strategies to regulate their body temperature at extremely variable ambient temperatures (Stabentheiner and Kovac 2014). Heinrich (1980a) reported that head temperatures of honey bees were above 7 °C at 17 °C ambient air temperature and below 3 °C at 43 °C ambient air temperature. Generally, foragers use solar heat to increase body temperature and save energy (Abou-Shaara et al. 2017), but during flight activities under lower ambient temperatures bees can respond it differently (Roberts and Harrison 1999, Woods et al. 2005, Stabentheiner and Kovac 2014). According to Heinrich (1979), the outgoing foragers of western honey bees elevate their thoracic temperature up to 38 °C by shivering action of flight muscles before exiting the hive. Even at an ambient temperature lower than 20 °C, *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) can maintain thoracic temperature above 30 °C by means of a combination of wing muscle activities (Dyer and Seeley 1987). In other cases, honey bees were foraging on warmer nectars (Norgate et al. 2010) and blossoms (Kevan 1975) to warm up themselves. However, the continuous exposure of foraging bees to high ambient temperatures could lead to the shorter life spans (Visscher and Dukas 1997, Remolina et al. 2007) and colony losses (Al-Ghamdi et al. 2016).

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Relative humidity (RH) is also important for both brood development and worker foraging. RH inside the nest is regulated by the workers bees (Li et al. 2016). According to Al-Ghamdi et al. (2016), RH under 50 % hindered the egg hatching, and the optimum RH range for normal egg hatching was from 90 to 95 %. Fluctuations in RH have impacts on foraging behavior of workers, as bees show specific behaviors such as foraging for extra water to increase RH inside the colonies (Human et al. 2006) and fanning behavior to reduce the RH to be within the optimal range (Kaftanoglu et al. 2011). However, air humidity might not have greater role in temperate climate, rather it has important role in dry and hot conditions (Abou-Shaara et al. 2017). Atmowidjojo et al. (1997) found that high body water loss in feral and domestic colonies of honey bees in Arizona with decreasing RH (0 to 35 %). Similar observation is made by Alqarni (2006) in Yemeni bees.

Light intensity is also important to foraging bees, as the active time of the foragers is day related (Eban-Rothschild and Bloch 2008). Osgood (1964) found that foraging initiation in the morning of the Alfalfa leaf-cutter bee depended upon the ambient temperature while foraging cessation in the evening was influenced mainly by the light intensity. There were no out-of-colony activities during the night, even in the presence of moonlight (Abou-Shaara et al. 2017) but resting time during the day can be modified by the foragers to fit with the forage availability and favorable environmental variables (Klein and Seeley 2011). Moore and Rankin (1993) found that light-off was the primary environmental cue for the Italian honey bees.

Goulson (2003) stated that the ecological success of competing honey bees species depends on their ability to thermoregulate so as to be able to forage at lower ambient temperatures. The internal nest temperature of *A. mellifera* ranges from 33 to 36 °C (Bujok et al. 2002, Kleinhenz et al. 2003, Petz et al. 2004, Aupinel et al. 2005, Jones et al. 2005, Silva et al. 2009). According to Ohashi et al. (2009), honey bees try to maintain their own ambient microclimate inside the nest, as bee broods are very sensitive to temperature fluctuations. Overheating of the brood nest is prevented by fanning (Southwick and Moritz 1987), evaporative cooling (Nicolson 2009), vacating the nest and clustering outside the hive entrance

(Abou-Shaara et al. 2017), and heat shielding (Starks and Gilley 1999). In case of ambient temperatures cooler than the desired brood temperature, honey bees workers maintain temperature by clustering and increasing endothermy (Harrison 1987, Stabentheiner et al. 2010, Szopek et al. 2013). Endothermy is maintained by younger bees older than 2 days which also enhanced the development of their flight muscles while engaging to clean warm brood cells (Stabentheiner et al. 2010). Worker bees with heated thoracic muscles further regulate the brood nest temperature by sitting motionless on the caps of the brood cells superficially (Silva et al. 2009) and also entering to the empty cells between the sealed brood cells (Kleinhenz et al. 2003, Humphrey and Dykes 2008).

Honey bees foragers are pre-warmed by relatively constant brood nest temperatures and can commence foraging earlier than most of the stingless bees and solitary bees (Jones et al. 2007), however foraging behavior is a great challenge in a wide range of temperatures. Abou-Shaara (2014) reported that *A. mellifera* foraging can take place from 10 to 40 °C. Tan et al. (2012) recorded 6.57 °C as the mean temperature for *A. mellifera* foraging initiation and 20 °C for the peak foraging activities, while Joshi and Joshi (2010) found reduction in foraging trips below 10 °C. Woyke et al. (2003) observed that 10 °C was the temperature at which foraging was initiated, and the numbers of *A. mellifera* foragers increased by ten-fold at 12 °C. On the other hand, Blažytė-Čereškienė et al. (2010) observed drastic reduction in the foraging activities of honey bees at higher temperature like 43 °C.

In this research, I explore foraging difference between Asian honey bees and European honey bees in relation to environmental variables, brood nest temperature, and thoracic temperatures of brood nest workers and departing foragers. The western honey bees, *A. mellifera*, lives in colonies ranging from approximately 15,000 to 60,000 individuals (Southwick and Moritz 1987). It is originally from Europe, the Middle East and Africa (Ruttner 1988a, Smith 1991, Franck et al. 1998), but has been introduced widely around the world (Alqarni et al. 2011). The Asian hive bee, *Apis cerana* Fabricius, is very similar in appearance to the western honey bees, though it has a smaller average body size and colonies range from approximately 2,000 to 20,000 individuals (Koetz 2013). The native home range of *A. cerana*

extends over tropical and moist rainforests, temperate forests, dry grasslands and taiga (Radloff et al. 2010). In the 1970s, *A. cerana* was deliberately introduced to Papua New Guinea; since then it has gone feral and spread over the Solomon Islands and recently, north-eastern Australia (Koetz 2013).

Several aspects of the Asian honey bees are still understudied in comparison to the European honey bees (Reddy, 1980). Thermoregulatory abilities of the Asian honey bees vary greatly by body size and nesting behavior (Dyer and Seeley 1987, Ruttner 1988b). For instance, the medium-sized *A. cerana* workers can forage earlier in the morning than the giant bees, *Apis dorsata* Fabricius, and dwarf bees *Apis florea* Fabricius and *Apis andreniformis* Smith (Oldroyd et al. 1992) because of their well thermoregulated cavity nest (Dyer and Seeley 1991). This ability of Asian honey bees is emphasized by Oldroyd and Wongsiri (2009), who argue there might be a resource partitioning among the species of honey bees to reduce interspecific competition.

It is just less than 30 years since *A. mellifera* was introduced to Nepal from northern India and Kashmir in 1992, after some prior unsuccessful introduction in 1978 (Allen 1995). Since then, commercial beekeepers have been attracted to *A. mellifera,* although the number of colonies of *A. cerana* in the country is still much higher due to presence of large numbers of wild and traditionally managed colonies (GoN 2016). Fortunately, there are no reported feral colonies of *A. mellifera* yet, but one can see the increasing trend of beekeeping with *A. mellifera* among the professional beekeepers of the country (FNBK 2016). This trend of apicultural shift to *A. mellifera* might not be good, as many recent studies warn that populations of *A. cerana* are drastically declined in several neighboring countries (Theisen-Jones and Bienefeld 2016). One of the several causes of *A. cerana* decline is suggested to be resource competition with exotic *A. mellifera* (Yang 2005, Tan et al. 2012).

Here I explore foraging differences between *A. cerana* and *A. mellifera* in Kathmandu valley, Nepal. I kept them in mixed-species apiaries and single-species apiaries throughout summer and rainy seasons in 2015 and spring, summer, and rainy seasons in 2016. I ask (a) Is there a difference in the foraging behavior (foraging initiation time, foraging peaks, mean number of foragers per minute

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throughout the day from 6 am to 6 pm, and foraging cessation) between the species. (b) Does foraging behavior of either species differ when they are kept in mixed-species apiaries *versus* single-species apiary? (c) Are there any environmental variables that best predict the foraging behavior of the either species?

Since *A. mellifera* is significantly larger in body size (Ruttner 1988a), several authors predicted that it can produce greater endogenous heat, enabling it to forage at lower ambient temperatures (Dyer and Seeley 1987, Bishop and Armbruster 1999). On contrary, several researchers from India and China (Verma and Dulta 1986, Partap et al. 2000, Yang 2005, Joshi and Joshi 2010) reported that *A. cerana* starts foraging earlier in the morning when *A. mellifera* is still warming up and continue foraging later in the evening, when *A. mellifera* has already ceased its foraging activities. In this study, I also investigate this puzzle by examining each species' relationships with temperature in more detail. Here, I also ask (d) Do *A. cerana* and *A. mellifera* maintain similar brood nest temperatures at the same ambient temperatures? (e) How about the thoracic temperatures of brood nest workers and departing foragers at the same ambient temperatures? (f) Do they commence foraging at similar air ambient temperatures in the colder month?

Materials and methods

Experimental apiary design

Apiaries were set up in four locations in the Kathmandu valley, Nepal, where honey bees forage throughout the year. Apiary A (mixed-species apiary, ap1) is located inside the Coronation Garden, Tribhuvan University, Kiritipur, Nepal (27.68 N latitude, 85.29 E longitude, and 1307 m elevation); apiary B (mixed-species apiary, ap2) is in the grounds of the National Agricultural Research Council (NARC), Khumaltar, Nepal (27.65 N latitude, 85.32 E longitude, and 1323m elevation); apiary C (single-species apiary with *A. cerana* only; ap3) is in the grounds of Knowledge Park, Godavari, Lalitpur, Nepal (27.59 N latitude, 85.39 longitude, and 1603 m elevation); and apiary D (single-species apiary with *A.*

mellifera only; ap4) is in Gokarna, Kathmandu, Nepal (27.76 N latitude, 85.40 E longitude, and 1418 m elevation). Apiaries A and B are 5 Km apart, while Apiaries C and D are 17 Km apart (see Figure 2-1). I sampled four queenright colonies of *A. cerana* and four of *A. mellifera* in apiaries A and B, and four queenright colonies of *A. cerana* or *A. mellifera* in the single species apiaries C and D, respectively. *A. mellifera* colonies were housed in standard Langstroth hives and *A. cerana* colonies were housed in standard Newton B type hives. All sampled colonies of each species were equalized periodically following the methods suggested by Delaplane et al. (2013).

Foraging activity

Foraging activity was observed in each apiary once every five days throughout the experimental period, from March 2016 to August 2018. This amounted to one apiary sampled per day, with one "extra" day in case of rainy weather. On test days, foraging activity was measured as the number of bees exiting a nest during a one-minute interval, every hour from 6:00 AM to 6:00 PM. The time of foraging initiation and cessation and associated environmental variables were recorded for each test day. To examine seasonal variation in foraging patterns, data were organized by season: spring (March-April), summer (May-June), and rainy (July-August).

Ambient temperature, relative humidity, and light intensity

The fluctuations in ambient air temperature and light intensity during the test period were recorded with the help of the Onset HOBO Data Logger Measuring Temperature and Light Intensity (Part Number: UA-002-08). The Onset HOBO Data Logger was deployed on the ground of experimental apiaries and data were pulled to the computer hourly for the analysis. Relative humidity was recorded manually every hour, using a Kestrel Meter 4000 Weather Meter.

Brood nest and worker bee body temperatures

Brood nest temperatures and thoracic temperatures of brood nest workers and departing foragers were measured from each test colony of both species throughout the month of March on the same experimental days when foraging activity was measured. The BAT-12 Microprobe Digital Thermometer

with a resolution of ±1°C was used to measure the brood nest and thoracic temperatures. It was associated with K-type thermocouple copper wire, 0.28 mm in diameter and provided very quick temperature readings. Thoracic temperature readings on worker bees were taken following the methods of Heinrich (1979). Ten brood nest workers directly from the brood nest and ten departing foragers from the sugar feeders in front of hive were captured. Worker bees were grasped with the help of rubber-covered forceps and the copper wire probe was thrust into the thorax about 3 mm deep until the highest stable temperature was observed. To obtain brood nest temperatures, the copper wire probe was thrust 3 mm deep to the center of the brood nest until the highest stable temperature was obtained. Observations were started as early as 5:00 AM to record initiation of foraging by each test hive. Ambient air temperature was recorded when the first forager exited from the entrance of a hive. These temperatures were recorded when ambient air temperature reached 9 °C in the morning, and when ambient air temperature reached 25 ° in the afternoon.

Statistical design

To study the foraging behavior of *A. cerana* and *A. mellifera*, the number of departing foragers from a colony was counted at different time points. The data was realized in the form of a nested, unbalanced "2 Species x 2 Setting x 13 Time" design. Factor levels were as follows:

Species=ac vs. am

Setting=Mixed vs. Single

Time=6 AM vs. ... vs. 6 PM

The 2-way design "Species x Setting" was replicated by Colony nested inside Location, where Location=ap1 vs. ap2 vs. ap3 vs. ap4. "ac x Single" was housed in ap3 and "am x Single" was housed in ap4 whereas both "ac x Mixed" and "am x Mixed" were housed in ap1 and ap2. Replications were achieved in the form of 4 Colony units in ap3 and ap4, and 8 Colony units (4 per species) in ap1 and ap2.

The Time factor was replicated by Date nested inside Month which, in turn, was nested inside Season.

Month=Mar vs. Apr vs. May vs. Jun vs. Jul vs. Aug

Season = Spring vs. Summer vs. Rainy

10 Spring + 11 Summer + 8 Rainy = 29 Dates were used in ap1 whereas 10 Spring + 11 Summer + 9 Rainy = 30 Dates were used in ap2, ap3, and ap4. An experimental unit, represented by a Date-Colony combination, was then labeled as Entity. At each Time factor, the number of foragers per minute per 10000 populations, labeled as "Per10KForagers" was measured for each "Entity" identifier. Measurements of three additional independent variables, namely, "Light Intensity", "Relative Humidity", and "Temperature", were observed at each Time level on each Date.

Statistical analysis

Welch two sample *t*-test (independent *t* tests) were used to test for differences between the species in minimum temperature of foraging initiation, brood nest temperatures, thoracic temperatures of departing workers, and brood nest workers.

A robust repeated-measures ANOVA on 20% trimmed means was conducted using the package "WRS2" in R (Mair and Wilcox 2018) to test the following effects: a) effect of species in overall foraging, b) the effects of time and the interaction of species and time in overall foraging, c) the effects of time and the interaction of species and time in *A. cerana* foraging only, and d) the effects of time and the interaction of species and time in *A. mellifera* foraging only. For *A. mellifera,* there were zero foragers at many instances of 6 AM and 6 PM. So, only the data from 7 AM to 5 PM were analyzed in order to avoid zero standard deviations.

For each species, a robust multiple linear regression was fit using time of the day, ambient air temperature, light intensity, and relative humidity as predictors using the package "MASS" in R (Venables and Ripley 2002). Since time was a categorical predictor with each level taking a binary value of either 1 (yes) or 0 (no), its first level, namely, "6 AM" became the baseline intercept while the

remaining levels were coded as difference from "6 AM" contrasts. For instance, the coefficient for "7 AM" was the sum of the intercept (coefficient for "6 AM") and the coefficient for "7 AM − 6 PM" and so on. The package "sfsmisc" was used to conduct Wald tests for the coefficients of the regression (Maechler 2018).

Prior to analysis, homogeneity of variances and normality of the data were examined using Levene's and Shapiro-Wilk's tests. Normality of data and homogeneity of variance were preserved by using a robust test.

Results

Foraging commencement, foraging cessation, and foraging duration

A. cerana commenced foraging earlier in the morning throughout all seasons than *A. mellifera* (Table 2-1). On contrary, cessation of foraging activities throughout the all seasons was earlier in the evening for *A. mellifera* than *A. cerana* (Table 2-1). It indicates that *A. cerana* had overall long foraging duration than *A. mellifera* at all seasons (Table 2-1). The change in foraging commencement, foraging cessation, and foraging duration throughout the seasons follow the mean measures of sun rise, sun set, and day length (see Table 2-1 and Figures 2-2, 2-3, and 2-4). On average, *A. cerana* had a lower minimum foraging commencement temperature (M=7.5146666667, SE=0.1169078391) than *A. mellifera* (M=8.826667, SE=0.1776925). This difference was highly significant (t (102.02) =-6.16826, p<0.001, r=0.5211922).

Foraging activity

The number of foragers was significantly non-normal for both *A. cerana*, W=0.93, p<0.001, and *A. mellifera*, W=0.94, p<0.001. The histograms and Q-Q plots supported the violation of normality. For the number of foragers, the variances were similar for *A. cerana* and *A. mellifera*, F (1, 9254) = 4.056, p=0.17, *ns*, but were significantly different for the mixed and single settings, F (1, 9254) =188.45,

p<0.001. Thus, a robust repeated-measure ANOVA on 20% trimmed means was conducted using the package "WRS2" in R to incorporate heterogeneous and non-normal number of foragers.

The effect of species was significant, F (1, 258.7403) = 11031.98, *p*<0.001. The effects of time and the interaction of species and time were also significant at *p*<0.001. For *A. cerana*, the effect of time was significant, F (12, 198.03) = 1524.75, *p*<0.001. However, the effects of setting and the interaction of setting and time were non-significant. For *A. mellifera,* the effect of time was significant, F (10, 199.99) = 5139.78, *p*<0.001. The effect of the interaction of setting and time was also seen slightly significant, F (10, 199.99) = 2.02, *p*=0.03. This significance, however, was only due to the fact that the effect of season was highly significant for either species at *p*<0.001. When the analyses were broken down by season, non-significance effects of setting and the interaction of setting and time were evident as shown in Tables 2-2 to 2-4. In overall, these results explain that *A. cerana* and *A. mellifera* follow significantly different patterns of foraging regardless of mixed-species and single-species settings. In other words, mixed-species settings didn't have significant effect in foraging behavior of either species (Figures 2-8 to 2-13).

A. mellifera foraging peaked only once at local noon, at which time the average ambient temperature was 26 °C with greater light intensity, and lower relative humidity. In contrast, *A. cerana* foraging peaked twice, earlier in the morning and later in the afternoon, at which times the average ambient temperature was 19 °C and 24 °C respectively with moderate light intensities and lower relative humidity (Figures 2-14 to 2-22).

The response variable for our data is the number of foragers for whom we have time of the day, temperature, light intensity, and relative humidity as possible predictors. Ignoring time of the day, a categorical variable for now, the results of the regression of the number of foragers on each other quantitative categorical variable is presented in Figure 2-23 for *A. cerana* and in Figure 2-24 for *A. mellifera.* These figures also report the correlation of each predictor variable and the number of foragers (the last column) as well as the histogram and density of each variable (the diagonal). The lack of

normality indicated by the histogram and density clearly direct us to use a robust method of regression analysis.

The results of a robust multiple linear regression fit for the two species with time of the day, temperature, light intensity, and relative humidity as predictors of the number of foragers are presented in Tables 2-5 and 2-6. This analysis shows that foraging activity of either species is best predicted by time of the day as evidenced by the fact that the constant or the intercept representing the categorical variable is highly significant with p-value < 0.001. We also note that some of the time levels are not significantly different from one another for *A. cerana*, namely, 6 AM, 10 AM, 11 AM, 4 PM, and 6 PM. As for the other quantitative environmental variables, relative humidity is highly significant for either species with p-value<0.001, light intensity is not significant with p-value=0.61 for *A. cerana* and p-value = 0.59 for *A. mellifera*. Temperature is fairly significant for either species with p-value=0.007 for *A. cerana* and p-value = 0.00975 for *A. mellifera*.

Upon removing time of the day as a predictor, we note that the other environmental variables, namely, light intensity, relative humidity, and temperature, become significant for either species thereby suggesting the fact that time of the day is the most dominating predictor for the number of foragers and masks the effect of all the other variables when present in the regression model (see Tables 2-7 and 2-8). Do note, however, that relative humidity is fairly significant (p-value = 0.01) for *A. cerana* and temperature is barely significant (p-value = 0.03) for *A. mellifera*.

Brood nest and workers bee body temperatures

On average, *A. cerana* had a lower brood nest temperature at both ambient air temperatures (M=32.98750000, SE=0.05618051 at 9 °C; M=33.19375, SE=0.07877645 at 25 °C) than *A. mellifera* (M=33.95625000, SE=0.03531849 at 9 °C; M=34.131250000, SE=0.068141244 at 25 °C). This difference was highly significant at both ambient air temperatures [t (25.255) =-14.598, p<0.001at 9 °C,

t (29.39) =-9.0007, *p*<0.001 at 25 °C]. It indicates that at the same ambient air temperature, *A. cerana* colonies maintained a significantly lower brood nest temperature than *A. mellifera* colonies (Figure 2-5).

There were same result in case of thoracic temperatures of departing foragers [*A. cerana*: M=23.79047619, SE=0.06502252; *A. mellifera*: M=26.21475, SE=0.0641253; t (103.53) = -31.622, *p*<0.001 at 9 °C and *A. cerana*: M=31.57460, SE=0.0398389; *A. mellifera*: M=32.71579, SE=0.04057771 ; t (117.44) =-20.068, *p*<0.001 at 25 °C] and brood nest workers [*A. cerana*: M=15.55882, SE=0.133686; *A. mellifera*: M=20.20000, SE=0.0645987480; t (95.901) = -31.259, *p*<0.001 at 9 °C and *A. cerana*: M=29.13636, SE= 0.05553874; *A. mellifera*: M=29.76429, SE=0.056143865; t (118.94) = -7.9511, *p*<0.001 at 25 °C] between *A. cerana* and *A . mellifera* foragers at the same ambient temperatures (Figures 2-6 & 2-7).

Discussion

Indigenous *A. cerana* of Nepal started foraging earlier in the morning and keep on foraging late in the evening even in the civil twilights. On contrary, exotic *A. mellifera* started foraging late in the morning at about or after the time of sun rise and ceased the foraging activities earlier in the evening with or before the sunset. It suggests that *A. cerana* can forage at lower ambient temperatures and might outcompete *A. mellifera* in cold morning or during cooler climate. This finding is consistent with the finding of Tan et al. (2012) in China and Joshi and Joshi (2010) in India. It indicates that if the *A. cerana* population drastically declined and driven to local extinction, some early-flowering plants will have effect on pollination services. Thus, the decline in *A. cerana* population will have serious effects on pollinating indigenous flora that is adopted to *A. cerana* pollination (Yang 2005).

Findings of this research show that foraging activities of the either species was best predicted by the time of the day. However, environmental variables such as light intensity, ambient air temperature, and relative humidity associated with the time of the day must have inter-played the role in foraging activities. For instances, foraging commencement of *A. mellifera* in cooler morning was temperature dependent while the cessation activities of both species at the evening was mainly controlled by decline in

the values of light intensity irrespective of other environmental factors. Foraging behavior of *A. mellifera* was positively correlated with light intensity and temperature, while *A. cerana* peaked its foraging at moderate temperatures and light intensity and declined its foraging at maximum temperature and light intensity. Foraging of both species was negatively correlated with relative humidity. This finding is consistent with the results of Abrol (2006) on path analysis of environmental factors influencing foraging behavior of four honey bees species.

Dyer and Seeley (1987) found that all species of honey bees maintain a thoracic temperature 5-6 °C above the ambient air temperature while foraging at cooler temperatures. Departing foragers of *A. mellifera* foragers actively vibrate their thoracic muscles prior to the commencement of foraging to achieve greater thoracic temperature than the brood nest workers (Heinrich 1980b). Thoracic temperature of brood nest workers was significantly lower than thoracic temperature of departing foragers in both *A. cerana* and *A. mellifera* at 6 °C ambient air temperature; however, which was not significantly lower at 20 °C ambient air temperature in China (Tan et al. 2012). In this study, I found that thoracic temperature of departing foragers was significantly higher than brood nest workers in both species of honey bees both at 9 °C and 25 °C air ambient temperatures. Also, both departing foragers and brood nest workers of *A. cerana* had significantly lower thoracic temperatures than *A. mellifera*. This study supports the view of Tan et al. (2012) that *A. mellifera* maintains a higher brood nest temperature than *A. cerana*. Thus, *A. mellifera* might have probable tradeoff between energy expenditure for stable brood nest and an elevated thoracic temperature for departing forager's temperature (Jones et al. 2005, Jones and Oldroyd 2006), however this allows them to carry heavier resource load back to the hive (Harrison and Fewell 2002). *A. cerana* on the other hand evolved with tolerable lower and variable brood nest temperature (Ruttner 1988a). Longer foraging hours observed in *A. cerana* should justify the tradeoff between foraging efficiency for metabolic efficiency.

Figures and figure legends

Figure 2-1. Experimental apiary design. Apiary A and apiary B are mixed-species apiaries located at university garden in Kiritipur and on the ground of National Agricultural Research Council (NARC), Khumaltar respectively. Apiary C and apiary D are single-species apiaries located at Godawari and Gokarna respectively.
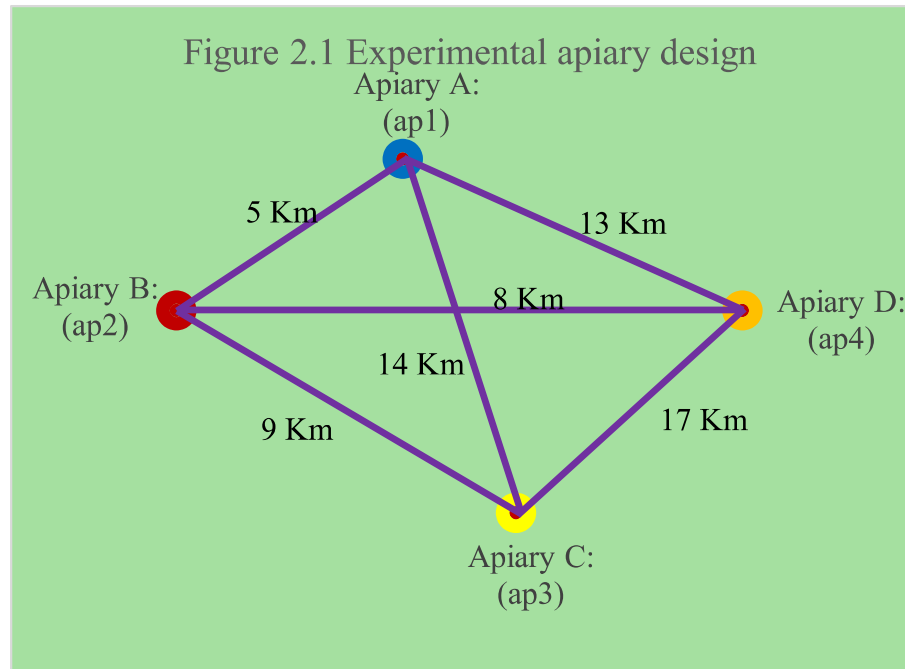

Figure 2-1

Figure 2-2. Foraging initiation time (FIT), foraging cessation time (FCT), and total foraging duration of *A. cerana* and *A. mellifera* in spring 2016.

Figure 2-2

Figure 2-3. Foraging initiation time (FIT), foraging cessation time (FCT), and total foraging duration of *A. cerana* and *A. mellifera* in summer 2016.

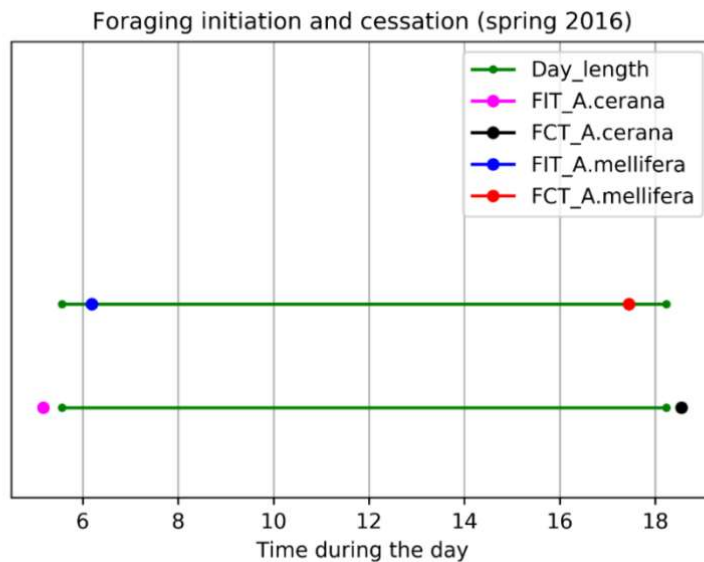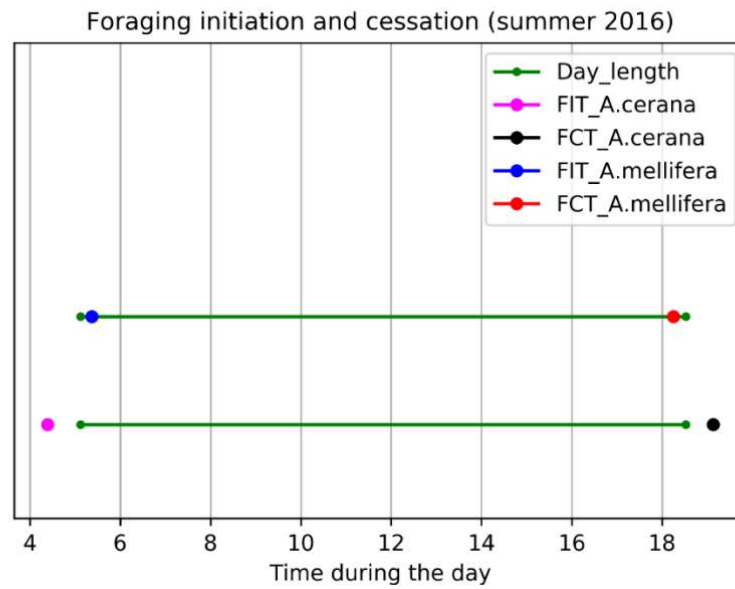
Figure 2-3

Figure 2-4. Foraging initiation time (FIT), foraging cessation time (FCT), and total foraging duration of *A. cerana* and *A. mellifera* in summer 2016.

Figure 2-4

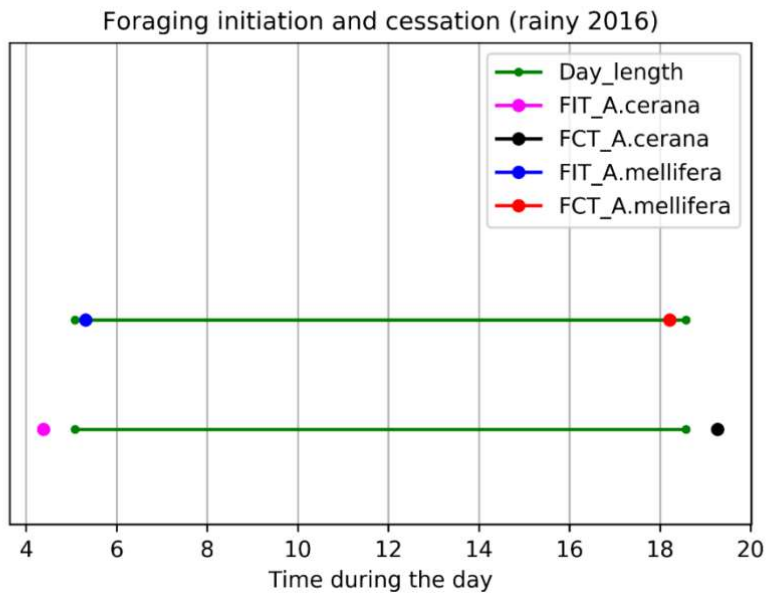**Figure 2-5.** Brood nest temperatures (BNT) of *A. cerana* and *A. mellifera* at 9 °C and 25 °C ambient air temperatures.
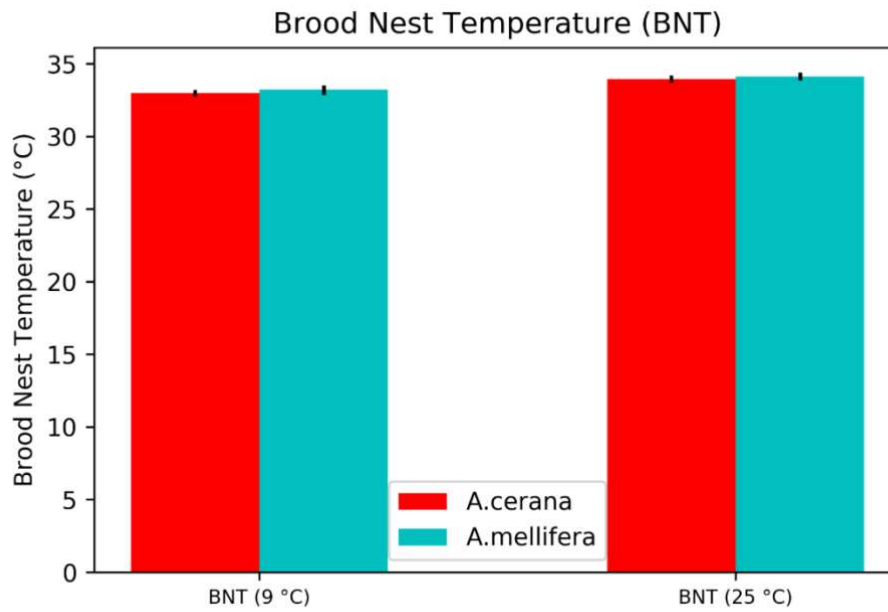
Figure 2-5

Figure 2-6. Thoracic temperatures of departing foragers (DF) of *A. cerana* and *A. mellifera* at 9 °C and 25 °C ambient air temperatures.
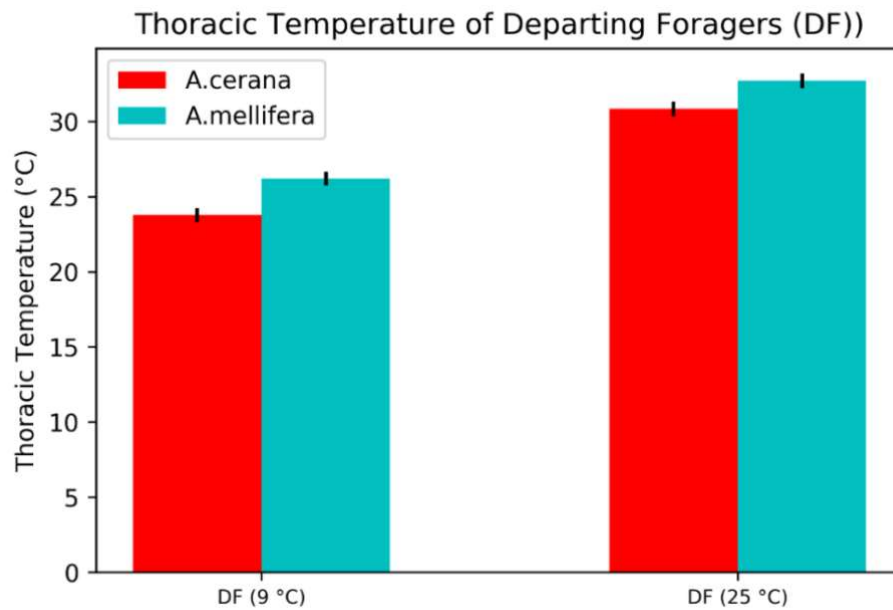
Figure 2-6

Figure 2-7. Thoracic temperatures of brood nest workers (BNW) of *A. cerana* and *A. mellifera* at 9 °C and 25 °C ambient air temperatures.

Figure 2-7

Figure 2-8. Box plot of numbers of foragers of *A. cerana* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August 2016). Numbers of foragers exiting per minute was counted in each experimental day on hourly basis from 6:00 AM to 6:00 PM.

Figure 2-8

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Figure 2-9. Box plot of numbers of foragers of *A. mellifera* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August 2016). Numbers of foragers exiting per minute was counted in each experimental day on hourly basis from 6:00 AM to 6:00 PM.

Figure 2-9

Figure 2-10. Box plot of numbers of foragers of *A. cerana* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August 2016) broken down into seasons (Spring: March and April, Summer: May and June, and Rainy: July and August). Numbers of foragers exiting per minute was counted in each experimental day on hourly basis from 6:00 AM to 6:00 PM.
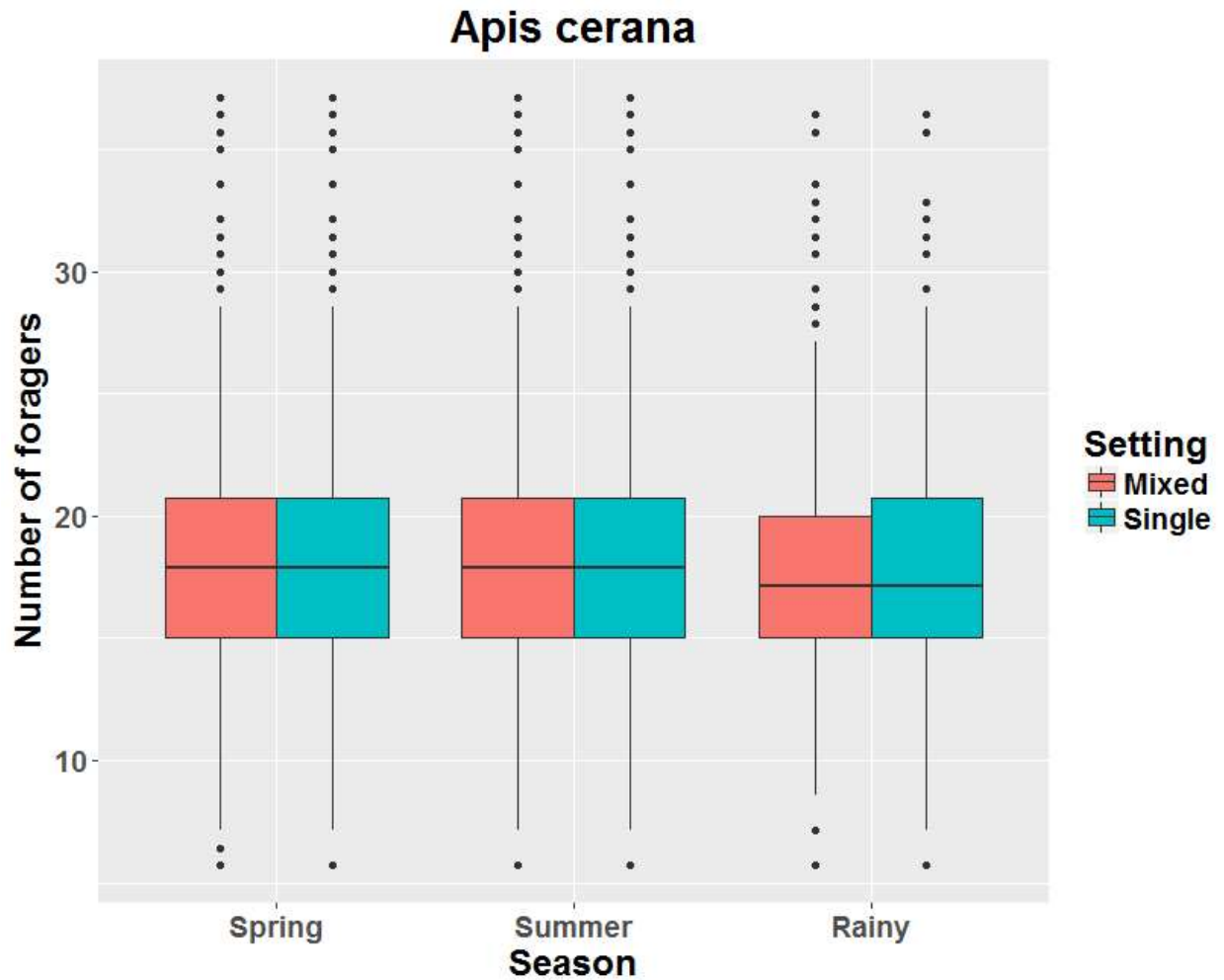
Figure 2-10

Figure 2-11. Box plot of numbers of foragers of *A. mellifera* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August 2016) broken down into seasons (Spring: March and April, Summer: May and June, and Rainy: July and August). Numbers of foragers exiting per minute was counted in each experimental day on hourly basis from 6:00 AM to 6:00 PM.
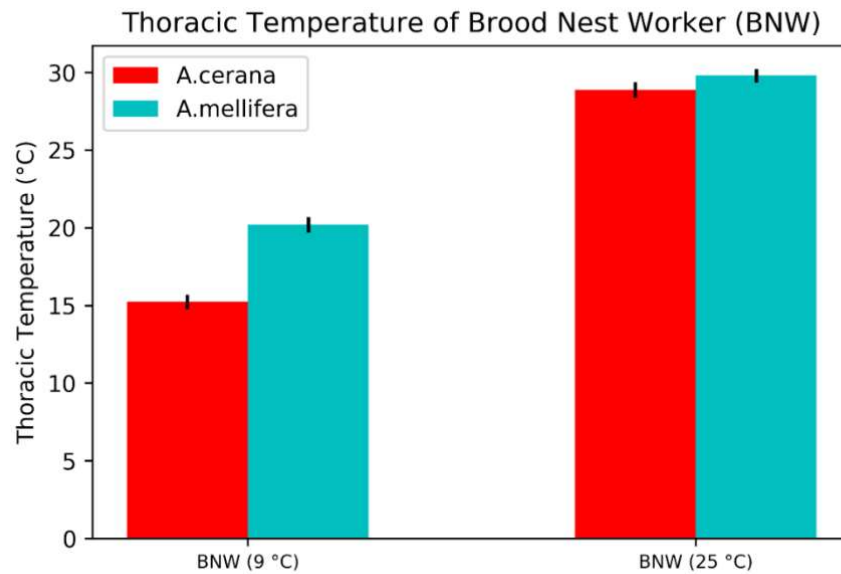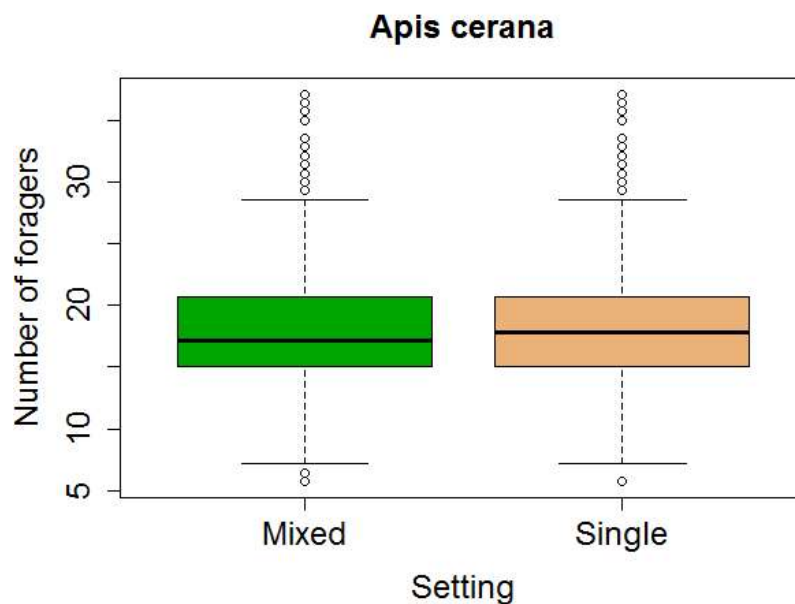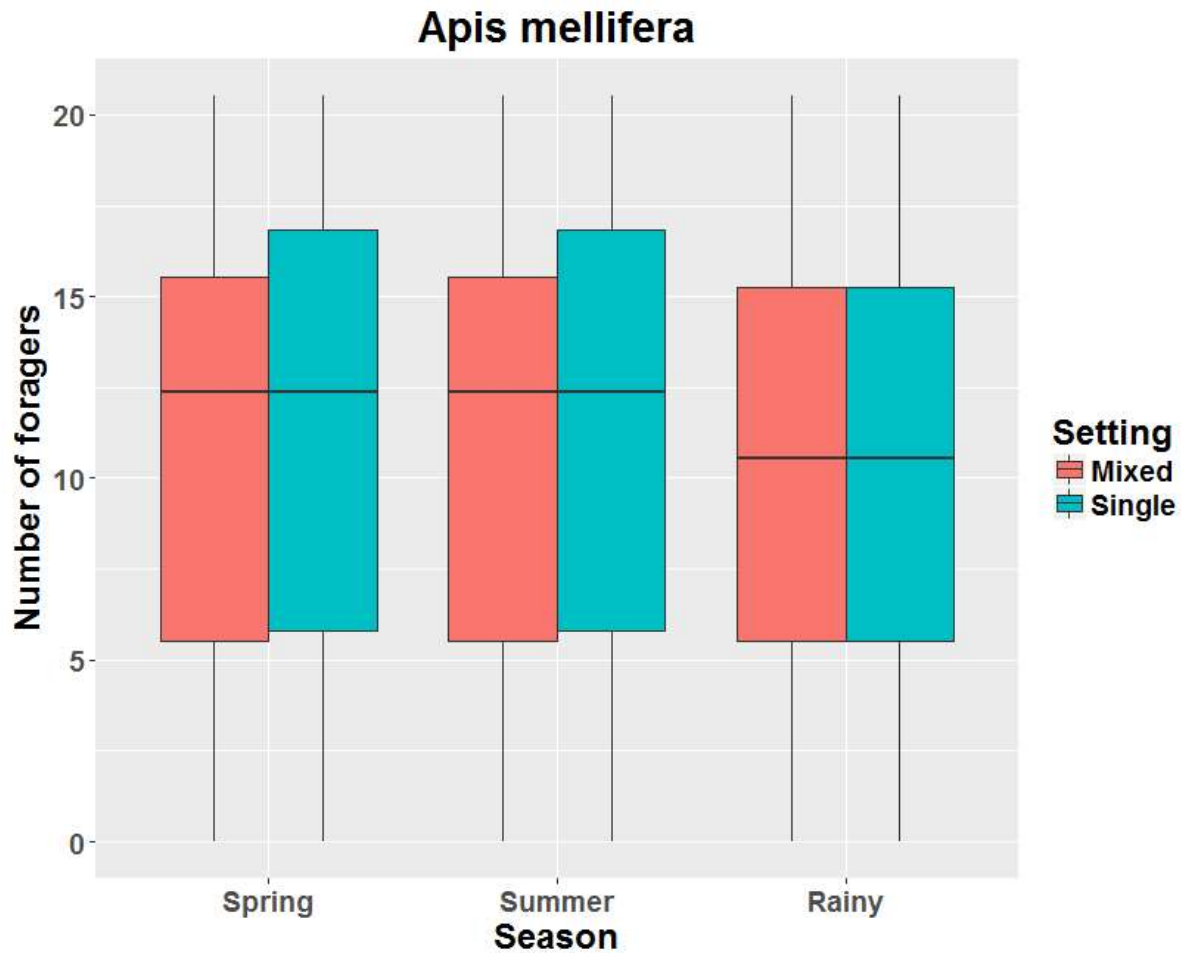

Figure 2-11

Figure 2-12. Box plot of numbers of foragers of *A. cerana* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August, 2016) broken down into seasons (Spring: March and April, Summer: May and June, and Rainy: July and August) and times of the day (6:00 AM to 6:00 PM).
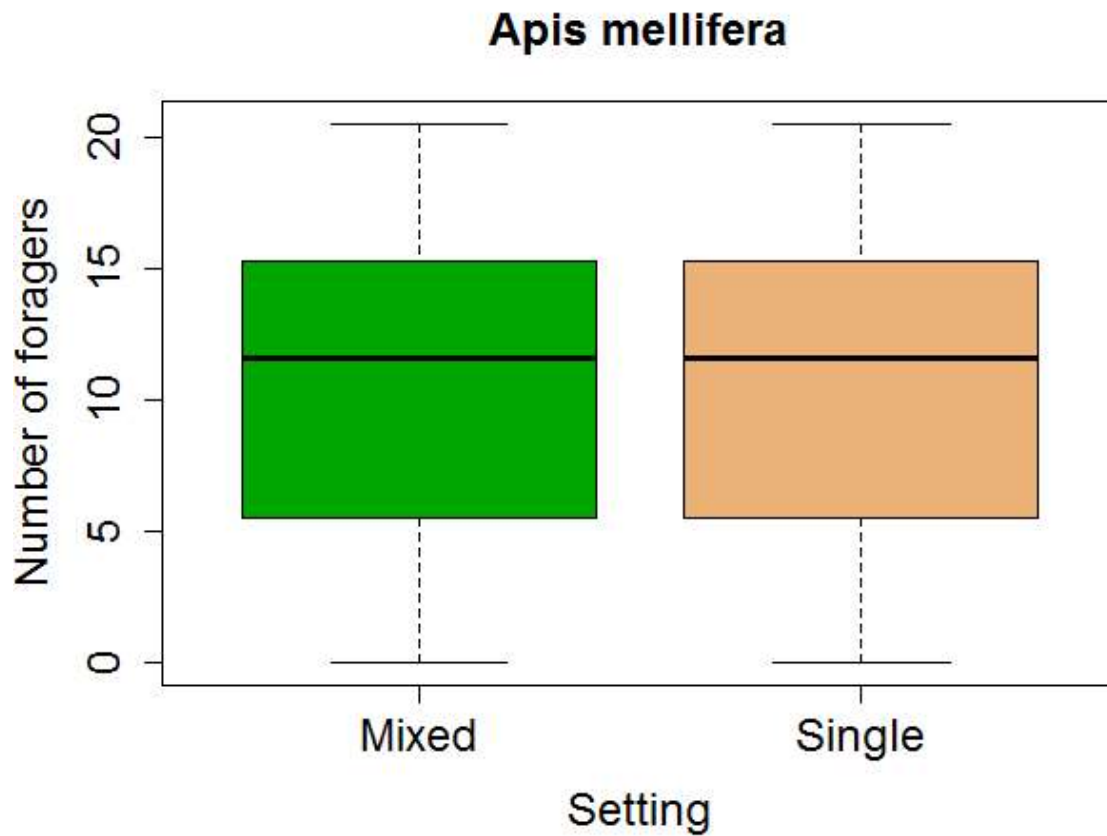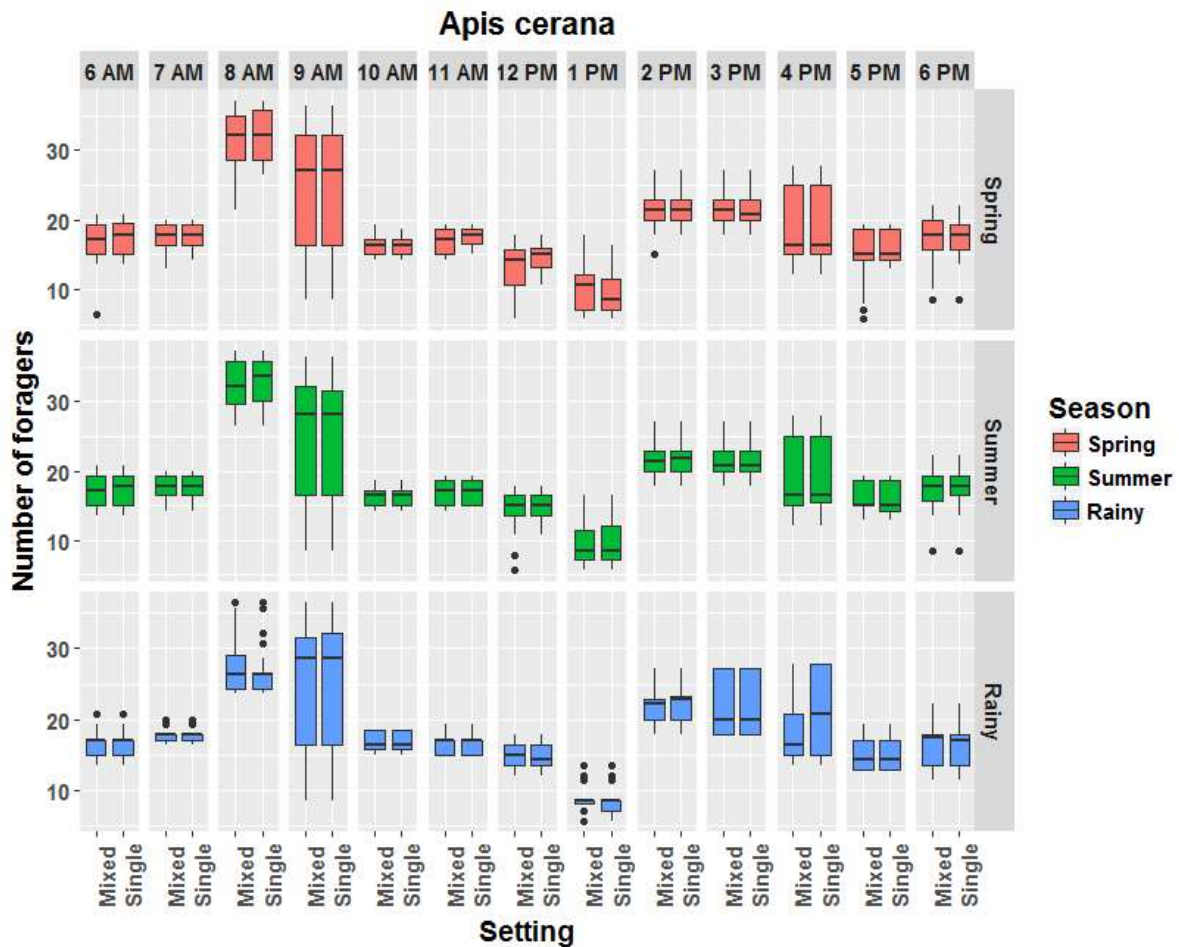

Figure 2-12

Figure 2-13. Box plot of numbers of foragers of *A. mellifera* exiting per minute (represented by per 10 K individuals of a colony) in mixed-species and single-species apiaries throughout the experimental period (March – August, 2016) broken down into seasons (Spring: March and April, Summer: May and June, and Rainy: July and August and times of the day (6:00 AM to 6:00 PM).
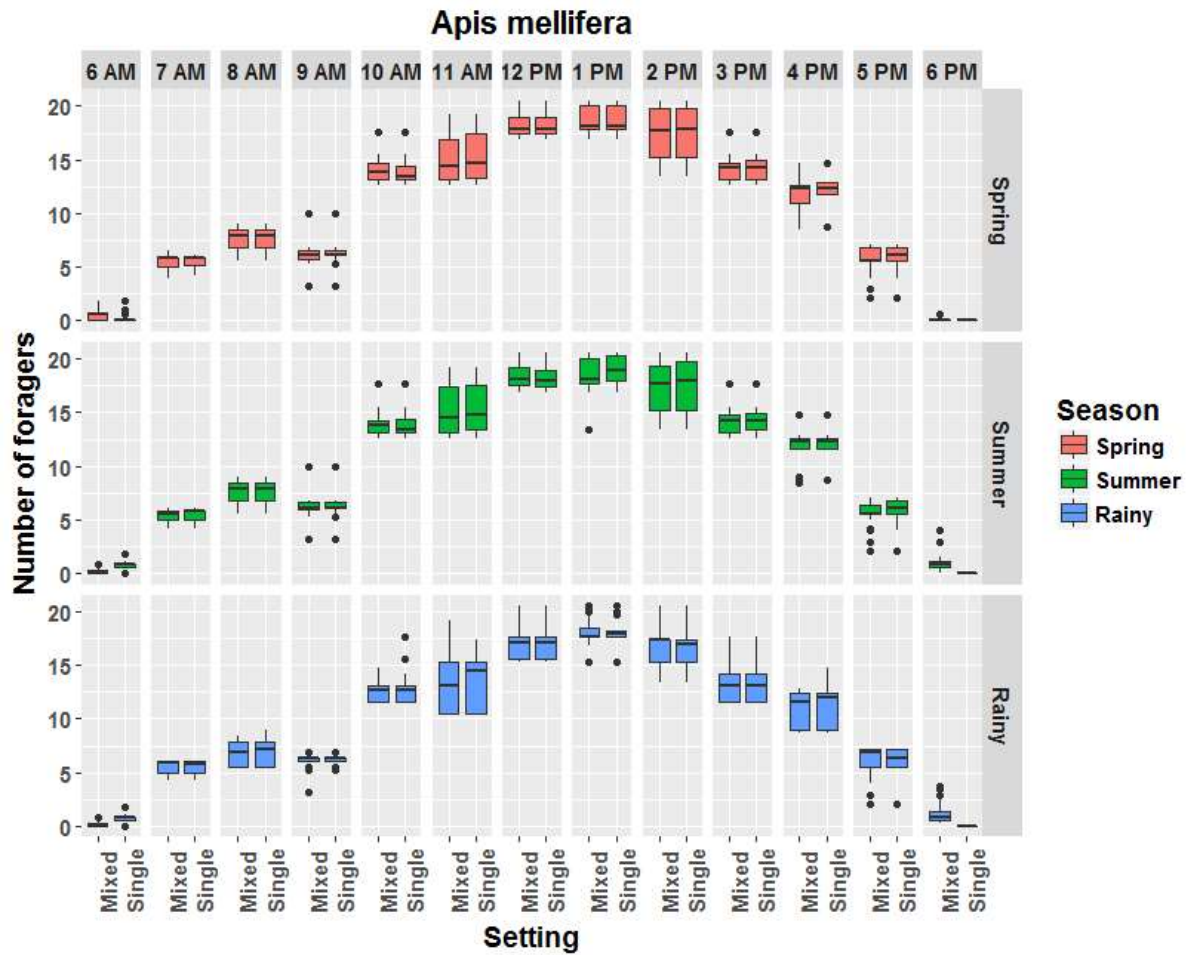
Figure 2-13

Figure 2-14. Foraging trend of *A. cerana* and *A. mellifera* in relation with temperature in mixed-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
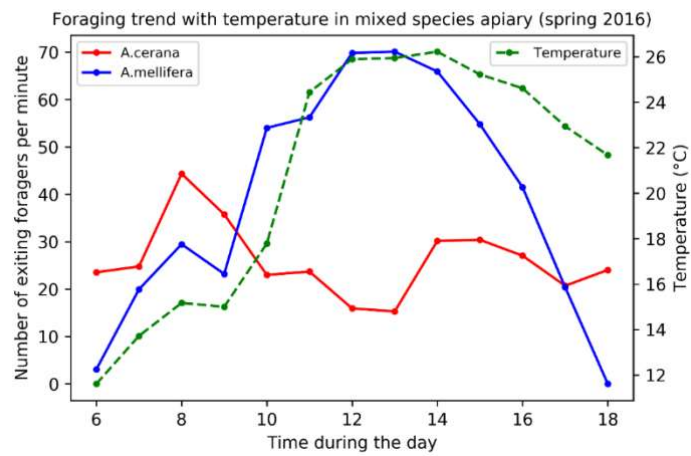
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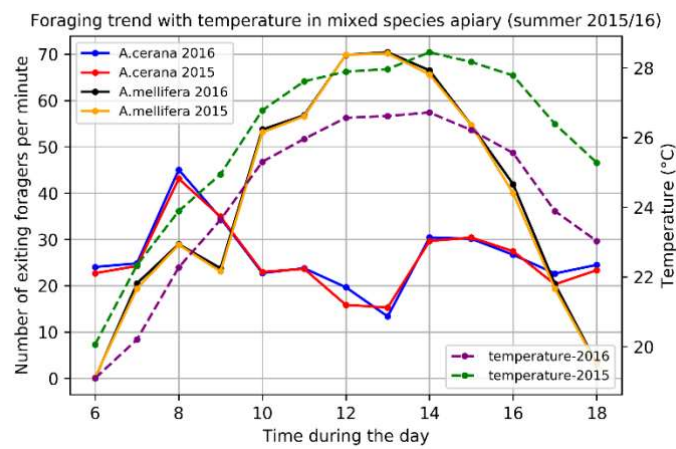
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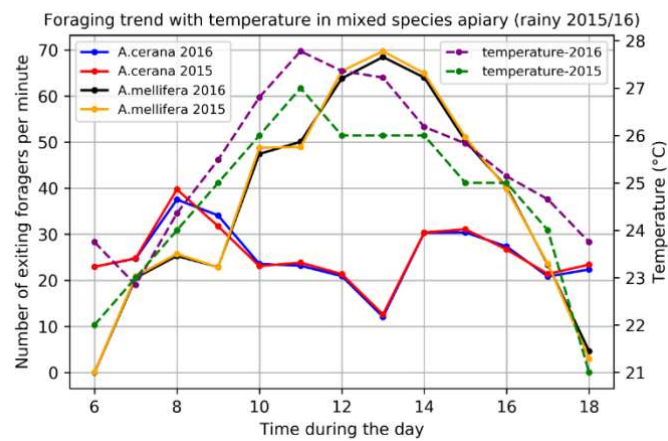
Figure 2-14 c

Figure 2-15. Foraging trend of *A. cerana* and *A. mellifera* in relation with light intensity in mixed-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
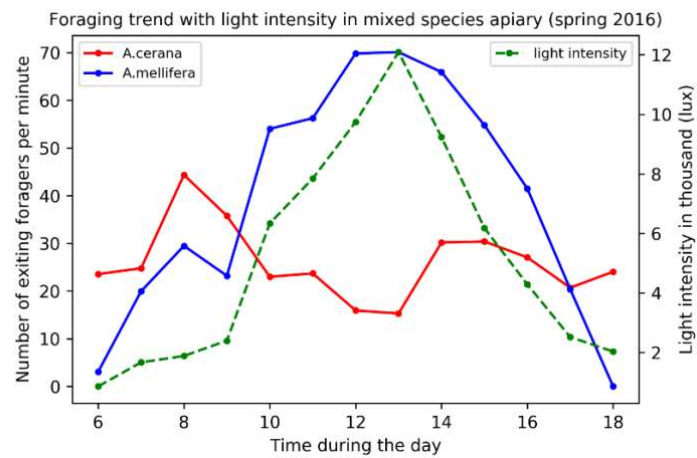
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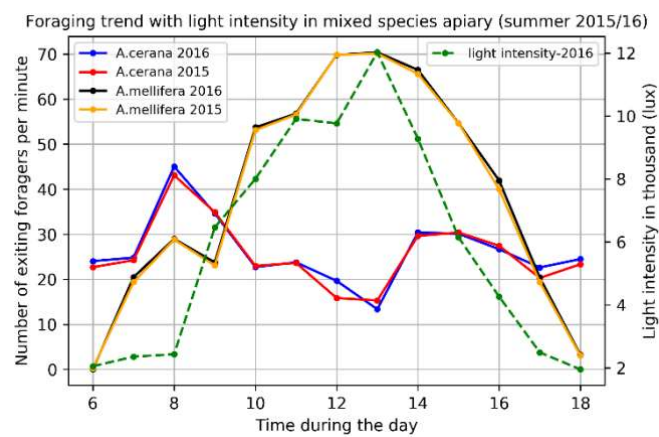
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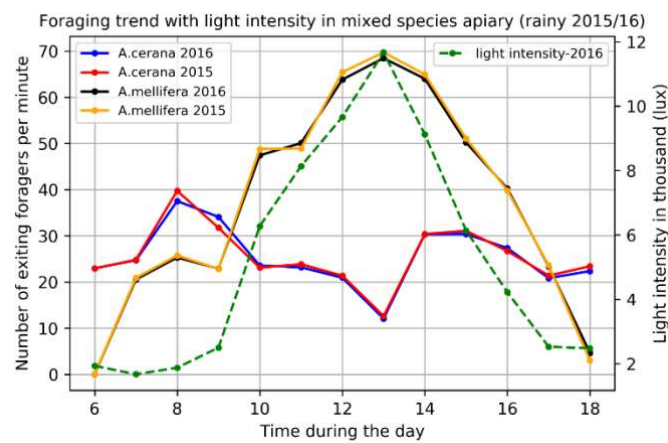
Figure 2-15 c

Figure 2-16. Foraging trend of *A. cerana* and *A. mellifera* in relation with relative humidity in mixed-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
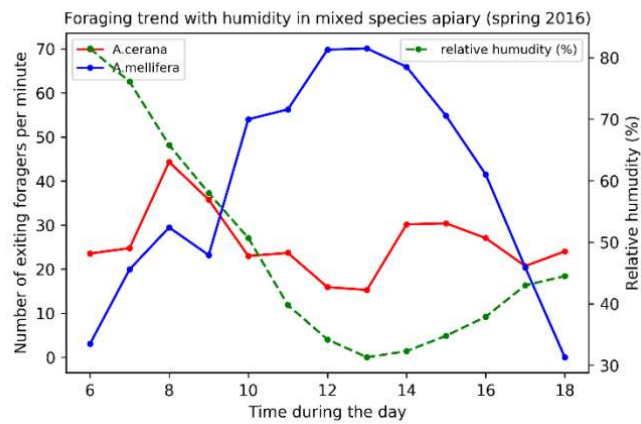
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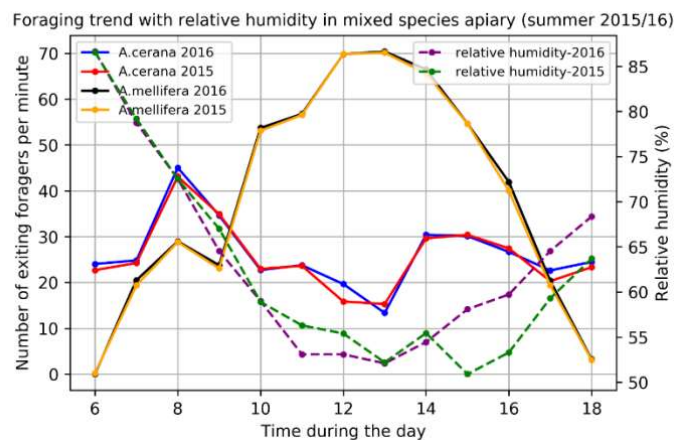
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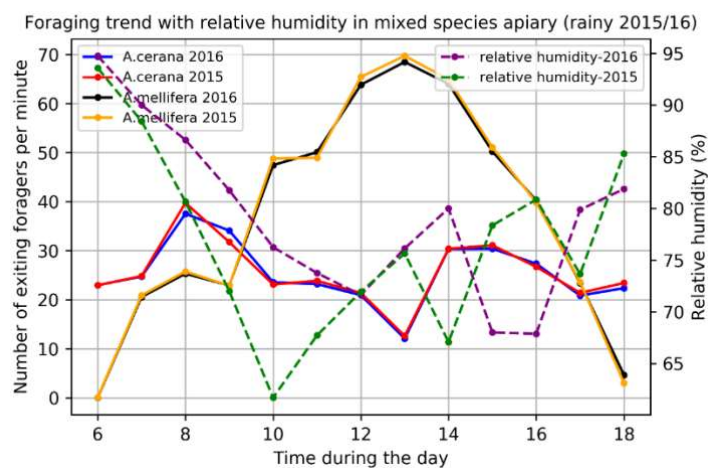
Figure 2-16 c

Figure 2-17. Foraging trend of *A. cerana* in relation with temperature in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
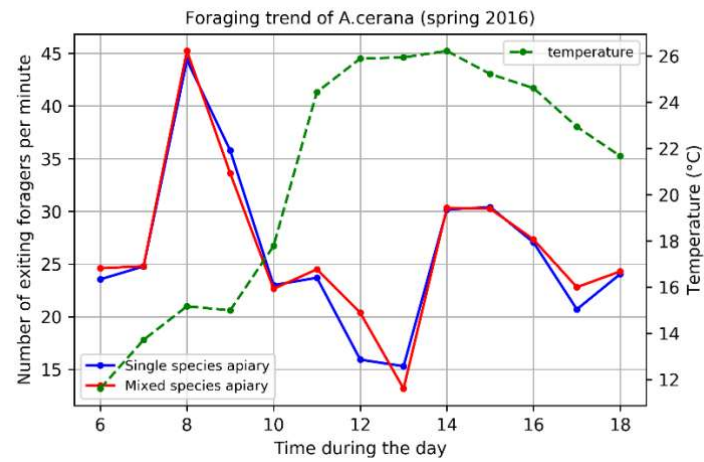
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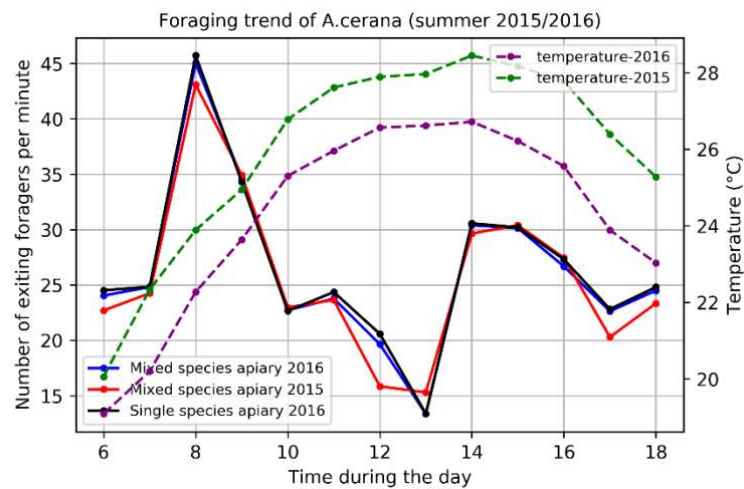
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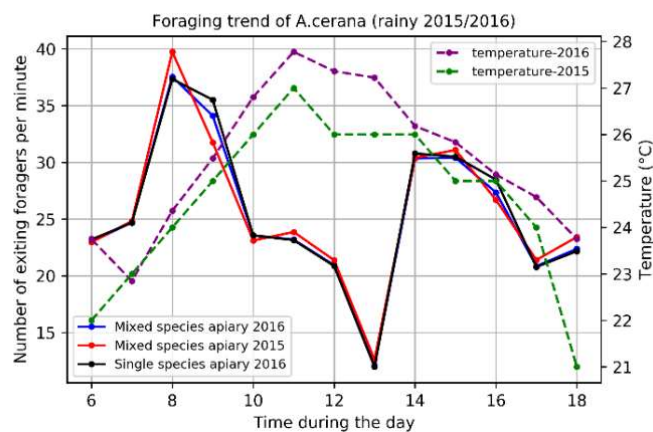
Figure 2-17 c

Figure 2-18. Foraging trend of *A. mellifera* in relation with temperature in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
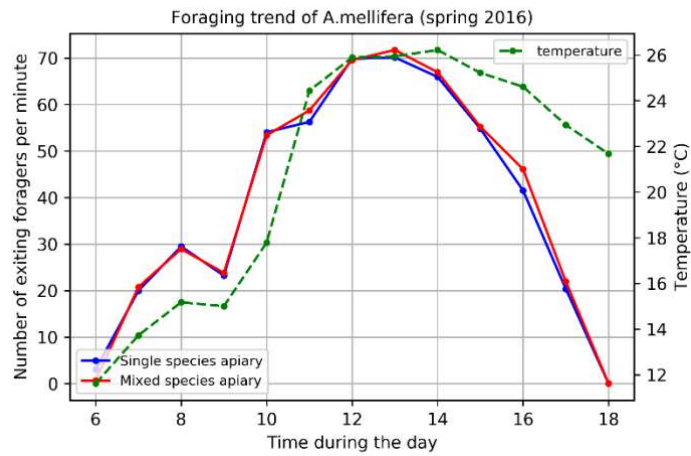
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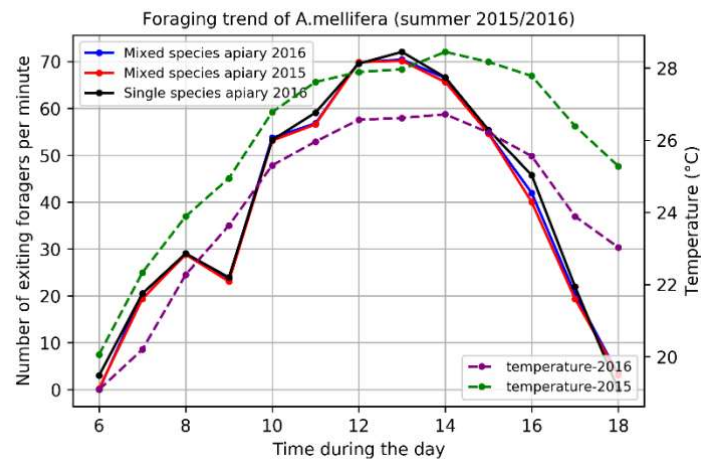
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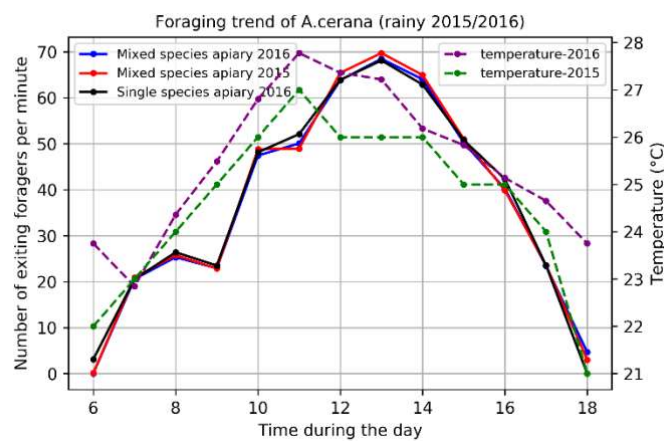
Figure 2-18 c

Figure 2-19. Foraging trend of *A. cerana* in relation with light intensity in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
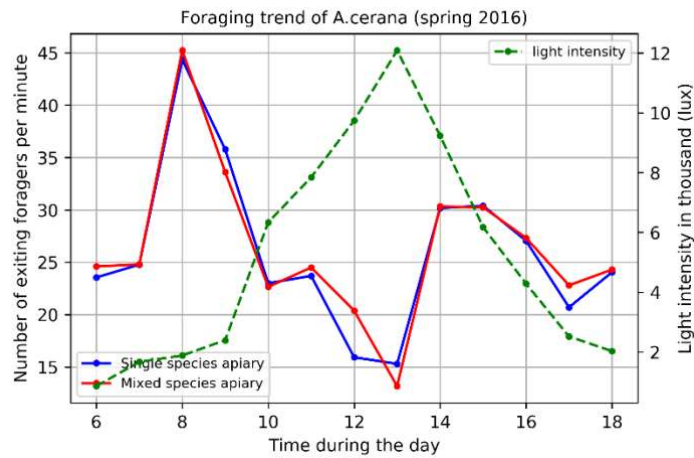
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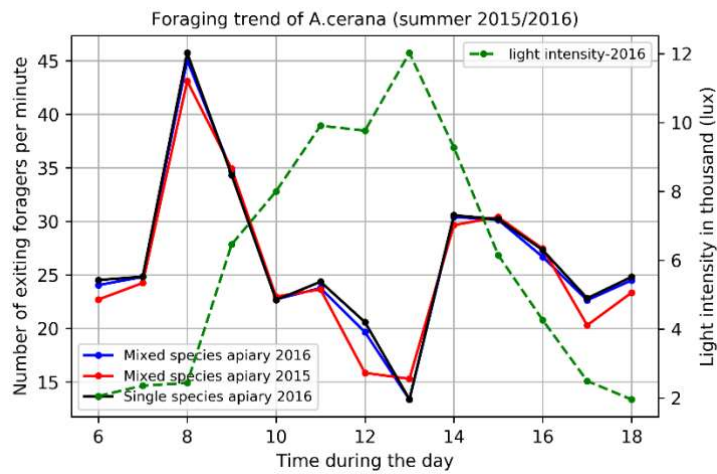
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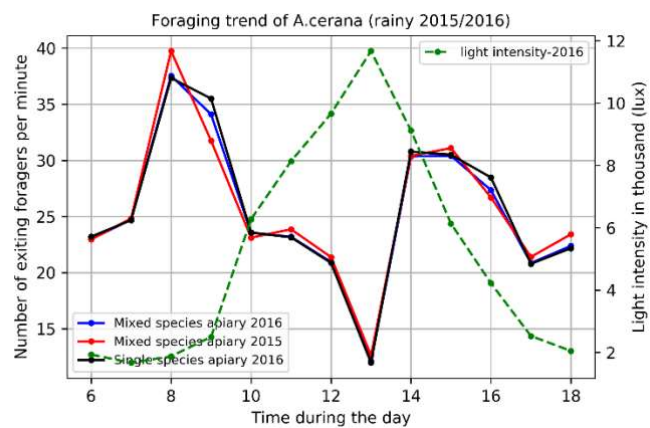
Figure 2-19 c

Figure 2-20. Foraging trend of *A. mellifera* in relation with light intensity in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
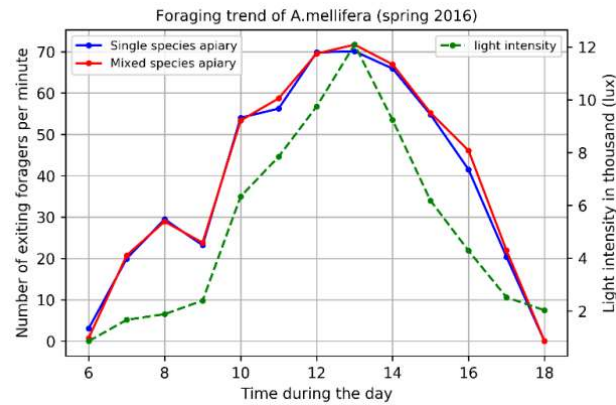
Figure 2-20 a
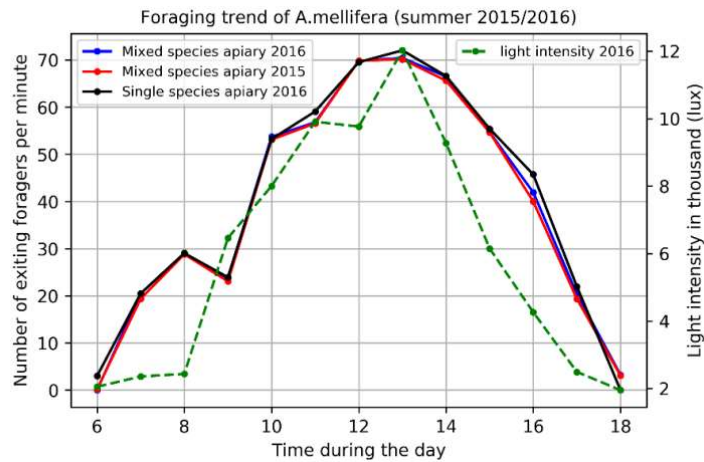
Figure 2-20 b
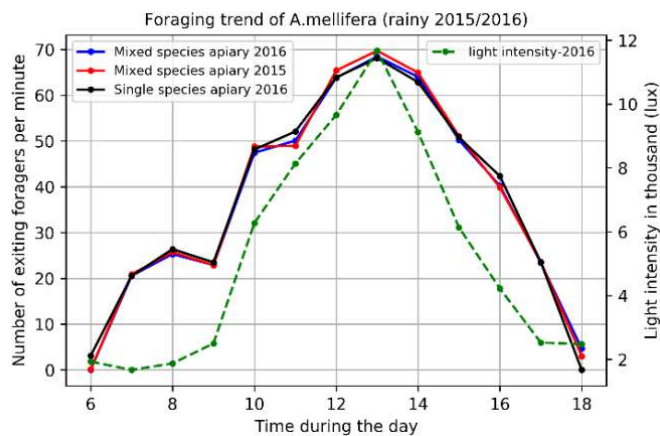
Figure 2-20 c

Figure 2-21. Foraging trend of *A. cerana* in relation with relative humidity in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
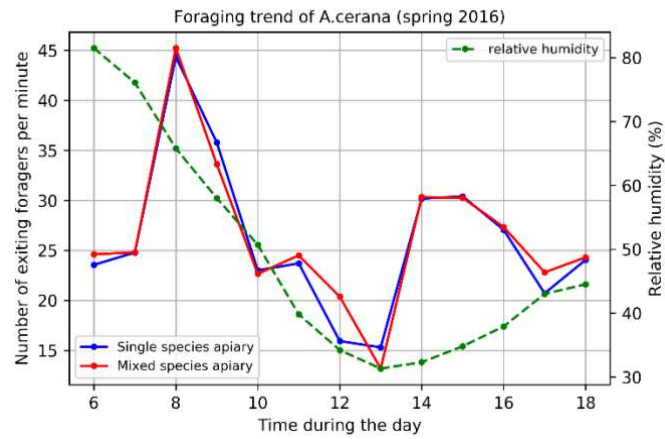
Figure 2-21 a
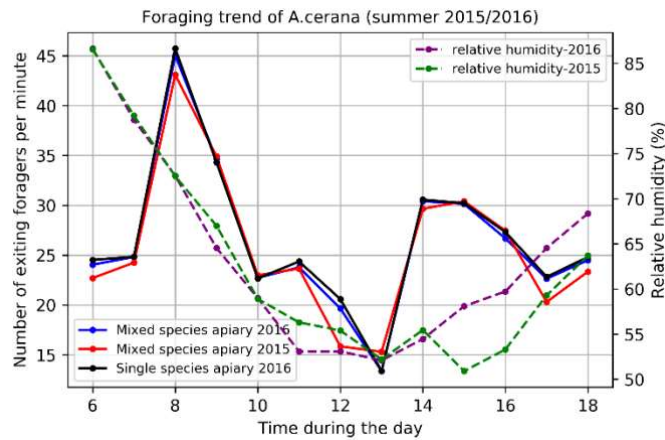
Figure 2-21 b
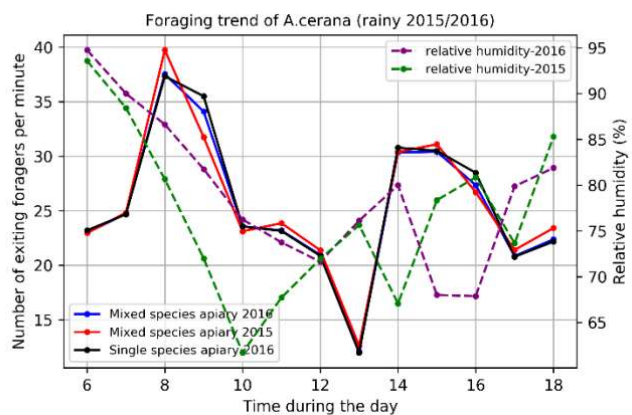
Figure 2-21 c

Figure 2-22. Foraging trend of *A. mellifera* in relation with relative humidity in mixed-species apiary and single-species apiary a) spring (2016), b) summer (2015 and 2016) and c) rainy (2015 and 2016).
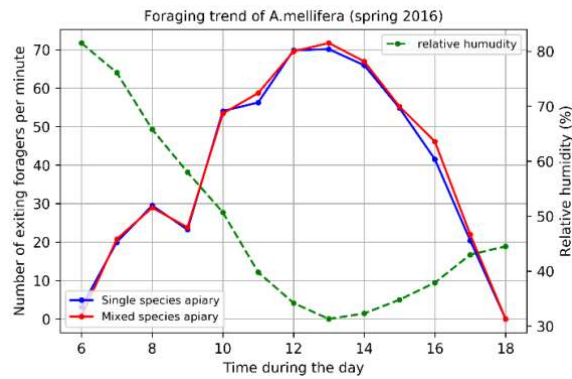
Figure 2-22 a
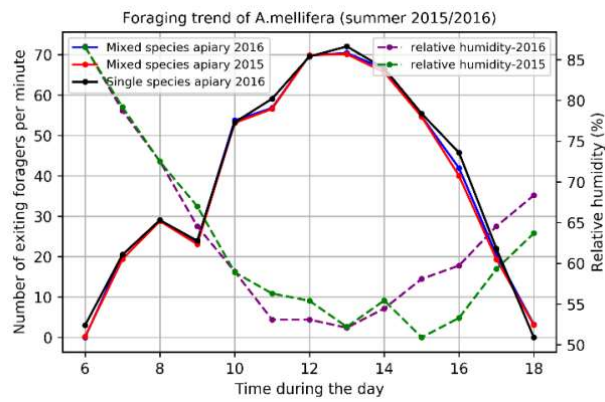
Figure 2-22 b
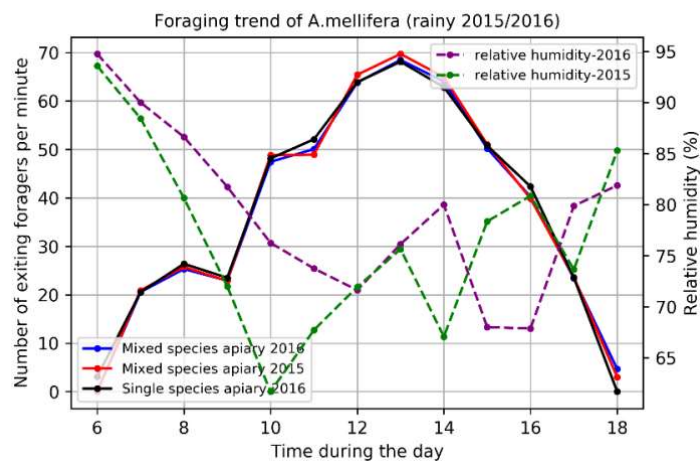
*Figure 2-22 c*

Figure 2-23. Pairwise correlation and regression of the number of foragers with each environmental variable (temperature, light intensity, and relative humidity) for *Apis cerana*. The last column of the plot represents a correlation of each variable and number of foragers, the last row represents regression of each variable and number of foragers, and the diagonal represents histogram and density of each variable. The center of the correlation ellipse is located at the sample means, that is, at the point (x-bar, y-bar). The ellipse collapses to a line if r = 1 or -1 (perfect correlation) and extends to a circle if r=0.
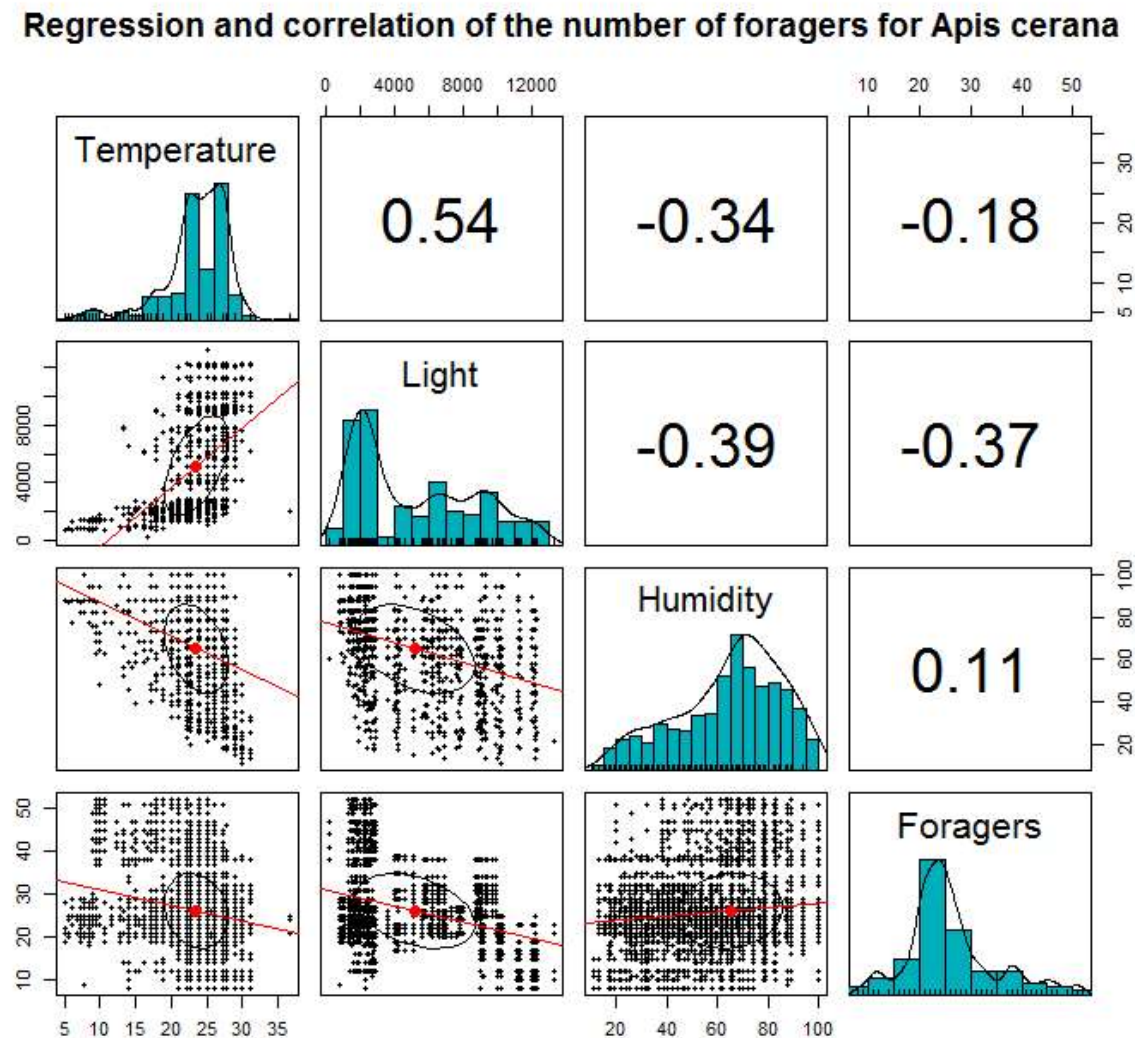
Figure 2-23

Figure 2-24. Pairwise correlation and regression of the number of foragers with each environmental variable (temperature, light intensity, and relative humidity) for *Apis mellifera*. The last column of the plot represents a correlation of each variable and number of foragers, the last row represents regression of each variable and number of foragers, and the diagonal represents histogram and density of each variable. The center of the correlation ellipse is located at the sample means, that is, at the point (x-bar, y-bar). The ellipse collapses to a line if r = 1 or -1 (perfect correlation) and extends to a circle if r=0.
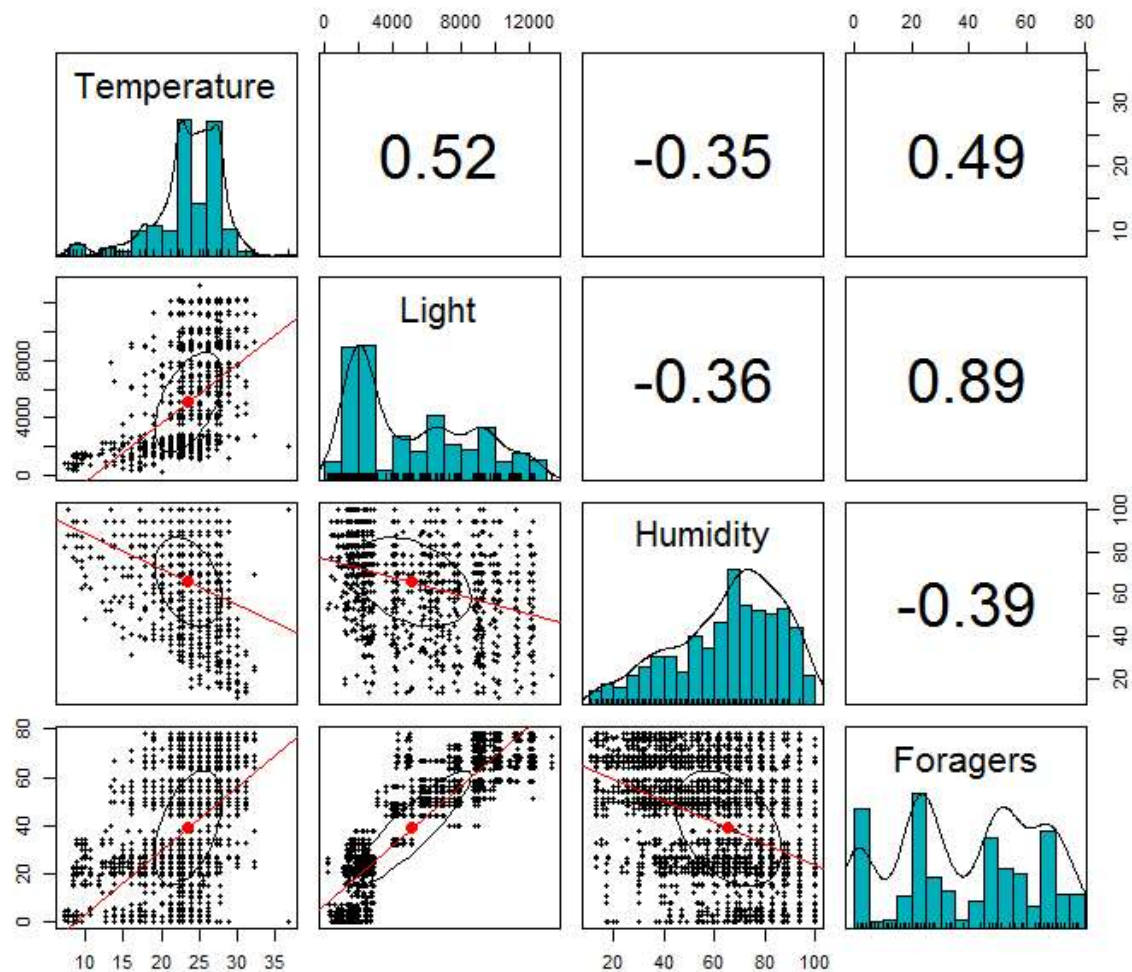
Figure 2-24

Tables and table legends

Table 2-1. Foraging commencement, foraging cessation, and foraging duration of *A. cerana* and *A. mellifera* foragers in different seasons in relation with sunrise and sunset.

Species	Season	Sunrise	Sunset	Foraging commencement	Foraging cessation	Foraging duration
Apis cerana	Spring	5:56±0:31	18:23±0:21	5:19±0:23	18:55±0:08	13:36±0:30
	Summer	5:12±0:06	18:52±0:14	4:40±0:11	19:11±0:12	14:31±0:20
	Rainy	5:23±0:11	18:53±0:12	4:38±0:15	19:27±0:09	14:49±0:26
Apis mellifera	Spring	5:56±0:31	18:23±0:21	6:19±0:08	17:45±0:14	11:22±0:15
	Summer	5:12±0:06	18:52±0:14	5:37±0:17	18:25±0:13	12:47±0:12
	Rainy	5:23±0:11	18:53±0:12	5:35±0:24	18:21±0:19	12:44±0:23

Table 2-1

Table 2-2. Robust repeated-measures ANOVA results of numbers of foragers of *A. cerana* and *A. mellifera* from 6 AM to 6 PM for the spring season (March and April) of 2016.

Species	Factor	F	Numerator DF	Denominator DF	p-value
Apis Cerana	Setting	0.40	1	81.61	0.53
	Time	705.74	12	65.87	<0.001
	Setting*Time	1.55	12	65.87	0.13
Apis Mellifera	Setting	3.50	1	90.86	0.06
	Time	4787.64	10	66.61	<0.001
	Setting*Time	0.66	10	66.61	0.75

Table 2-2

Table 2-3. Robust repeated-measures ANOVA results of numbers of foragers of *A. cerana* and *A. mellifera* from 6 AM to 6 PM for the summer season (May and June) of 2016.

Species	Factor	F	Numerator DF	Denominator DF	p-value
Apis cerana	Setting	0.69	1	93.14	0.41
	Time	752.41	12	74.34	<0.001
	Setting*Time	0.23	12	74.34	0.996
Apis mellifera	Setting	3.21	1	101.89	0.08
	Time	5254.65	10	75.06	<0.001
	Setting*Time	0.76	10	75.06	0.67

Table 2-3

Table 2-4. Robust repeated-measures ANOVA results of numbers of foragers of *A. cerana* and *A. mellifera* from 6 AM to 6 PM for the rainy season (July and August) of 2016.

Species	Factor	F	Numerator DF	Denominator DF	p-value
Apis Cerana	Setting	0.42	1	69.78	0.52
	Time	2145.89	12	56.17	<0.001
	Setting*Time	0.28	12	56.17	0.99
Apis Mellifera	Setting	0.29	1	76.59	0.59
	Time	2738.94	10	57.00	<0.001
	Setting*Time	0.53	10	57.00	0.86

Table 2-4

Table 2-5. Results of the robust linear regression of the number of foragers for *Apis cerana* using time of the day, temperature, light intensity, and relative humidity as predictors. Residual standard error is 2.68 on 4612 degrees of freedom.

Predictor	Estimate	Std. Error	F	p-value
Intercept=6 AM	18.28	0.33	3022	<0.001
7 AM – 6 AM	0.65	0.21	11	0.001
8 AM – 6 AM	13.77	0.22	4065.9	<0.001
9 AM – 6 AM	13.86	0.23	3048.7	<0.001
10 AM – 6 AM	-0.81	0.44	3.57	0.06
11 AM – 6 AM	-0.27	0.58	0.23	0.63
12 PM – 6 AM	-2.74	0.71	15.58	<0.001
1 PM – 6 AM	-8.31	0.88	93.52	<0.001
2 PM – 6 AM	4.23	0.68	40.99	<0.001
3 PM – 6 AM	4.08	0.44	88.27	<0.001
4 PM – 6 AM	0.02	0.32	0.005	0.94
5 PM – 6 AM	-1.46	0.24	39.8	<0.001
6 PM – 6 AM	0.34	0.22	2.32	0.13
Light Intensity	0	0.0001	0.26	0.61
Relative Humidity	-0.009	0.002	15.33	<0.001
Temperature	-0.03	0.01	7.18	0.007

Table 2-5

Table 2-6. Results of the robust linear regression of the number of foragers for *Apis mellifera* using time of the day, temperature, light intensity, and relative humidity as predictors. Residual standard error is 1.132 on 3902 degrees of freedom.

Predictor	Estimate	Std. Error	F	*p*-value
Intercept=7 AM	6.53	0.19	12.59.6	<0.001
8 AM – 7 AM	1.97	0.11	369.22	<0.001
9 AM – 7 AM	0.62	0.11	33.4	<0.001
10 AM – 7 AM	7.83	0.18	1909.2	<0.001
11 AM – 7 AM	8.67	0.23	1390.4	<0.001
12 PM – 7 AM	12.16	0.27	2157.5	<0.001
1 PM – 7 AM	12.83	0.35	1404.3	<0.001
2 PM – 7 AM	11.95	0.27	1981.2	<0.001
3 PM – 7 AM	8.14	0.18	2013.5	<0.001
4 PM – 7 AM	6.29	0.14	2054.2	<0.001
5 PM – 7 AM	0.5	0.11	20.38	<0.001
Light Intensity	0	0	0.3	0.59
Relative Humidity	-0.01	0.001	65.14	<0.001
Temperature	-0.02	0.01	6.69	0.00975

Table 2-6

Table 2-7. Results of the robust linear regression of the number of foragers for *Apis cerana* using only environmental variables as predictors. Residual standard error is 4.341 on 4624 degrees of freedom.

Predictor	Estimate	Std. Error	F	*p*-value
Intercept	19.49	0.6	1024.4	<0.001
Light	-0.001	0	347.29	<0.001
Humidity	-0.01	0.004	6.16	0.01
Temperature	0.07	0.02	10.3	0.001

Table 2-7

Table 2-8. Results of the robust linear regression of the number of foragers for *Apis mellifera* using only environmental variables as predictors. Residual standard error is 2.673 on 4624 degrees of freedom.

Predictor	Estimate	Std. Error	F	*p*-value
Intercept	3.34	0.32	106.03	<0.001
Light	0.002	0	10668	<0.001
Humidity	-0.02	0.002	104.05	<0.001
Temperature	0.03	0.01	4.72	0.03

Table 2-8

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Chapter 3

Forager assessments, competitive effects, and resource overlap between Western honey bees

(*Apis mellifera* L.) and Asian honey bees (*Apis cerana* F.)

Abstract

The western honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) plays a complicated role in biological invasions. It is native to Europe, Africa, and the Middle East and has been introduced throughout most of the world, potentially competing with indigenous bee species. It has been introduced to Asia, which is home to at least eight other *Apis* species. *Apis cerana* Fabricius is the most widespread species of Asian honey bees; it is very similar to *A. mellifera* in habits and appearance and is frequently cultivated by bee-keepers. Thus, the potential for competition between these two species is expected to be very high. Nepal is a particularly interesting place to investigate impacts of *A. mellifera* on indigenous bees as it is still a relatively new introduced species with small population. This study compares floral resources —pollen and nectar—used by these two honey bees to explore resource overlap and competition. Results from this study show that *A. cerana* collects significantly greater pollen loads when they are in a single-species apiary, than in mixed-species apiary with *A. mellifera*. Nectar volume, nectar concentration, and mg of sugar in nectar collected by *A. cerana* are also significantly higher in single-species apiary than mixed-species apiary. In contrast, *A. mellifera* does not show significant differences in the volume and concentration of nectar and weight of pollen loads collected in single-species and mixed-species apiaries. *Pianka's index of niche overlap* between *A. mellifera* and *A. cerana* from this study is 0.73. This suggests that there is extensive overlap in the resources used by the two species.

Key words

Apis cerana, *Apis mellifera*, **exotic species, pollen, nectar, pianka's index**

Introduction

Biological invasions and the impact of exotic species on native species have received attention from both applied and theoretical ecologists (Holway 1999, Thomson 2004). There is also growing recognition of the importance of native bees as pollinators and the threats they face from invasive species (Buchmann and Nabhan 1996, Paton 1996, Brown and Paxton 2009, Potts et al. 2010). The western honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini), plays a complicated role in this story. It is originally from Europe, the Middle East and Africa (Ruttner 1988, Smith 1991, Franck et al. 1998), but has been introduced widely around the world (Alqarni et al. 2011). It is probably the single most recognized pollinator in the world, and is under threat from diseases, parasites and pesticides, particularly in Europe and the Americas (Pirk et al. 2017). On the other hand, it is also a highly successful invader which has naturalized over most of its introduced range, and its impact on native ecological system is poorly quantified and controversial (Bronstein 1995, Paini 2004, Potts et al. 2010). While there are no native honey bees in the Americas, many honey bees species are indigenous to Asia, many with habits very similar to those of the western honeybees (Hepburn and Radloff 2011). Therefore, the introduced western honey bees in Asia may be in very close competition with indigenous honey bees as well as with non-*Apis* bees.

In this study, I examine the potential for competition between *A. mellifera* and *Apis cerana* Fabricius in Nepal. *A. cerana* is the most widespread of the Asian honey bees species, is frequently cultivated by bee-keepers, and is similar to *A. mellifera* in habits and appearance (Buchmann 1996). The documented history of *A. mellifera* introductions into the range of *A. cerana* began more than a century ago. *A. mellifera* was introduced to Japan in 1877 (Sakai and Okada 1973, Watanabe 1981, Yoshida et al. 1994, Solignac et al. 2005), to China in 1920 (Kuang and Kuang 2002) and soon after they were imported to many other Asian countries for commercial beekeeping (Theisen-Jones and Bienefeld 2016). *A. mellifera* colonies now flourish in many regions of Asia (FAO 2016), but in most of the Asia they are expensive to maintain, as they are susceptible to many local predators and parasites (Verma 1990, Joshi et

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al. 2002, Sharma 2004, Oštir 2014). It has long been suspected that *A. mellifera* might have serious impacts on native honey bees species, especially *A. cerana* [Japan: Sakagami (1960); India: Atwal and Sharma (1971); China: Yang (2005)]. Therefore, an investigation of competition between the *A. mellifera* and *A. cerana* is timely.

Nepal is a particularly valuable place to carry out this research as *A. mellifera* is still newly introduced to this region, and the population of *A. mellifera* in Nepal is still small relative to the indigenous *A. cerana* population (FNBK 2016). *A. mellifera* was unsuccessfully introduced to Nepal in 1978; successful introduction by commercial beekeepers took place in 1992 after an epidemic of Thai sac brood virus killed over 90% of indigenous *A. cerana* colonies across the entire Hindu Khus Himalayan region (Allen 1995, Thapa et al. 2000). The recent establishment of *A. mellifera* in Nepal makes it an interesting site to study early stages of resource overlap and potential competition between closely related indigenous and introduced species. It is also logical to gather base-line data on interactions between these two species near the beginning of their interactions. This study can be a useful model for invading species in general and for the possible impacts of western honey bees on native bees worldwide. Also, this information will benefit those who manage the honey bee species for their products and pollination services to understand the foraging preferences of these species.

Introduced *A. mellifera* could affect indigenous *A. cerana* in many ways, such as direct competition for floral resources, nesting sites, disruption of drone congregations and mating, and introduction of parasites and pathogens. Competition between introduced and indigenous species for floral resources or reduction of indigenous pollinator populations may also have effects on the species composition of plant communities. In this study, I focus on forager-assessments and potential competition between *A. mellifera* and *A. cerana* in Nepal for the floral resources of pollen and nectar. East European *A. mellifera* from temperate climates and *A. cerana* from tropical and semi-tropical climates evolved under very different seasonal patterns of nectar and pollen availability. Average colony size in *A. mellifera* is larger than average colony size in *A. cerana*, and European *A. mellifera* generally

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store more honey and pollen (Ruttner 1988). In addition, workers of east European *A. mellifera* are generally larger than tropical and semi-tropical *A. cerana* workers (Dyer and Seeley 1987). Difference in body size and colony size between these two species makes this study more interesting to propose the questions about the potential competition.

The foraging biology and strategies of both *A. mellifera* and *A. cerana* in Nepalese landscape could either minimize or exacerbate competitive interactions between the species (Sakagami 1960, Koeniger 1982, Verma 1990, Sharma 2004). The impacts of competitively-driven changes in foraging behavior on *A. cerana* populations will depend on the degree to which their plant preferences for nectar and pollen overlap with those of *A. mellifera,* the duration and timing of nectar availability, and the ability of *A. mellifera* to exclude *A. cerana* from resource patches or plant species. Similarly, indirect effects of *A. mellifera* on native bee plant-pollinator interactions will vary depending on whether native bees respond to competition from *A. mellifera* primarily by shifting daily patterns of foraging, by moving to different resource patches, or by utilizing different plant species.

Pollen is the main source of protein and fat for honeybees and developing bee larvae are raised on a diet largely composed of pollen (Dietz 1975, Pesante et al. 1987, Nicolson 2011). Because *A. mellifera* colonies are significantly larger and body size is bigger than *A. cerana*, their colony demands for pollen must be higher. In this research, I ask the following questions about the pollen foraging behavior of *A. cerana* and *A. mellifera* when they are kept in mixed and single-species apiaries: (1) Do individual *A. cerana* foragers collect pollen loads of similar weight regardless of the presence or absence of *A. mellifera?* (2) Is the total weight of pollen collected by *A. cerana* colonies similar regardless of presence or absence of *A. mellifera?*

Nectar is the primary source of carbohydrates for bees and the primary food for adult bee (Standifer 1980, Brodschneider and Crailsheim 2010, Nicolson 2011). As noted above, nectar sources may differ in the duration and timing of nectar production. In addition, nectars from different sources

may differ in both volume and sugar concentration. Nectar secretion and availability follow different seasonal patterns in both temperate (Crane 1975, Paton 1996) and tropical ecosystems (Roubik 1979). Flowering patterns should result in detectable differences in foraging behavior by bees adapted to each environment. For example, Winston and Katz (1982) described the specific differences in foraging, honey storage and absconding behaviors of southern African *A. mellifera* (*A. mellifera scutellata*) and eastern European *A. mellifera* (*A. mellifera ligustica, A. m. carnica,* and others). Likewise, Seeley (1989) found that an *A. mellifera* colony exploits only highly profitable patches of flowers when the colony's food reserves are good but when-food reserves are low, the colony's foragers will exploit both highly profitable and less profitable flower patches.

In this study, I compare the nectar foragers of *A. cerana* and *A. mellifera* over the spring, summer, and rainy seasons in Nepal. I measure Volume and Concentration of the nectar and milligrams (mg) of sugar collected by foragers and ask the following questions: (1) Do individual *A. cerana* foragers collect similar Volume of nectar in mixed-species and single-species apiaries? (2) Do *A. cerana* foragers collect similar Concentration of nectar in all apiaries regardless of presence and absence of *A. mellifera*? (3) Is the total amount of sugar collected by *A. cerana* similar in mixed-species apiaries and single-species apiary?

Coexisting species must utilize resources differently in some aspects (Hardin 1960). The concept of the competitive exclusion principle was later expanded as mathematical theories of limiting similarity (MacArthur and Levins 1967) and ecological niche (Chase and Leibold 2003). Utilization of food resources is considered as a major niche axis to measure potential resource competition (Schoener 1974, Gotelli et al. 2015). Ecologists predict that coexisting species should exhibit relatively low overlap in the use of resources, while there should be higher overlap in the niches of two species if they compete for resources (Connell 1980, Gotelli et al. 2015). These fundamental concepts of ecology are very relevant in the case of potential resource competition between *A. cerana* and *A. mellifera* after introduction of *A.*

mellifera to Asian landscapes. In this research, I analyze the pollen resources used by both species to observe the overlap in the species of pollen resources used by them.

Materials and Methods

Experimental apiary design

Apiaries were set up in four locations of Kathmandu valley, Nepal where honey bees forage throughout the year. Apiary A (mixed-species apiary; ap1) is located inside the Coronation Garden, Tribhuban University, Kiritipur, Nepal (27.68 N latitude, 85.29 E longitude, and 1307 m elevation); apiary B (mixed-species apiary; ap2) is on the grounds of the National Agricultural Research Council (NARC), Khumaltar, Nepal (27.65 N latitude, 85.32 E longitude, and 1323 m elevation) ; apiary C (single-species apiary with *A. cerana* only; ap3) is on the grounds of Knowledge Park, Godavari, Lalitpur, Nepal (27.59 N latitude, 85.39 longitude, and 1603 m elevation); and apiary D (single-species apiary with *A. mellifera* only; ap4) is in Gokarna, Kathmandu, Nepal (27.76 N latitude, 85.40 E longitude, and 1418 m elevation). Apiaries A and B are 5 Km apart, while Apiaries C and D are 17 Km apart (see Figure 2-1). I sampled four queenright colonies of each species in apiaries A and B while only four queenright colonies of *A. cerana* in apiary C and only four queenright colonies of *A. mellifera* were sampled in apiary D. All *A. mellifera* colonies were housed on standard Langstroth hives and *A. cerana* colonies were housed on standard Newton B type hives. All sampled colonies of each species were equalized periodically following the methods suggested by Delaplane et al. (2013). This involves the estimation of numbers of adult honeybees and capped brood cells by lifting each frame in the colony and estimating, to the nearest one eighth of frame side, how much capped brood cells and adult bees are present. All apiaries are in similar semi-urban agricultural landscapes of Kathmandu valley with sparsely available agricultural and horticultural crops. There are considerable numbers of deciduous tall trees adjacent to agricultural and horticultural lands within the distance of honey bees forage.

Forager assessments

On each test day, thirty returning foragers were sampled from each test hive three times a day (09:00 hrs., 12:00 hrs., and 16:00 hrs.) and forager loads were examined. This experiment was conducted in all apiaries on every 5th day throughout the experimental period from the month of March 2016 to August 2016. For example, the hive entrances of the test colonies were closed using entrance blocks from 08:45 to 9:00 and returning foragers were collected using hollow cylinders provided with a plastic bag to the bottom from 9:00 to 9:10. When 25-30 returning foragers per colony had been collected, the bags were removed from the cylinder and placed in a cooler to chill the bees. This made it possible to handle the bees for nectar and pollen measurements while they were paralyzed by the cold. Captured honey bees were classified as pollen collectors (P), nectar collectors (N), both nectar and pollen collectors (P & N), water collectors (W), and empty bees (E).

Returning foragers collecting a full load of pollen in their corbicula and with less than 5 μl of honey stomach contents were considered pollen collectors, foragers who collected more than 5 μl of honey stomach contents with more than 5 % sugar concentration and no pollen loads in corbicula were considered nectar collectors, foragers with any amount of honey stomach contents at less than 5% sugar concentration and without pollen loads in their corbicula were considered-water collectors, foragers who collected pollen loads in their corbicula and with more than 5 μl of nectar in their honey stomach were considered both nectar and pollen collectors, and foragers who collected nothing were considered empty bees. Same sampling procedures were repeated at 12:00 hrs. and 04:00 hrs. on each test day at all apiaries. In addition, *A. cerana* foragers were further assessed in both mixed-species apiaries at 5:30 AM in all experimental day of May 2016 to August 2016 when foraging activities of *A. mellifera* were yet to commence.

Body mass of individual bees and their pollen load

Body mass of captured forager bees that had collected only pollen were measured with a Veritas M124A Analytical Balance, first along with their pollen loads and later without loads. The mean difference in mass is considered the pollen load collected by individual returning foragers. The scraped off pollen load was placed in a micro-centrifuge tube, stained with Calberla's fluid, photographed at 100X and 400 X magnifications, and matched up with the pollen library to identify the taxonomy of pollen species collected (Figure 3-1).

Calberla's solution and pollen preparation

Pollen grains were stained with Calberla's fluid using a smear-slide technique (Calberla 1878, Bernhardt et al. 2013, Bernhardt et al. 2014, Stolze 2015). Calberla's solution was prepared by mixing 5 ml glycerin, 10 ml 95 % ethanol, 15 ml distilled water, and 2–3 drops of a saturated aqueous basic fuchsin solution as described by (Gay et al. 1941). As basic fuchsin solution is considered to have potential health effects (Stolze 2015) it was handled carefully, wearing appropriate personal clothing. The sporopollenin in the outer exine layer of the pollen is easily stained by Calberla's fluid (Watson et al. 2007, Fraser et al. 2011). Pollen grains from each colored pollen balls were mounted in a glass slide and observed under a compound microscope to confirm the plant origin of a pollen ball. 100X and 400 X magnified pollen grains were photographed with the help of the *Image Focus 4* software and *euromex* camera (https://www.euromex.com/en/products/branches/education-digital-solutions/cmex/). These pollens were matched with plant species with the help of a reference pollen library of the bee flora around the apiaries.

Reference pollen library

Pollen grains of the bee flora blooming within 1 km radius of the study apiaries were collected once a week throughout the experimental period. These bee plants were identified with the help of the reference library collection at Godavari Botanical Garden and confirmed by a plant taxonomist, Mr.

Kuber Jung Malla. Pollen grains were mounted on a glass slide, stained with a Calberla's fluid, and observed under a compound microscope. Pollen grains from taxonomically identified plants were photographed at 100X and 400 X magnifications. The photographs were used to make a reference atlas of pollen found around each apiary (Figure 3-2). This aids the identification of the plants visited by *A. cerana* and *A. mellifera* for pollen resources.

Pollen traps and pollen samplings

Pollen traps were fitted at the hive entrances of two colonies of each species in mixed-species apiary A and two colonies in each single-species apiary (apiary C and Apiary D) once a week from March 2016 to August 2016. Full-day pollen loads collected by each experimental colony were collected as suggested by Nagamitsu and Inoue (1999). Pollen traps and the pollen collecting box used were made and by the local beekeeping workshop at Godavari and tested on each species of honey bees before experimentation (http://beekeepingnepal.com.np/). Each trap of a yellow plastic board (1 mm thick) with holes through which bees were forced to pass so that pollen balls were scraped off and collected in the collecting tray of the trap. The diameter of the holes was 4.5 mm for *A. cerana*, and 5.0 mm for *A. mellifera*. The traps were placed over the hive entrance the night before a collection day and removed in the evening of the collection day to collect the full-day pollen loads. It is very interesting that most honey bees visit only one flower species during one foraging trips so that differently colored pollen balls usually come from different plant species (Betts 1920, Grant 1950, Free 1963). Pollen loads from each experimental hive were weighed, and the pollen balls were sorted by color to record the species diversity of the pollen collected. Finally, pollen balls were remixed, and 20 % by weight of full-day pollen loads from each test colony sorted by color and used to calculate the relative abundance of pollen balls from different plant species. Pollen grains from five pollen balls of each color type were stained with Calberla's fluid, mounted in five separate glass slides, and photographed at 100X and 400 X magnifications (Figure 3-3). These pollen photographs were matched up with the aid of reference pollen library described earlier.

Nectar sampling

As stated above, foragers were chosen, examined and indexed according to the presence, volume and concentration of sugar in the nectar load. This was done by squeezing the honey stomach in the bee's abdomen between the thumb and forefinger so that the contents of the honey stomach would flow through the esophagus, to the mouthparts and into a 50 μl micro-capillary tube calibrated to 5 μl (Roubik 1982, Roubik and Buchmann 1984). Its Sugar concentration is measured using a hand-held refractometer designed to measure sugar concentration in small volumes of nectar (Bailey/Stanley 45-03). Sugar concentration was calculated as the total amount of dissolved solids per volume of nectar. If the contents of the honey stomach had a concentration of more than 5% solids, it was considered nectar if it contained

Statistical design for potential resource competition

Case I

To study resource competition between *A. cerana* (ac) and *A. mellifera* (am), the quantity of foraging resources acquired by an individual forager of a colony was recorded at a given time point. Four dependent variables, namely, pollen weight, nectar volume, nectar concentration, and mg. of sugar in nectar, were of interest in our study. The effect of competition for a given species means the significant change in its acquired quantity of foraging resources between the two conditions of single-species and mixed-species apiary settings. The data thus are to be analyzed separately for each species in the form of a nested, unbalanced "2 Setting x 3 Time" design. Factor levels were as follows:

Setting=Mixed vs. Single

Time=9 AM vs. 12 PM vs. 4 PM

The factor "Setting" was replicated by Colony nested inside Location. "ac x Single" was housed in ap3 and "am x Single" was housed in ap4 whereas both "ac x Mixed" and "am x Mixed" were housed in both ap1 and ap2.

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Location=ap1 vs. ap2 vs. ap3 vs. ap4

Replications were achieved in the form of 4 Colony units in ap3 and ap4, and 8 Colony units in ap1 and ap2, 4 Colony units per species. The Time factor was replicated by Date nested inside Month which, in turn, was nested inside Season.

Month=Mar vs. Apr vs. May vs. Jun vs. Jul vs. Aug

Season = Spring vs. Summer vs. Rainy

10 Spring + 11 Summer + 8 Rainy = 29 Dates in ap1 whereas 10 Spring + 11 Summer + 9 Rainy = 30 Dates in ap2, ap3, and ap4 were used. An experimental unit, represented by a Date-Colony combination, was then labeled as Entity. At each Time factor, 30 random individual bees were observed from each Entity and then measured for the quantity of foraging resources (pollen weight, nectar volume, nectar concentration, and mg of sugar in nectar). This yielded the pollen weight data from 11110 bees out of which 5497 were for *A. cerana* and 5613 for *A. mellifera*. Averaging over the individual bees from each Entity at a given time point, there were 1068 observations for *A. cerana* and 1072 for *A. mellifera*. In the same manner the datasets for the nectar volume, the nectar concentration, and the mg. of sugar in nectar consisted of 12999 bees out of which 6623 were for *A. cerana* and 6376 for *A. mellifera*. Averaging over the individual bees from each Entity at a given time point, there were 1077 observations for *A. cerana* and 1064 for *A. mellifera*.

Case II

6 Spring + 7 Summer + 6 Rainy = 19 Dates in ap1 whereas 6 Spring + 8 Summer + 6 Rainy = 20 Dates in ap3 and ap4 were used to assess the full day pollen loads collected by two colonies of each species.

Case III

In addition to case I and case II, 11 Summer + 8 Rainy = 19 Dates were used in ap1 whereas 11 Summer + 9 Rainy = 20 Dates in ap2 were used to assess 30 random *A. cerana* foragers at 5:30 AM when *A. mellifera* foragers were yet to commence.

Statistical analysis

All dependent variables, namely, pollen weight, nectar volume, nectar concentration, and mg. of sugar in nectar, exhibited significant non-normality for both *A. cerana* and *A. mellifera*. It was consistent across the three seasons. The histograms and Q-Q plots supported this violation of normality.

For *A. cerana*, all four response variables violated the homoscedasticity assumption across the two settings, namely, the assumption that the variance in the case of the single apiary setting is the same as that in the mixed apiary setting. This assumption, however, held true for *A. mellifera*. Broken down by the three seasons, these results were almost always consistent. The only exceptions were the followings: nectar volume of *A. cerana* in the summer and rainy seasons, nectar volume of *A. mellifera* in the summer and rainy seasons, nectar concentration of *A. cerana* in the spring season, nectar concentration of *A. mellifera* in the spring season, and mg. of sugar in nectar of *A. mellifera* in the spring season. A robust repeated-measures ANOVA on 20% trimmed means was conducted using the package "WRS2" in R to test the significance difference of all four variables in between settings at different times of the day of each species (Mair and Wilcox 2018).

Independent t tests (Welch two sample *t*-test) were used to test: a) for differences between two species in body mass and individual forager load, b) for differences of full day pollen load collected by a colony of the same species between mixed-species apiaries and single-species apiary, and c) for differences in pollen load, nectar volume, nectar concentration, and mg of sugar collected by individual *A. cerana* foragers between 5:30 AM and 9:00 AM at mixed species apiaries.

Niche overlap study

I used package "EcoSimR" (Gotelli et al. 2015) to run the niche overlap module with "RA3" algorithm (Winemiller and Pianka 1990) and Pianka (1973) index of overlap to calculate observed niche overlap index and simulated niche overlap indices. According to this model, prediction of resource overlap is always relative to the null expectation and this EcoSimR tests for overlap in resource use among a set of coexisting species (*A. cerana* and *A. mellifera*) using a set of unordered, discrete resource categories (pollen abundance). This analysis is meant to reveal that whether an observed niche overlap, calculated between the coexisting *A. cerana* and *A. mellifera* in mixed species apiary, is more or less than would be expected if they used resource categories independently of one another.

The data for this niche overlap analysis consists of a "2 X 43" data frame in which 2 rows represent *A. cerana* and *A. mellifera* respectively and 43 columns represent a discrete, unordered pollen resource category used by both species in mixed-species apiary (ap1) during the spring season. This data frame is obtained by combining full day pollen trap data from two colonies of each species in apiary ap1 collected in six sampling days in Spring season.

I used "pianka" index as basic metrics to calculate the niche overlap between *A. cerana* and *A. mellifera* which is similar to the competition coefficient "α" in Lotka-Volterra equations. This index ranges from "0" to "1" where "0" means no overlap and "1" means complete overlap (Gotelli et al. 2015). As explained in Gotelli et al. (2015), "RA3" was used as default algorithm which reshuffles the row values, retains the observed niche breadth of each species at relative degree of utilization, but randomly alters the particular resource categories used.

Results

Foragers' assessments

Regardless of mixed-species and single-species settings, both *A. cerana* and *A. mellifera* foragers follow similar, consistent patterns in the proportion of the resource specific foragers. For example, the

proportion of nectar foragers was highest in the spring months (March and April), moderate in the summer months (May and June), and lowest in the rainy months (July and August) (Figures 3-4 & 3-5). In contrast, the proportion of pollen foragers was highest in the summer months and moderate in spring and rainy months (Figures 3-6 & 3-7). More interestingly, 40 % or more foragers of both species returned empty in the rainy moths (Figures 3-8 & 3-9).

Body mass of individual bees and their foraging load

The mass of *A. cerana* foragers (66.49± 0.031mg) was significantly lower (t (3097.5) = -538.4, p<0.001 than that of *A. mellifera* foragers (88.20±0.025 mg). The load carried by *A. mellifera* foragers (48.57±0.08 mg) was also significantly greater than that carried by *A. cerana* (31.37 ±0.07 mg) foragers (t(3224.2) = -144.3, p<0.001).

Potential resource competition

Case I

The overarching results were that the effect of setting ("mixed-species apiary *versus* single-species apiary") was significant for *A. cerana* and not significant for *A. mellifera*. These results stayed consistent when broken down by season as shown in Tables 3-1 to 3-4 and the box plots (Figures 3-10 to 3-25) also support the same conclusions. There were a few noteworthy exceptions. The effect of setting on pollen weight turned out barely significant, F (1, 79.14) = 4.99, p=0.0283, for *A. mellifera* in the summer. The effect on nectar concentration for *A. cerana* in the spring was barely non-significant, F (1, 66.1) = 3.72, p=0.0581. Finally, the effect on nectar concentration was not significant, F (1, 126.44) = 1.35, p=0.247, for *A. cerana* in the rainy season.

Case II

Interestingly, *A. cerana* foragers performed significantly better in mixed-species apiary for all resource variables (pollen weight, nectar volume, nectar concentration, and mg of sugar) at 5:30 AM, before *A. mellifera* began foraging than at 9:00 AM when both *A. cerana* and *A. mellifera* were actively

foraging (Table 3-5). These results stayed consistent when broken down by season and time, as shown by the error bar plots (Figures 3-26 to 3-29).

Case III

On average, the full-day pollen weight collected by colonies of *A. cerana* in the single-species apiary (M=82.84783, SE=1.96391785) was heavier than the pollen weight collected in the two mixed-species apiaries (M=.72.22093, SE=1.585537). This difference was highly significant t (100.23) =-4.2102, *p*<0.001. In contrast, full-day pollen weight collected by colonies of *A. mellifera* in the single-species apiary (M=138.9250, SE=3.81551951) was slightly lighter than colonies' full-day pollen weight in mixed-species apiaries (M=140.1364, SE=2.55136275, t (74.956) =0.26392, *p*=0.7926). This difference was not statistically significant (Figures 3-30 & 3-31).

Niche overlap

The "EcoSimR" module for niche overlap offered two graphic outputs. Firstly, *plot (type= "hist")* generated a standard histogram of simulated metric values (blue bars in Figure 3-32) based on the "RA3" algorithm and "pianka" metric. The vertical red line in the Figure 3-32 indicates the observed overlap index from the original data, the pair of vertical long-dash lines indicates the 95% one-tailed cut points, and the short-dash lines indicate the 95% two-tailed cut points. The latter would constitute the proper 95% confidence interval for the null distribution. Result indicated that observed overlap index ($O_{obs.}$) of pollen categories between *A. cerana* and *A. mellifera* is 0.73039 and mean of simulated overlap index ($O_{sim.}$) is 0.41193. The $O_{obs.}$ metric is greater than all $O_{sim.}$ metrices. It means there is no single $O_{sim.}$ metrics which greater than or equal to $O_{obs.}$ metric [P ($O_{obs.} \leq O_{sim.}$) < 0.001] indicating high overlap in pollen resources used by *A. cerana* and *A. mellifera* in mixed species apiary. Secondly, *plot (type= "niche")* generated a rendering of the species × utilization matrix for the original data matrix (red in Figure 3-33) and one simulated data matrix (blue in Figure 3-33). The area of each circle depicted is proportional to the utilization of a pollen category by either *A. cerana* or *A. mellifera* and if there is no circle that means there is no utilization of that pollen category.

Discussion

The ecotype of *A. cerana* that is indigenous to Nepal was significantly smaller in body size than the introduced *A. mellifera* in this region. I also observed that *A. cerana* collected significantly lighter pollen load than *A. mellifera*. This supports the findings from earlier studies from Thailand and China (Dyer and Seeley 1987, Tan et al. 2012). Given that the average colony size of *A. mellifera* is significantly larger than the average colony size of *A. cerana* (Ruttner 1988) and it collects the heavier load of food resources than *A. cerana*, this study supports that *A. mellifera* should outcompete *A. cerana* if both species are kept together in resource limited condition. On the contrary, a finding from my second chapter of this dissertation shows that *A. cerana* can forage at lower ambient temperatures and might outcompete *A. mellifera* in cold morning or during cooler climate. However, there are instances of many species of honey bees living together in harmony if sufficient resources are available for their survival (Koeniger et al. 1999).

The results on the resource specific foraging of both species indicated that peak nectar collection was in spring months (March and April) and it gradually decreased in summer and rainy months (Figure 3-4 & 3-5). This might be due to greater availability of flower blossoms in spring, which is also reported as honey flow season in Nepal (Thapa et al. 2000, Verma, 1990). The high proportion of foragers of both species collected pollen in summer months (Figure 3-6 & 3-7). This justifies the summer months as time for greater brood development, higher bee population and swarm preparation (Mattu and Verma, 1985). More interestingly, a high proportion of foragers returned home empty in the rainy months (Figure 3-8 & 3-9). Continuous monsoon, foggy weather, and scarcity of bee flora during rainy months might have caused bees to return home empty. I also observed high proportion of empty foragers in the later afternoon (4 PM) even during the spring and summer months. This might be related with nectar secretion and pollen dehiscence timing of the available bee flora in the region.

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Results from all three cases of potential resource competition in this study show that performance of *A. cerana* on the collection of pollen and nectar resources was negatively affected by the presence of *A. mellifera*. On contrary, there were no significant effect of *A. cerana*'s presence on the performance of *A. mellifera*. This observation supports that exotic *A. mellifera* should outcompete indigenous *A. cerana* if both species are foraging for the same specific limited resources in the given time and space. However, before this conclusion can be reached, more complex studies are needed. Such studies must observe the foraging behavior and resource collection by these honey bees species in different landscapes with limited and unlimited floral resources.

The observed niche overlap calculated between the co-existing *A. cerana* and *A. mellifera* in this study was more than expected if they used resource categories independently of one another. Most pollen sources were shared between the two honey bees species. This finding is consistent with the finding of Nagamitsu and Inoue (1999) who compared the plant taxa of pollen resources for *A. cerana japonica* and *A. mellifera linguistica* at a primary beech forest in Japan. This suggests extensive overlap in the categories of pollen species used by both species which should cause competition between them under resource limited conditions. However, detailed survey on pollen abundance within the foraging range of both species and defining the different preference in pollen species used by each species would be necessary to make this conclusion.

Figures and figure legends

Figure 3-1. Pollen ball scraped off from the corbicula of returning foragers and placed in a micro-centrifuge tube (A and B), pollen grains stained in Calberla's fluid and magnified at 100 X (C), and pollen was identified as *Ocimum basilicum* L. of family Labiateae (D).
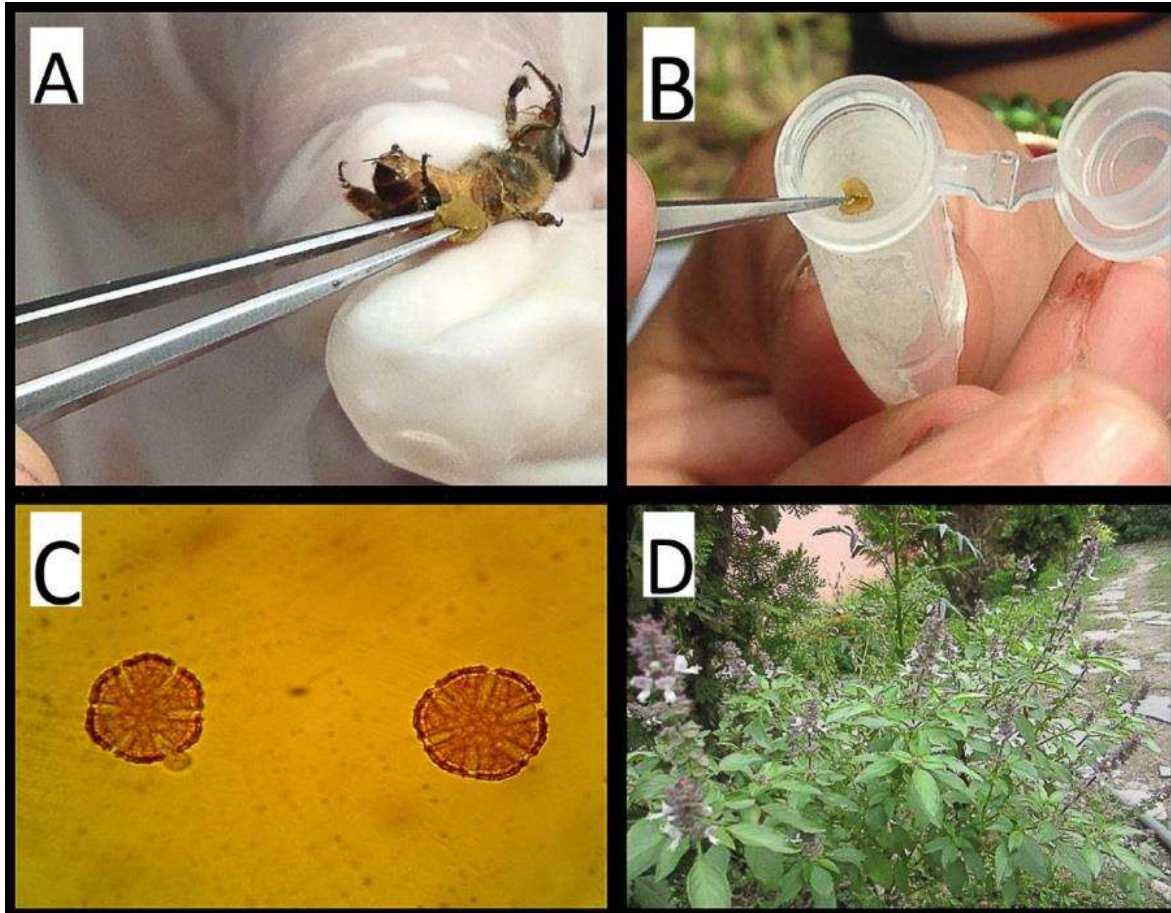
Figure 3-1

Figure 3-2. Sample pollen library (photo of plant, stained pollen grains, and plant ID) of bee flora blooming around the apiaries (A-I), *Cirsium wallichi* DC. of family Asteraceae (A-C), *Malus domestica* Borkh.of family Rosaceae (D-F), and *Lagerstroemia indica* L. of family Lythraceae (G-I).

Figure 3-2

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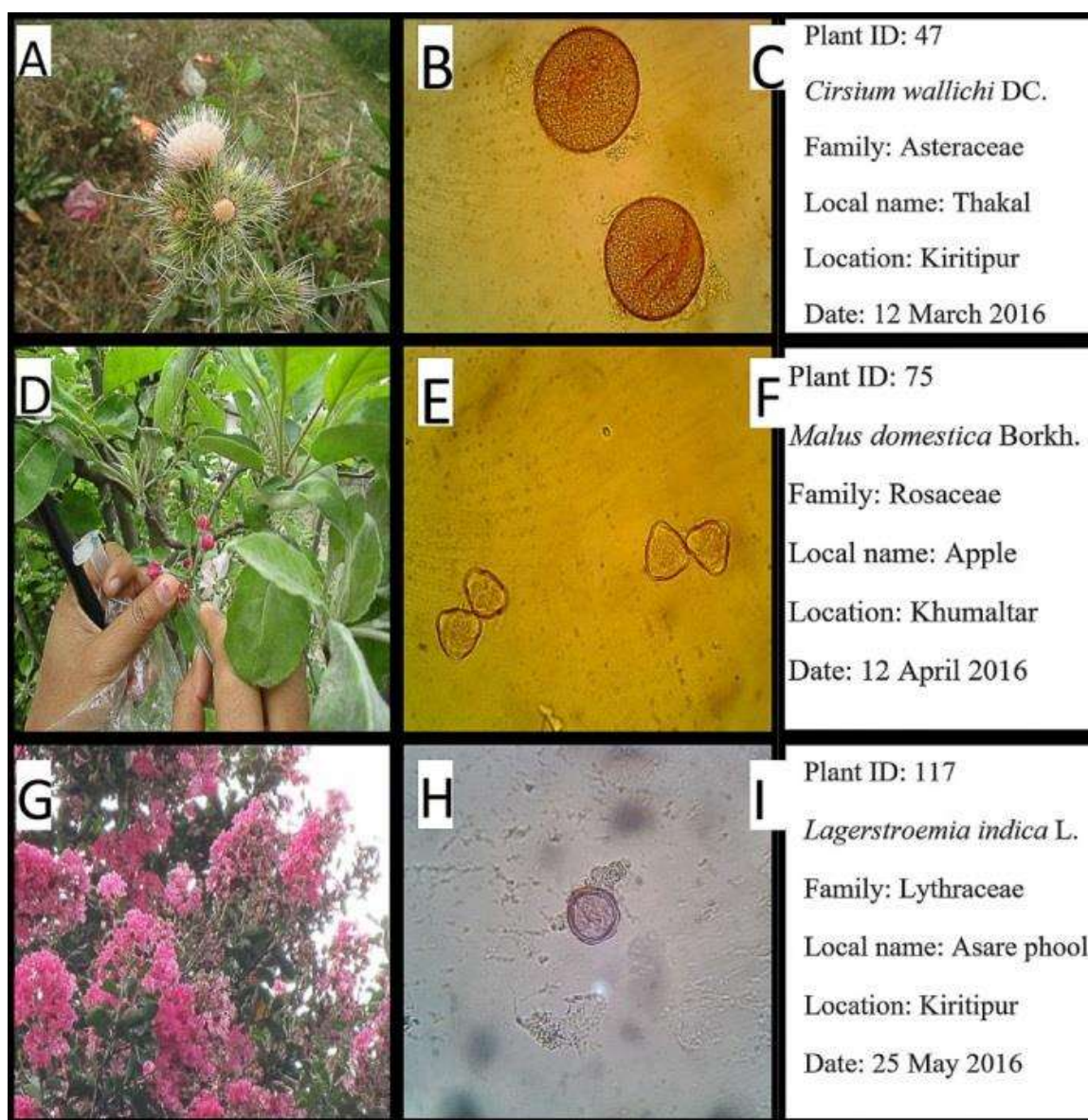
Figure 3-3. Pollen trap was fitted at the entrance of beehive (A), full day pollen balls collected by a colony was trapped in a pollen trap, diversity of the pollen was recorded based on the color of pollen balls and 20 % of thoroughly mixed pollen balls were sampled to record the abundance of each pollen morpho-species (B-D), pollen grains from each pollen morpho-species were stained with Calberla's fluid, pollen photographs were taken, and matched with pollen library to identify the pollen plant species(E-J), *Bauhinina variegata* L. of family Fabaceae (E), *Cuphea micrantha* Kunth. Of family Lythraceae (F), *Callistemon citrinus* (Curtis) Skeels of family Myrtaceae (G), *Calendula officinalis* L. of family Asteraceae (I), an unidentified pollen (H), and *Raphanus sativus* L. of family Brassicaceae (J).
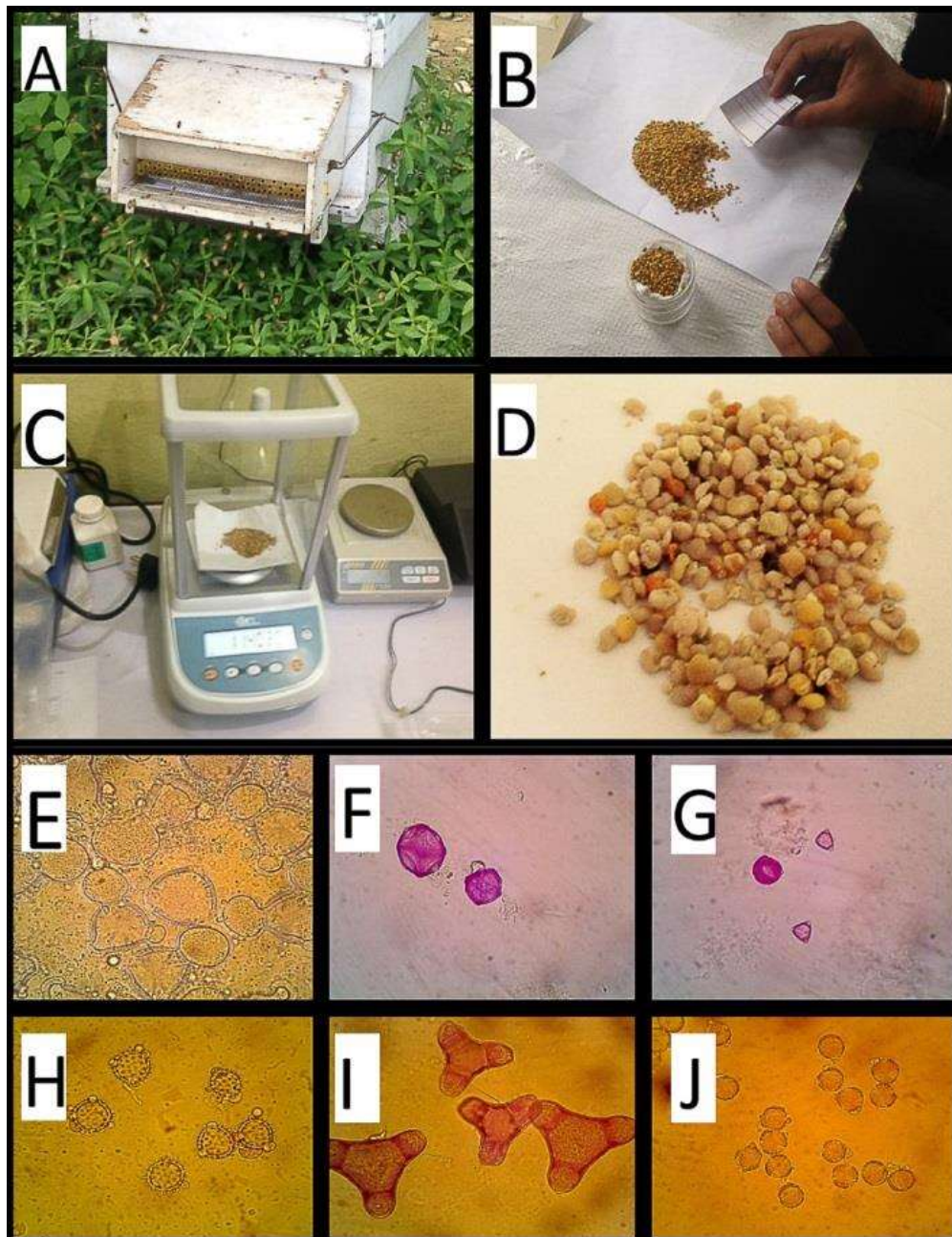
Figure 3-3

Figure 3-4. Proportion of nectar foragers (N) of *A. cerana* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.
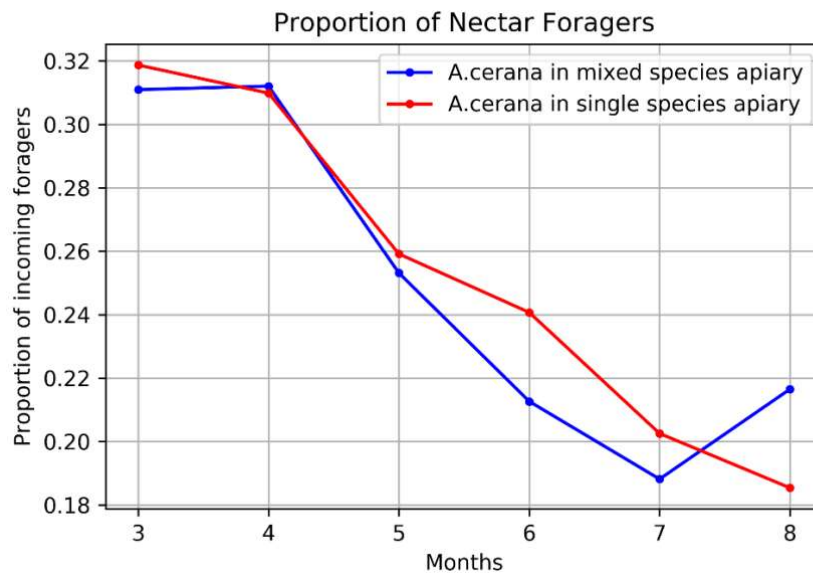
Figure 3-4

Figure 3-5. Proportion of nectar foragers (N) of *A. mellifera* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.
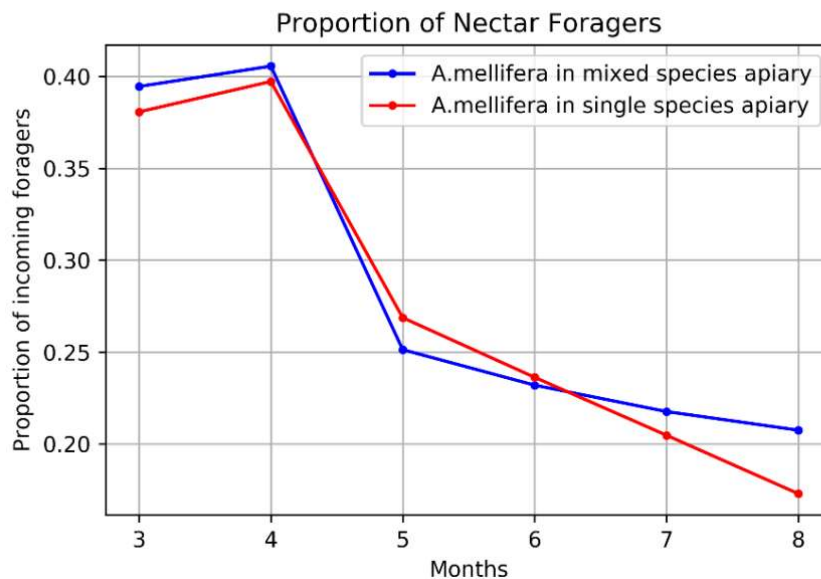
Figure 3-5

Figure 3-6. Proportion of pollen foragers (P) of *A. cerana* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.
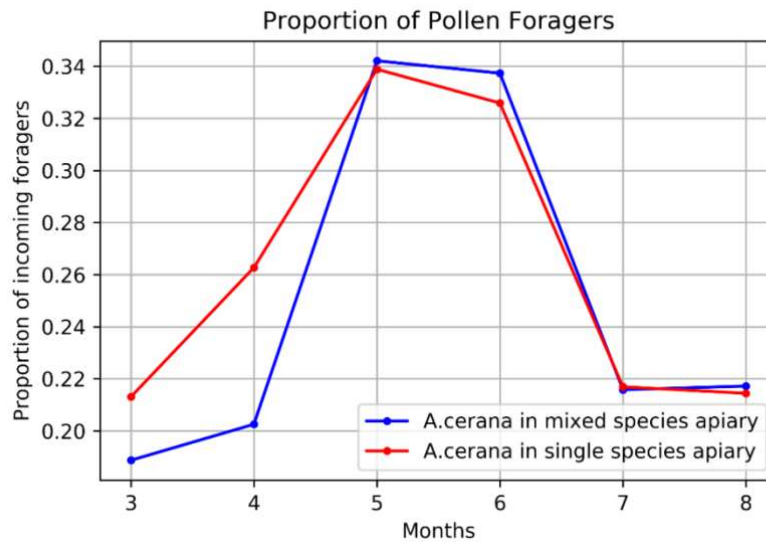
Figure 3-6

Figure 3-7. Proportion of pollen foragers (P) of *A. mellifera* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.
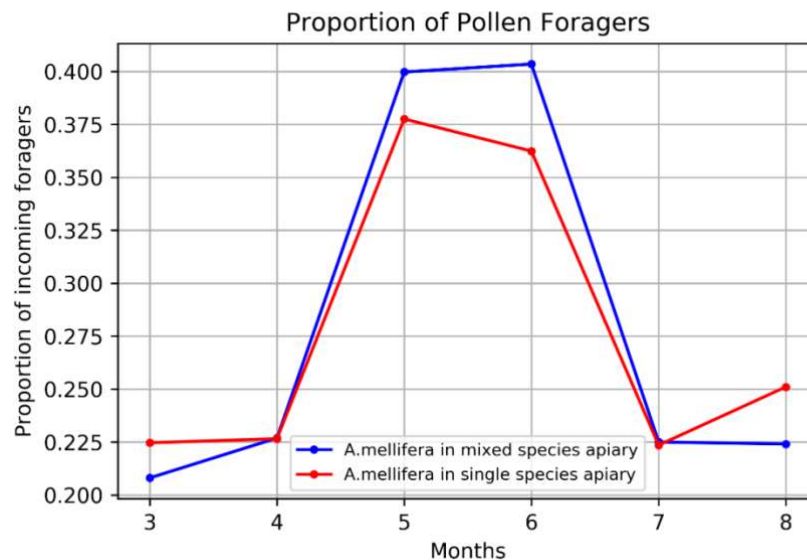
Figure 3-7

Figure 3-8. Proportion of empty foragers (E) of *A. cerana* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.
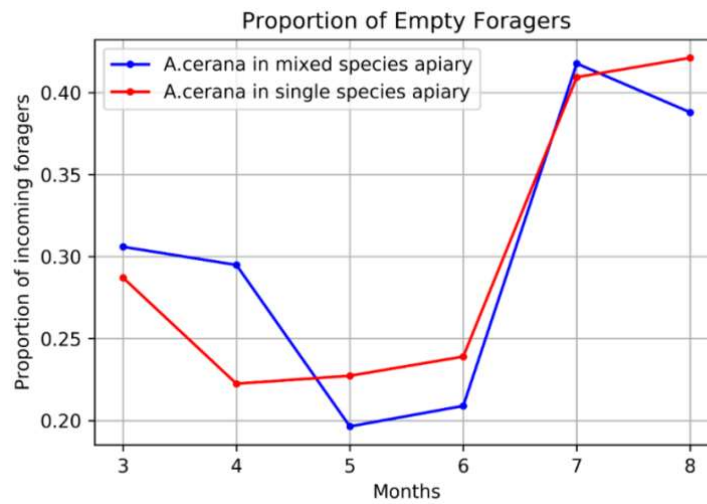
Figure 3-8

Figure 3-9. Proportion of empty foragers (E) of *A. mellifera* measured among 30 returning foragers of four colonies observed through March (3) to August (8) in mixed-species and single-species apiaries.

Figure 3-9

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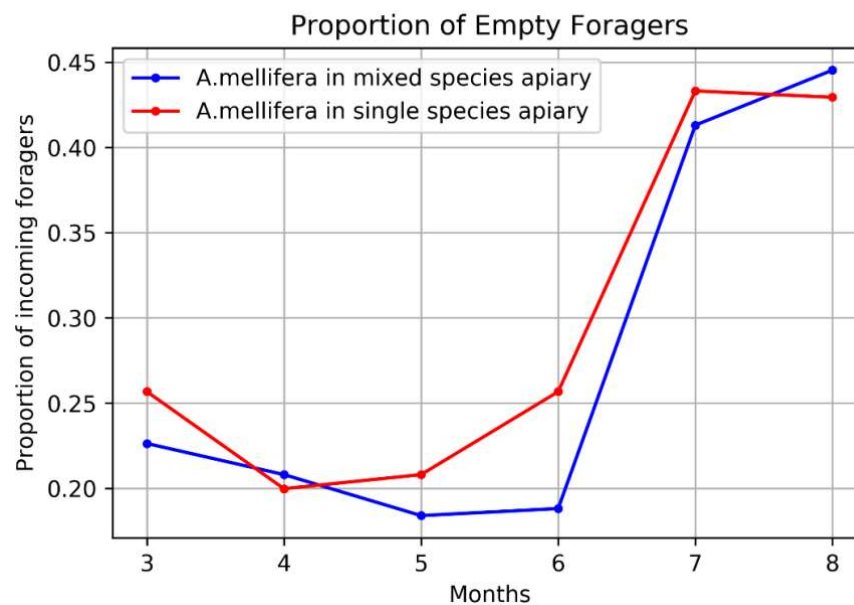
Figure 3-10. Box plot of pollen weight (mg) collected by pollen foragers (P) of *A. cerana* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
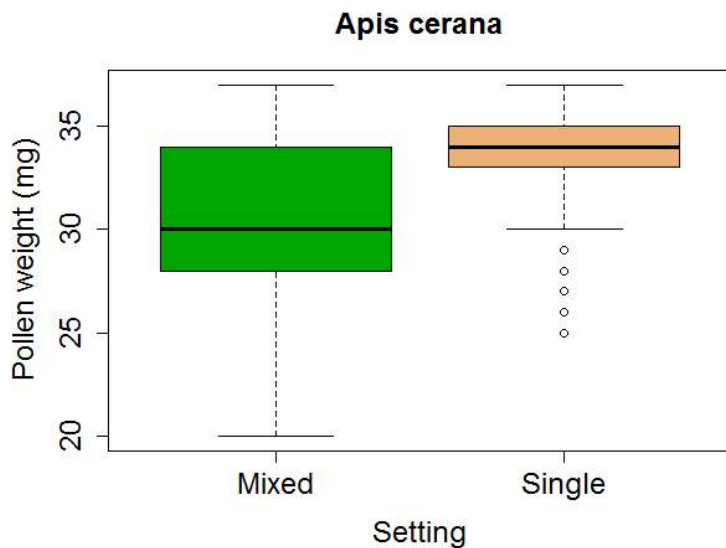
Figure 3-10

Figure 3-11. Box plot of pollen weight (mg) collected by pollen foragers (P) of *A. mellifera* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
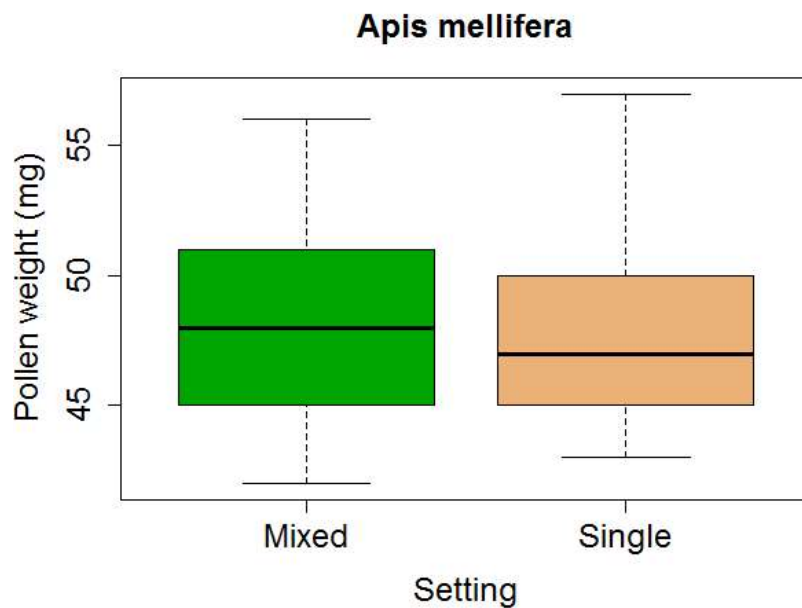
Figure 3-11

Figure 3-12. Box plot of pollen weight (mg) collected by pollen foragers (P) of *A. cerana* foragers in mixed-species and single-species apiary settings broken down into all seasons and all sampling times.
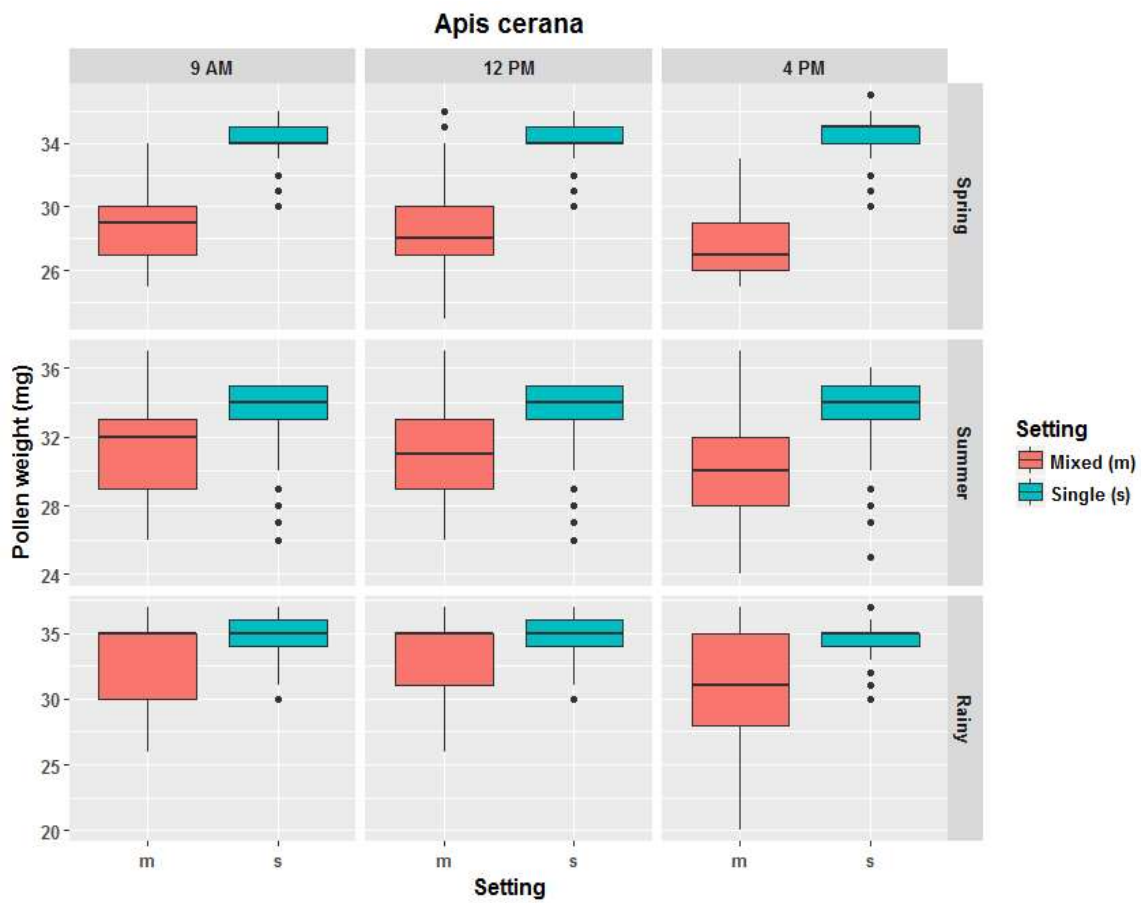
Figure 3-12

Figure 3-13. Box plot of pollen weight (mg) collected by pollen foragers (P) of *A. mellifera* in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
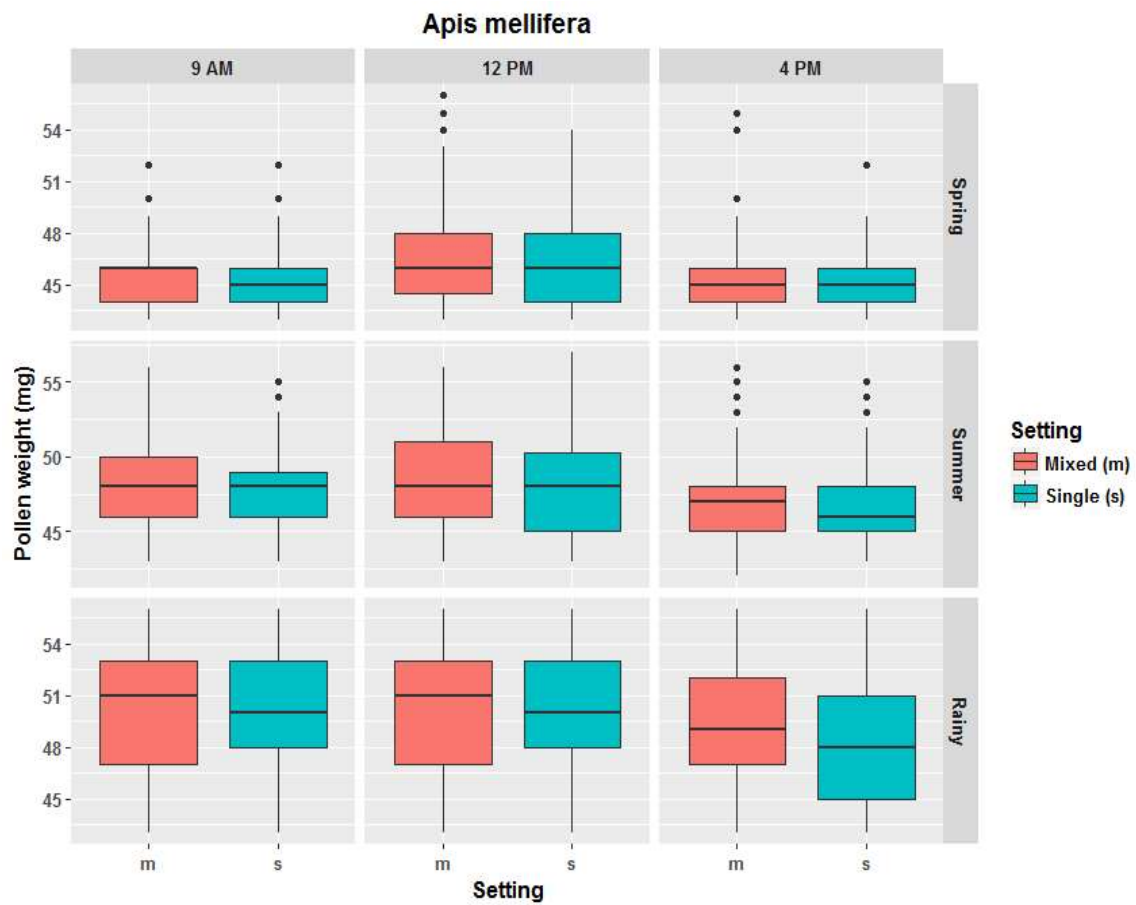
Figure 3-13

Figure 3-14. Box plot of nectar volume (μl) collected by nectar foragers (N) of *A. cerana* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
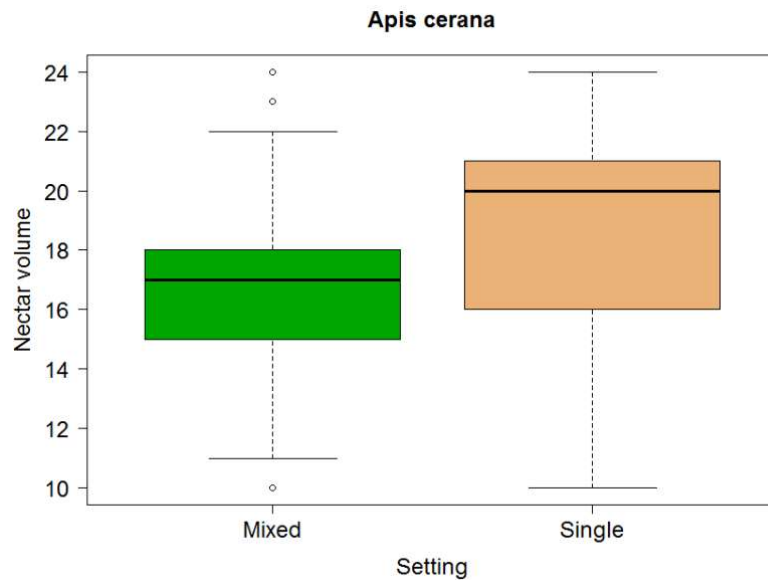
Figure 3-14

Figure 3-15. Box plot of nectar volume (μl) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
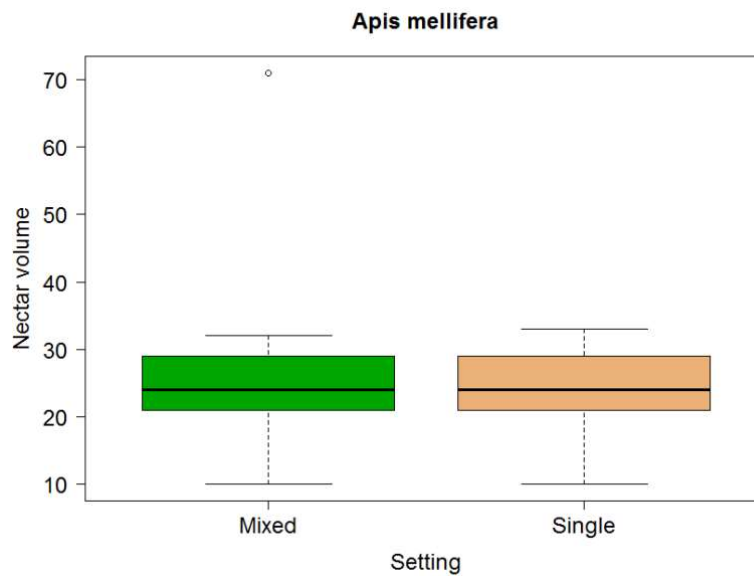
Figure 3-15

Figure 3-16. Box plot of nectar volume (μl) collected by nectar foragers (N) of *A. cerana* foragers in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
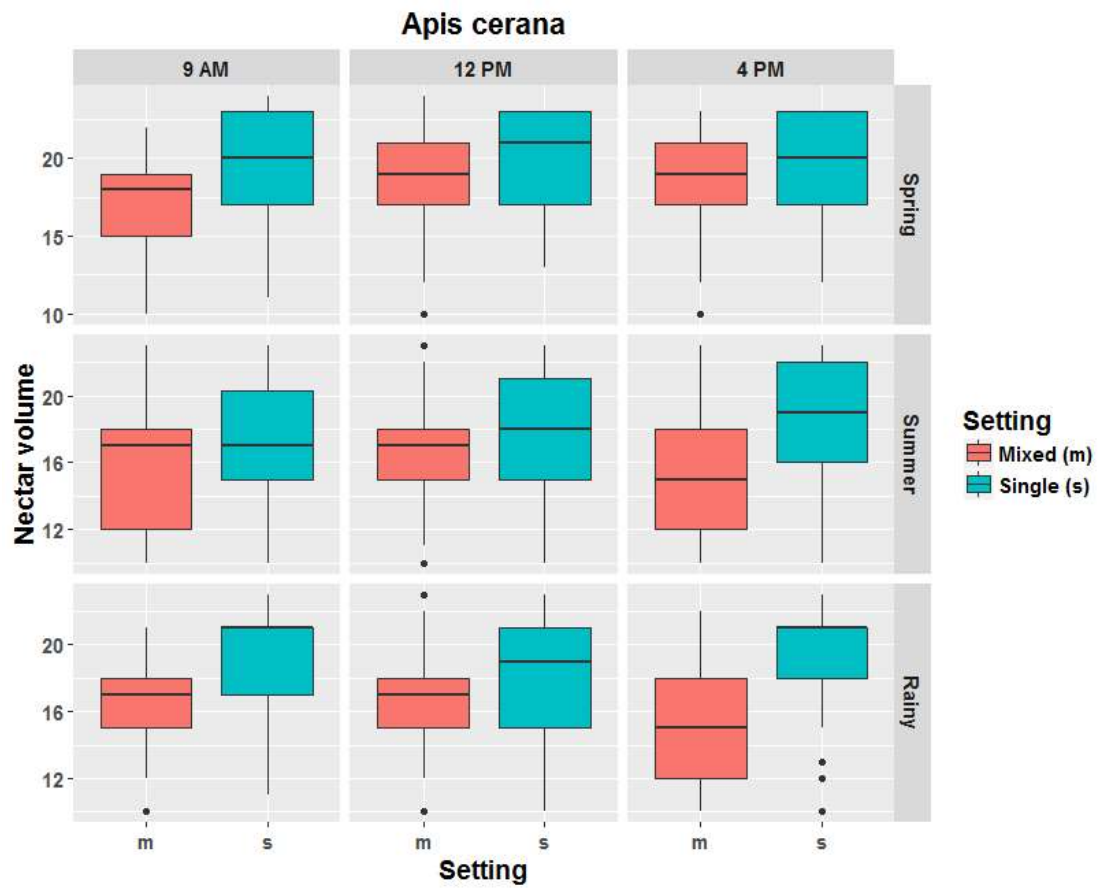
Figure 3-16

Figure 3-17. Box plot of nectar volume (μl) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
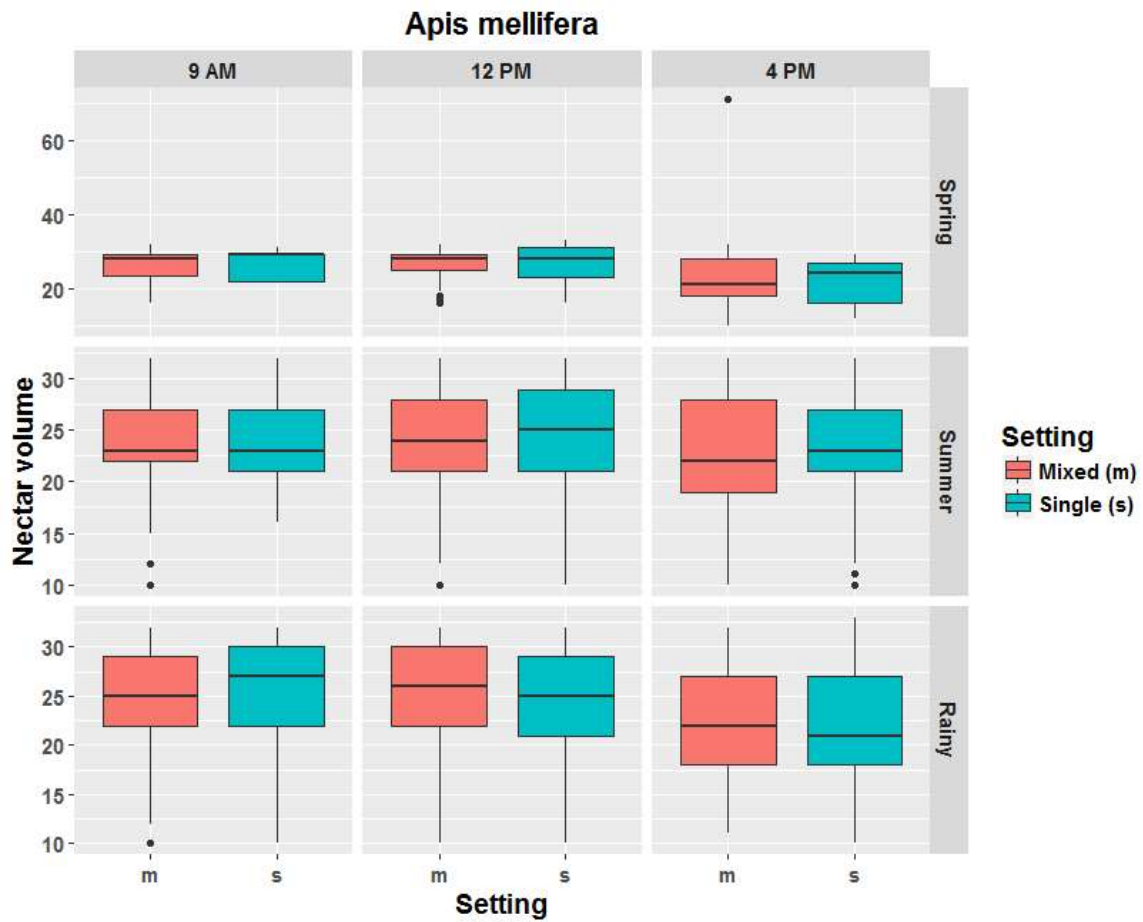
Figure 3-17

Figure 3-18. Box plot of nectar concentration (% Brix) collected by nectar foragers (N) of *A. cerana* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
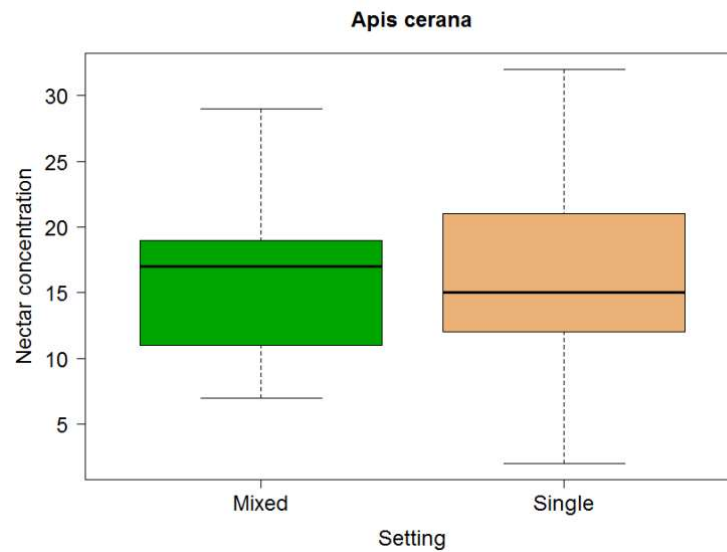
Figure 3-18

Figure 3-19. Box plot of nectar concentration (% Brix) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
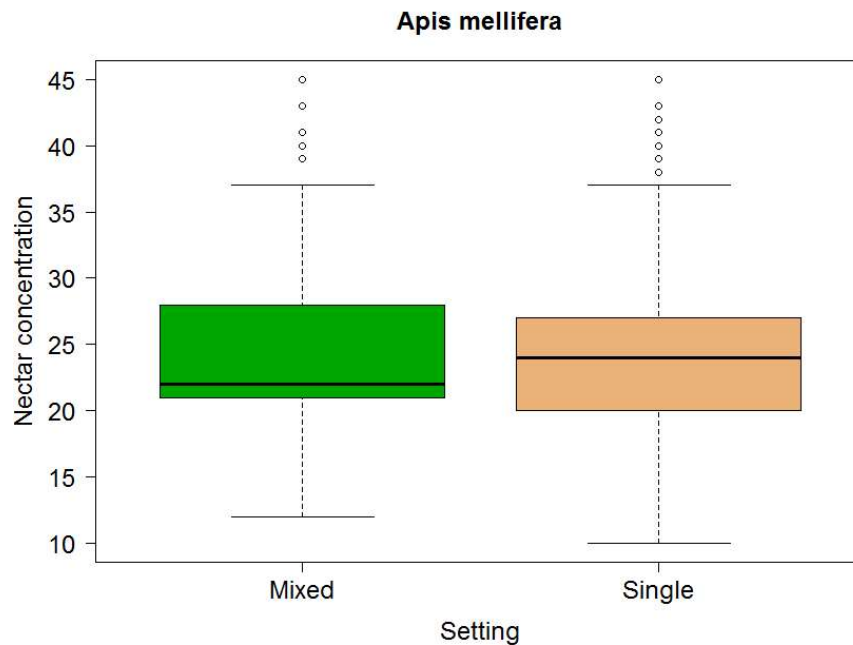
Figure 3-19

Figure 3-20. Box plot of nectar concentration (% Brix) collected by nectar foragers (N) of *A. cerana* foragers in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
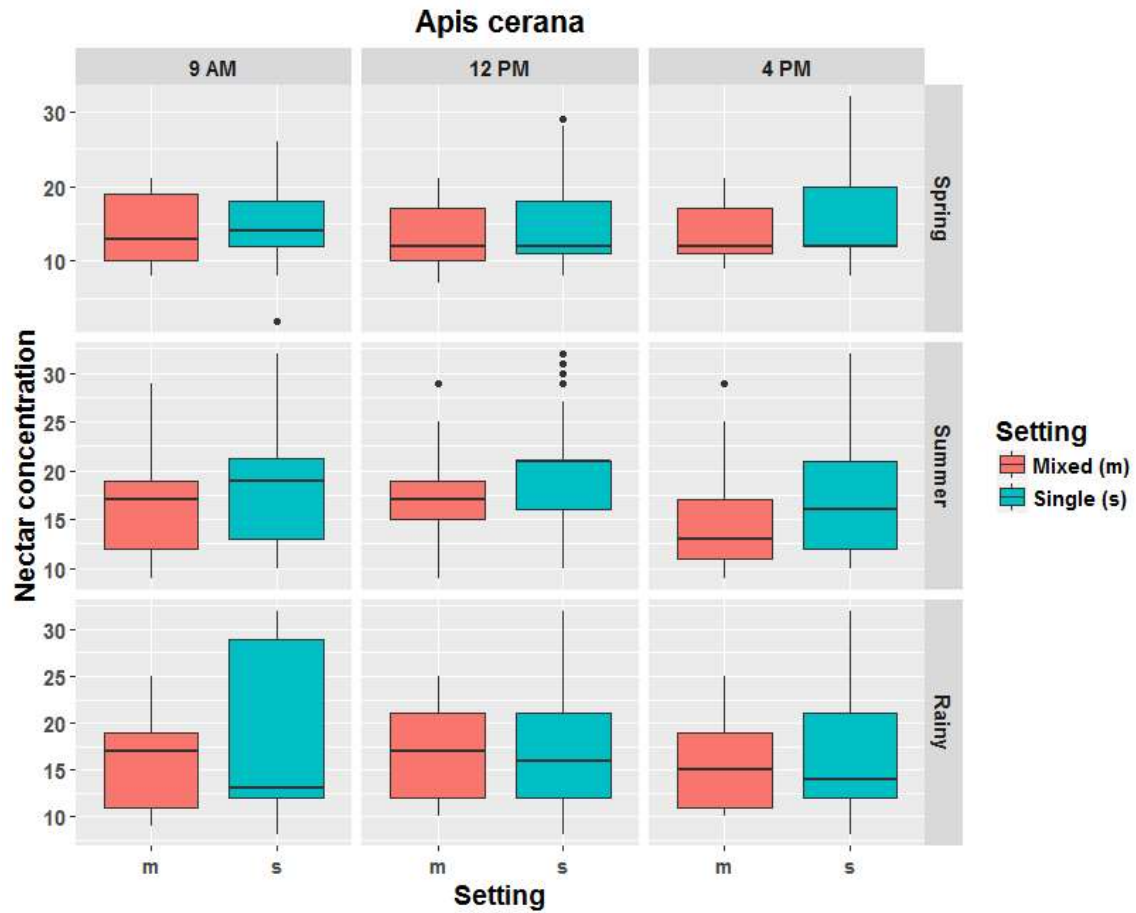
Figure 3-20

Figure 3-21. Box plot of nectar concentration (% Brix) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
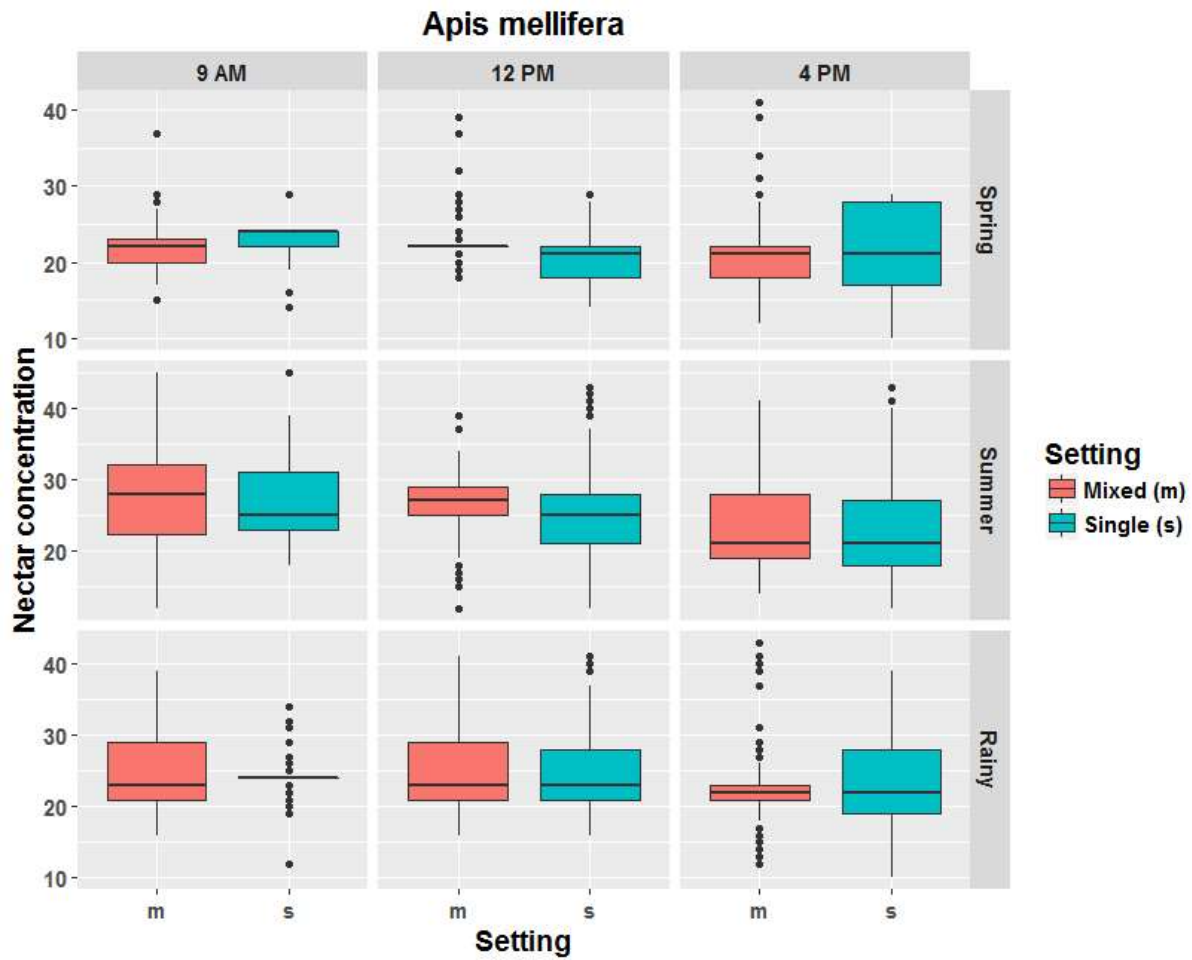
Figure 3-21

Figure 3-22. Box plot of amount of sugar in nectar (mg) collected by nectar foragers (N) of *A. cerana* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
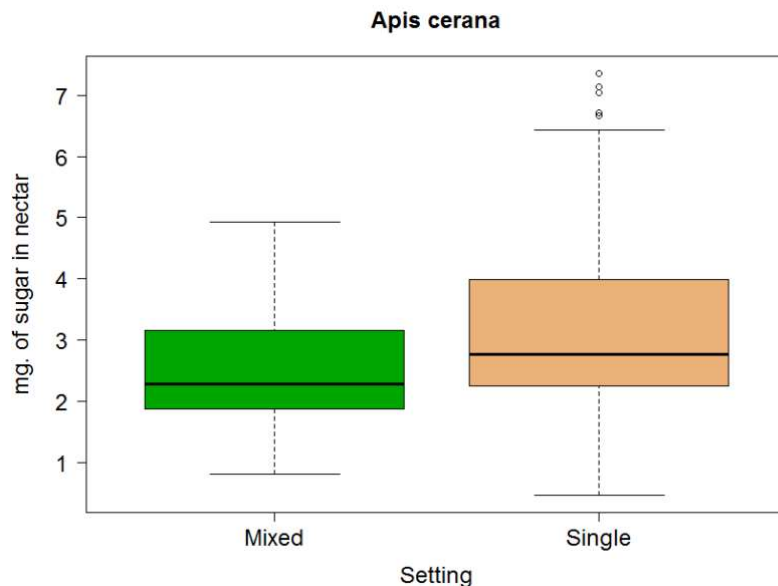
Figure 3-22

Figure 3-23. Box plot of amount of sugar in nectar (mg) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings throughout the experimental period (March-August) in all seasons (spring, summer, and rainy) and at all sampling times (9:00 AM, 12:00 PM, and 4:00 PM).
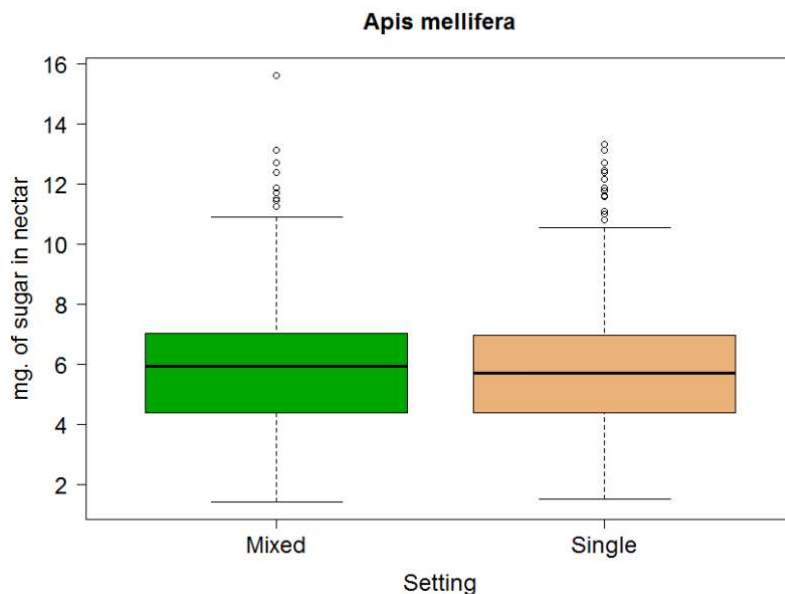
Figure 3-23

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Figure 3-24. Box plot of amount of sugar in nectar (mg) collected by nectar foragers (N) of *A. cerana* foragers in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
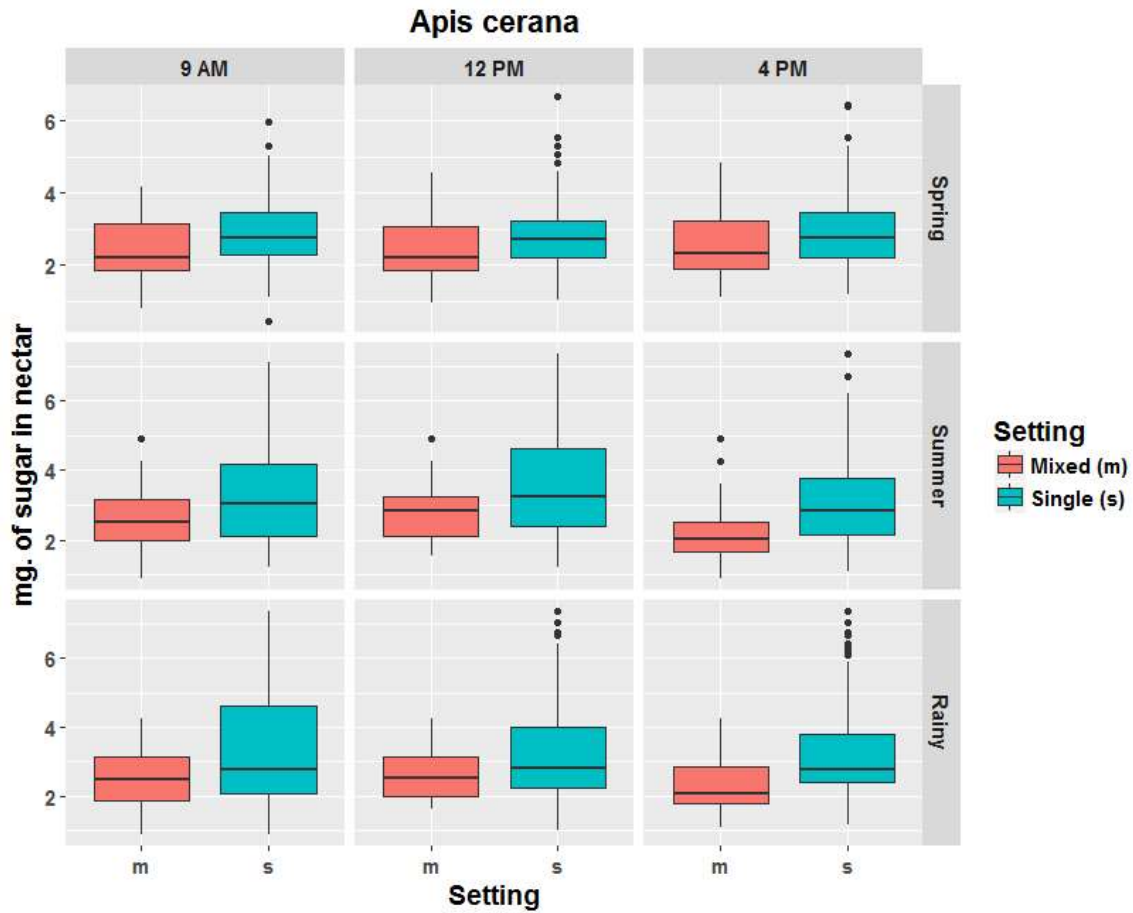
Figure 3-24

Figure 3-25. Box plot of amount of sugar in nectar (mg) collected by nectar foragers (N) of *A. mellifera* in mixed-species and single-species apiaries settings broken down into all seasons and all sampling times.
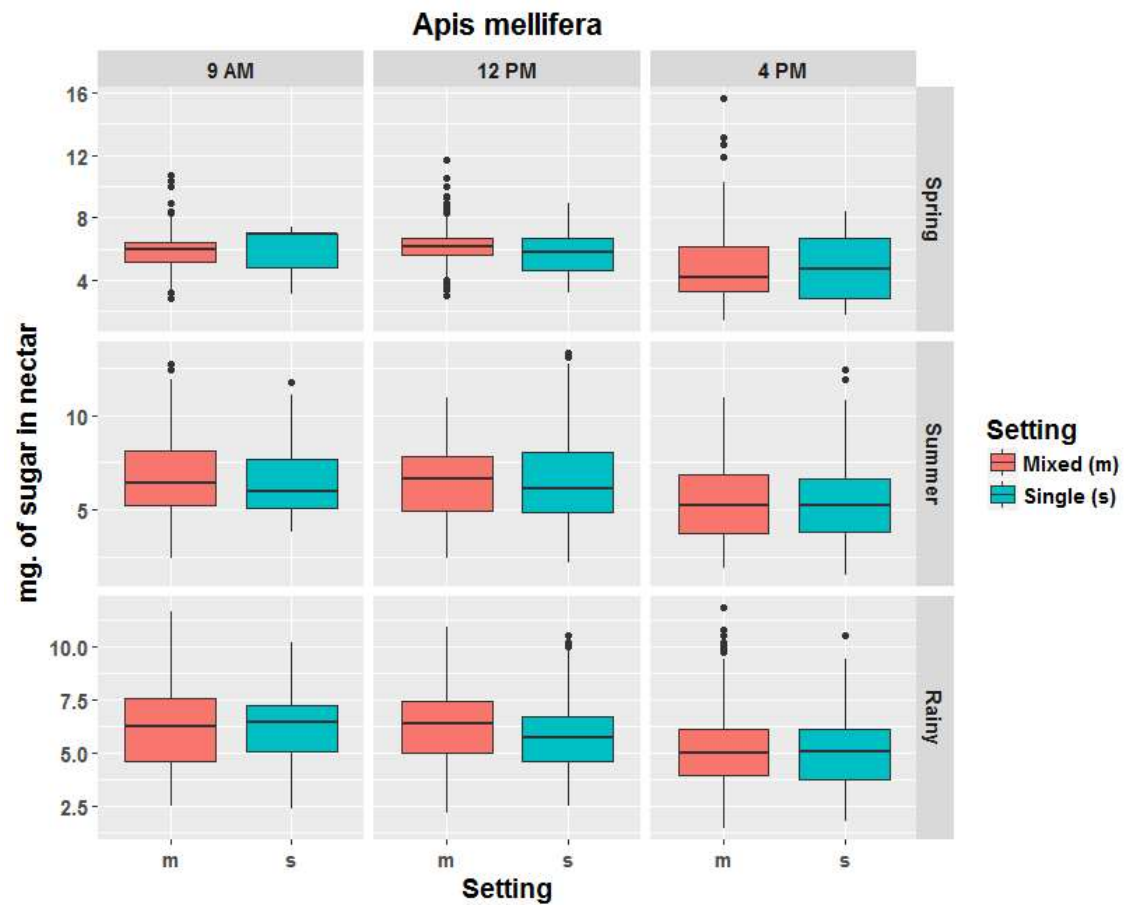
Figure 3-25

Figure 3-26. Average weight of pollen (mg) collected by *A. cerana* foragers at 5:30 AM when *A. mellifera* foragers were yet to commence and at 9:00 AM when both species were actively foraging in summer and rainy seasons.
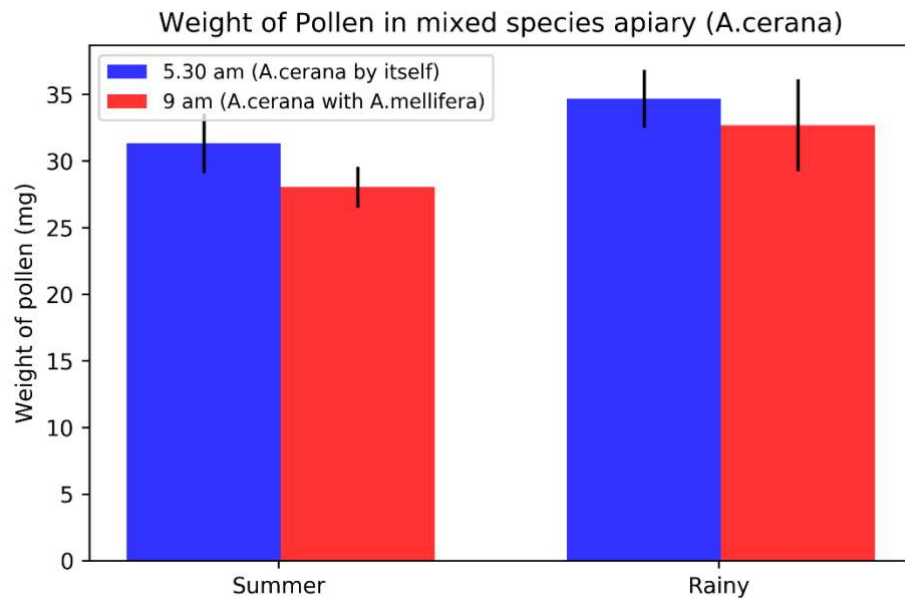
Figure 3-26

Figure 3-27. Average volume of nectar (µl) collected by *A. cerana* foragers at 5:30 AM when *A. mellifera* foragers were yet to commence and at 9:00 AM when both species were actively foraging in summer and rainy seasons.
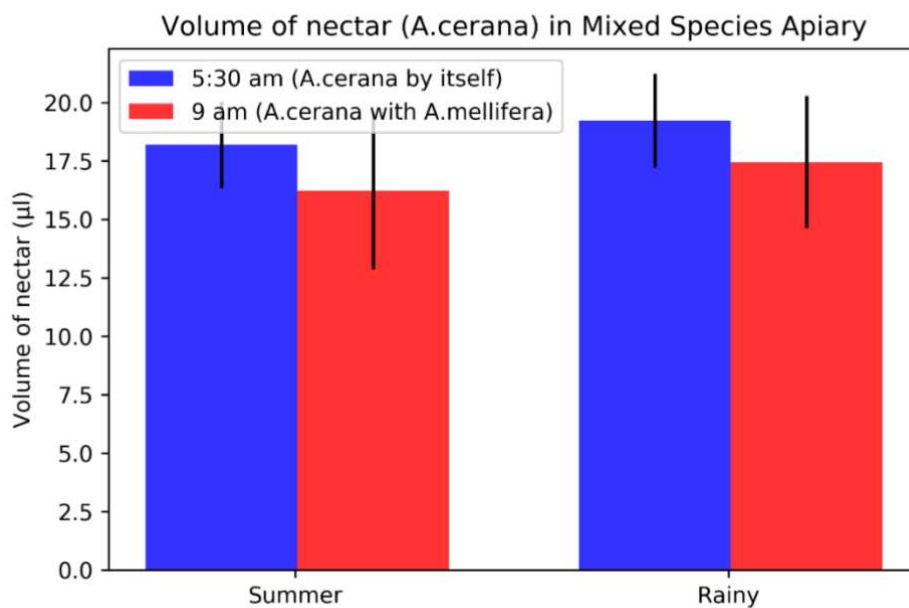
Figure 3-27

Figure 3-28. Average nectar concentration (% Brix) collected by *A. cerana* foragers at 5:30 AM when *A. mellifera* foragers were yet to commence and at 9:00 AM when both species were actively foraging in summer and rainy seasons.
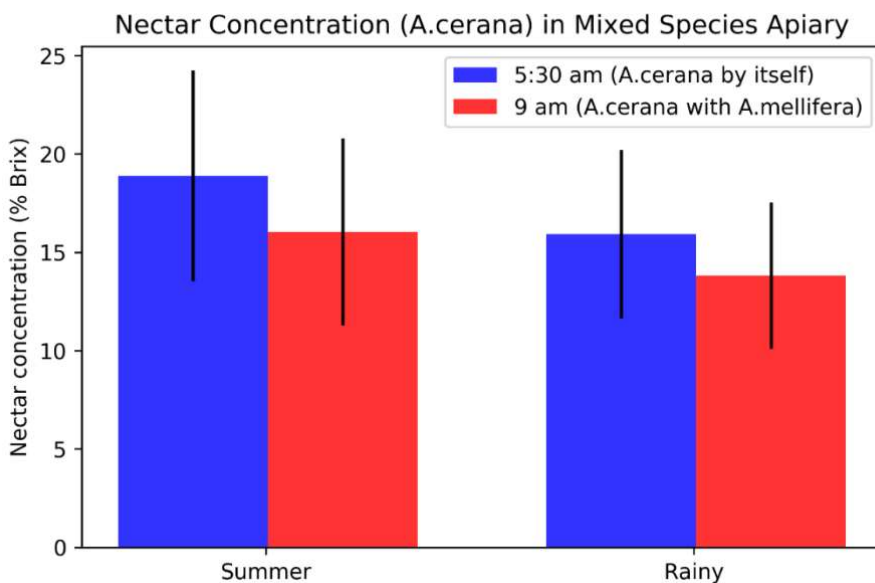
Figure 3-28

Figure 3-29. Average amount of sugar in nectar (mg) collected by *A. cerana* foragers at 5:30 AM when *A. mellifera* foragers were yet to commence and at 9:00 AM when both species were actively foraging in summer and rainy seasons.

Figure 3-29

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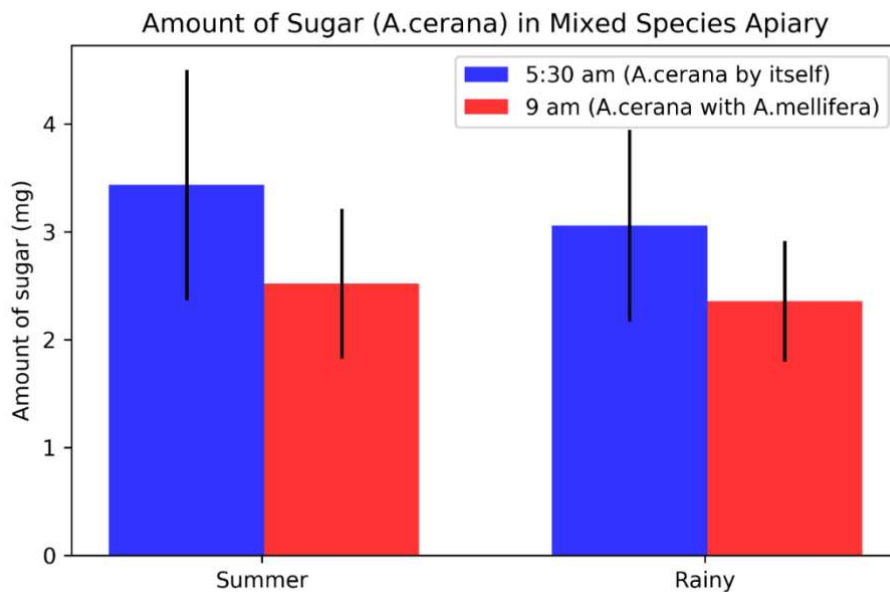
Figure 3-30. Average full-day pollen weight collected in pollen traps by two colonies of *A. cerana* at mixed-species apiaries and single-species apiary in spring, summer, and rainy seasons.
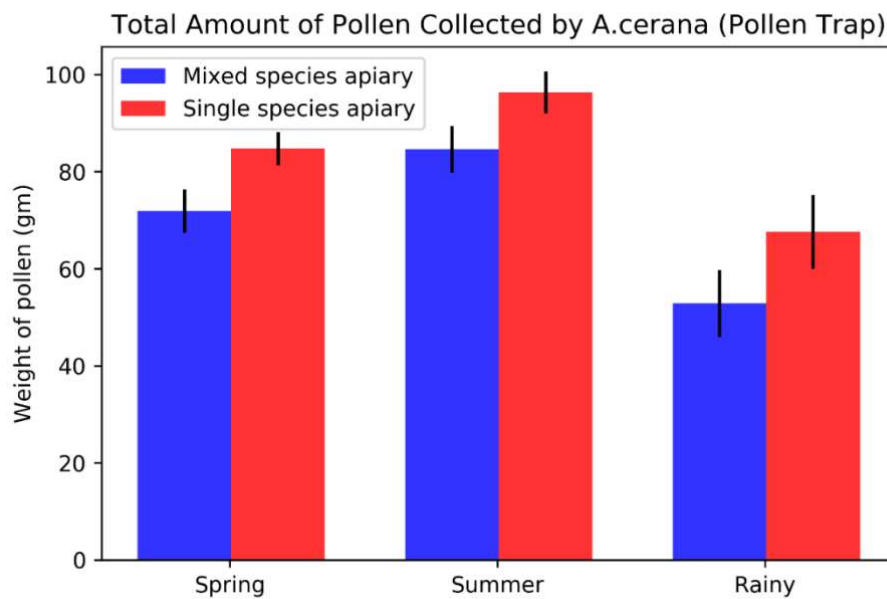
Figure 3-30

Figure 3-31. Average full-day pollen weight collected in pollen traps by two colonies of *A. mellifera* at mixed-species apiaries and single-species apiary in spring, summer, and rainy seasons.
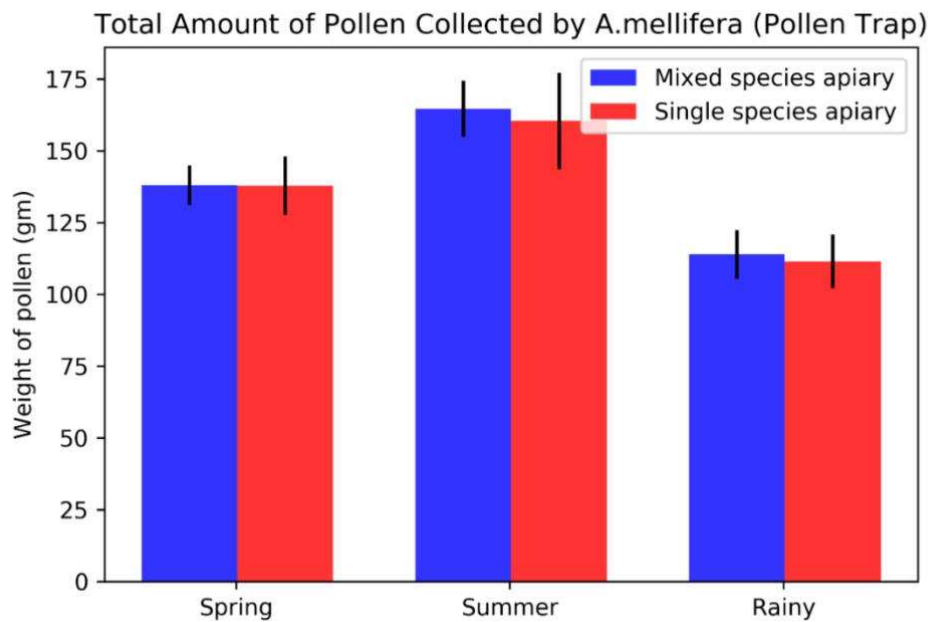
Figure 3-31

Figure 3-32. Histogram of 1000 simulated niche overlap values (blue bars). The vertical red line indicates the observed niche overlap value from the original data, the pair of vertical long-dash lines indicates the 95% one-tailed cut points, and the short-dash lines indicate the 95% two-tailed cut points.
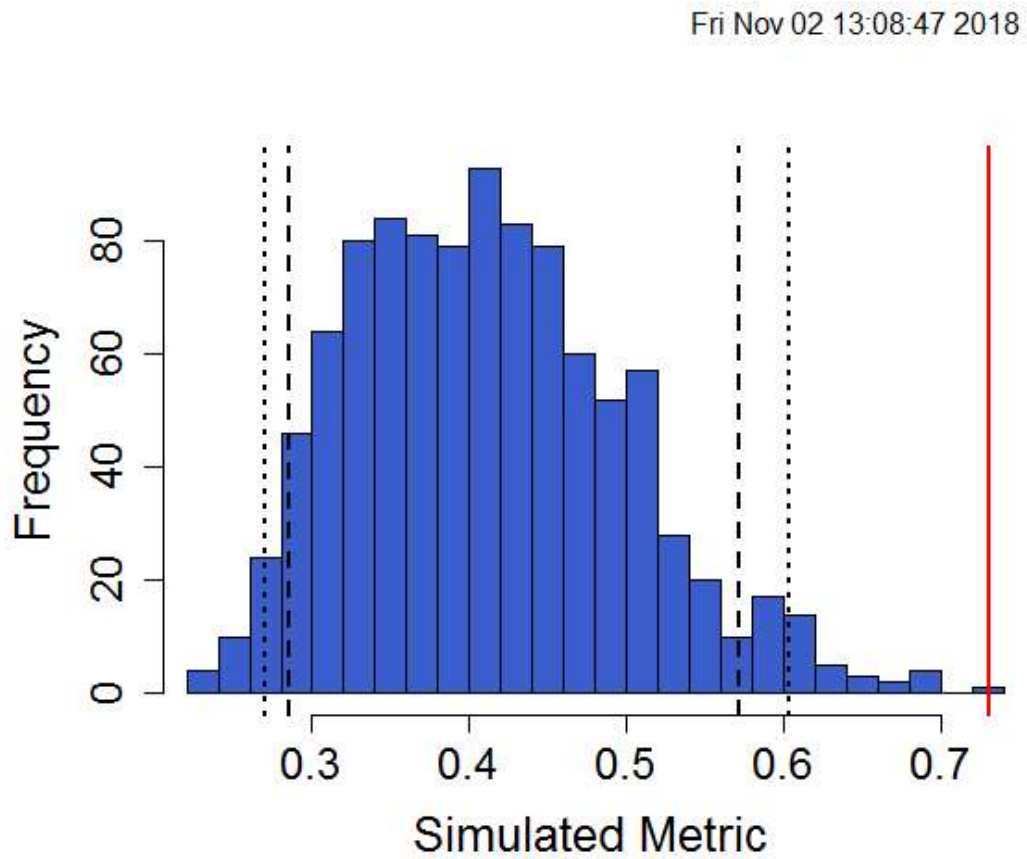
Figure 3-32

Figure 3-33. A rendering of the species × utilization matrix for the original data matrix (red) and one simulated data matrix (blue). The area of each circle depicted is proportional to the utilization of a pollen category by either *A. cerana* (Species 1) or *A. mellifera* (Species 2) and if there is no circle that means there is no utilization of that pollen category.
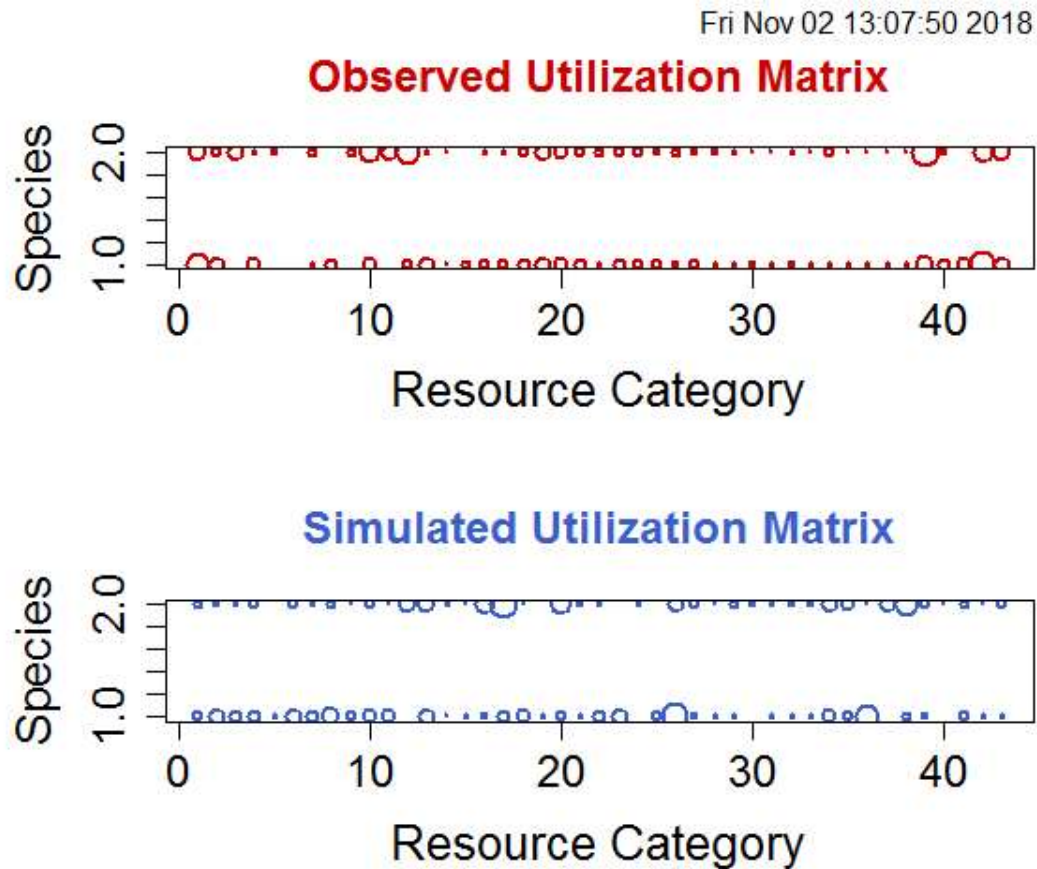
Figure 3-33

Tables and table legends

Table 3-1. Robust repeated-measures ANOVA results for pollen weight collected by *A. cerana* and *A. mellifera* in mixed-species apiaries and single-species apiaries.

Species	Season	F	Numerator DF	Denominator DF	p-value
Apis cerana	spring	3750.72	1	81.95	<0.001
	summer	258.97	1	107.11	<0.001
	rainy	41.68	1	113.66	<0.001
Apis mellifera	spring	0.95	1	77.77	0.33
	summer	4.99	1	79.14	0.0283
	rainy	0.19	1	125	0.66

Table 3-1

Table 3-2. Robust repeated-measures ANOVA results for nectar volume weight collected by *A. cerana* and *A. mellifera* in mixed-species apiaries and single-species apiaries.

Species	Season	F	Numerator DF	Denominator DF	p-value
Apis cerana	spring	37.94	1	75.1	<0.001
	summer	47.14	1	60.42	<0.001
	rainy	179.49	1	106.66	<0.001
Apis mellifera	spring	0.02	1	95.29	0.88
	summer	0.09	1	115.4	0.76
	rainy	0.16	1	127.78	0.69

Table 3-2

Table 3-3. Robust repeated-measures ANOVA results for concentration of nectar collected by *A. cerana* and *A. mellifera* in mixed-species apiaries and single-species apiaries.

Species	Season	F	Numerator DF	Denominator DF	p-value
Apis cerana	spring	3.72	1	66.1	0.0581
	summer	14.27	1	61.39	<0.001
	rainy	1.35	1	126.44	0.247
Apis mellifera	spring	0.03	1	92.19	0.85
	summer	1.96	1	111.98	0.16
	rainy	3.04	1	115.97	0.08

Table 3-3

Table 3-4. Robust repeated-measures ANOVA results for mg of sugar in nectar weight collected by *A. cerana* and *A. mellifera* in mixed-species apiaries and single-species apiaries.

Species	Season	F	Numerator DF	Denominator DF	p-value
Apis cerana	spring	22.92	1	57.46	<0.001
	summer	38.45	1	54.59	<0.001
	rainy	33.98	1	126.42	<0.001
Apis mellifera	spring	0.06	1	86.49	0.81
	summer	0.54	1	109.19	0.46
	rainy	0.11	1	121.18	0.74

Table 3-4

Table 3-5. Mean pollen weight (mg±SE), nectar volume (µl±SE), nectar concentration (%brix±SE), and mg of sugar in nectar (mg±SE) collected by *A. cerana* colonies in mixed-species apiaries at 5:30 AM when *A. mellifera* foragers were yet to commence foraging activities and at 9:00 AM when both species were actively foraging.

Species	measurement	5:30 AM	9:00 AM	p-value (t-test)
	Pollen weight (mg)	33.07±0.10	31.15±0.13	t (1554.9) = -11.83, p< 2.2e-16
Apis cerana	Nectar volume (µl)	18.78±0.09	16.81±0.14	t (978.17) = -11.968, p< 2.2e-16
	Nectar concentration (%brix)	17.01±0.23	14.82±0.19	t (981.73) = -7.3894, p = 3.15e-13
	mg of sugar in nectar (mg)	3.19±0.05	2.42±0.03	t (824.43) = -14.275, p < 2.2e-16

Table 3-5

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Chapter 4

Nesting biology, niche modelling, and traditional uses of stingless bees in Nepal

Abstract

Indigenous and non-indigenous peoples in tropical and subtropical areas of the world use stingless bees for diverse purposes. Scattered records indicate that people keep stingless bees in Nepal, but there is no published information on their identity or natural history and no record of traditional knowledge about them. I investigated stingless bees in Nepal through field surveys and ethnographic research across the Terai and Pahad regions (21 districts and 12 zones) and identified a single species, provisionally identified as *Tetragonula iridipennis* Smith (Hymenoptera: Apidae, Meliponini), in nine districts and seven zones. Nesting biology, local indigenous nomenclature, uses, traditional management practices, and knowledge of its behavior were documented for the first time from the country. In addition, the relative cultural importance of this species for each ethnic community (Chhetri, Brahmin, Tharu, and Kirat) that participated in the study was determined. Nests of *T. iridipennis* were found inside the cavities of medium to very large canopy trees of economic and cultural importance, such as trees used as timber and animal feed. I documented eighteen specific uses of bee products in food, medicine, crafts, and religious beliefs, which people largely exploit through an extractive management practice. Niche models suggest a broad distribution of *T. iridipennis* in southern Nepal and, in combination with my field data, areas where it may be locally extinct and areas where it may have been introduced by humans. Finally, the conservation status and future directions for the sustainable use of this bee species in the country was discussed.

Key Words

Tetragonula, relative cultural importance, ethnobiology, distribution

Introduction

Stingless bees (Apidae: Meliponini) are among the most significant groups of bees in terms of their economic, ecological, and cultural importance. They live in perennial colonies, have a complex social organization, and are often the most commonly encountered bees in tropical and subtropical areas (Michener 2007). They play a substantial role in maintaining the world's biodiversity through pollination services, even though they represent less than three percent of the bee species worldwide (Klein et al. 2007). They are the main pollinators of both native and cultivated plants, including important global commodities such as coffee (Slaa et al. 2006). One-fourth of the more than 1000 plant species cultivated in the tropics for food, beverages, fiber, spices, and medicines (Purseglove 1968, 1972, Roubik 1995) are likely pollinated by stingless bees. Recent trends of the global pollinator crisis (Brosi et al. 2007) suggest that human activities are negatively affecting the diversity and abundance of stingless bees (Kearns et al. 1998, Liow et al. 2001, Samejima et al. 2004). This has raised concerns in science and society (Reyes-González et al. 2014) due to the evident consequences to global food security, crop-based economies, and people's livelihoods (Potts et al. 2010, Potts et al. 2016).

Stingless bees are also an integral part of many cultures, both past and present. The nesting provisions, including honey and wax, are used by many people for a variety of purposes (Michener 2007) Several species are deeply embedded in the indigenous knowledge of many societies, as they represent a natural source of food, craft materials, folk medicine, and alternative income e.g. (Stearman et al. 2008, Ayala et al. 2013, Villanueva-Gutiérrez et al. 2013, Reyes-González et al. 2014, Vit et al. 2015). Stingless bees have been important in the social and religious life of Mesoamerican people since pre-Columbian times. These people created and practiced stingless bee management techniques that are now commonly known as meliponiculture (Crane 1992, Cortopassi-Laurino et al. 2006). The ancient traditional practices of the Mayan of the Yucatán Peninsula and the Nahuas and Totonac of the Sierra Norte in the state of Puebla are still in practice today (Foster 1942). Comparatively little is known of the history and ethnography of stingless bee usage in Asia.

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The nesting habits and nest architecture of stingless bees vary greatly not only among genera, but also among species within a genus (Roubik 1982, Sakagami et al. 1993). Stingless bees occur primarily at low or mid-elevations, although a few species inhabit high altitudes in the Andes (Gonzalez and Engel 2004). Unlike the honeybees, stingless bees nests are primarily found inside the forest cover (Brown et al. 2016). Approximately 500 species of stingless bees are known from tropical and sub-tropical regions of the world (Michener 2007). The Eastern Hemisphere is home to only about 20% of the total number of stingless bees, most them distributed across the Indo-Malayan and Australasian Regions. Despite the relatively small number of species in the Eastern Hemisphere, this stingless bee fauna remains largely unexplored (Engel et al. 2017, Rasmussen et al. 2017) and the indigenous and local knowledge of these bees are poorly documented. For example, although Nepal and other countries of the Indian sub-continent are home to several species of *Apis* L. honey bees and *Apis* beekeeping is an ancient and well documented tradition (Batra 1977, Bhatta and Tamrakar 2009), there is no comprehensive study of stingless bees in this region. To date, only brief records exist on the occurrence of stingless bees in Bangladesh (Hannan 2007) and Nepal (Uma 1999, Gurung et al. 2003, Bhatta 2005) primarily in the context of beekeeping activities with *Apis*. Crane (1999) reported the existence of meliponiculture in Nepal, and Uma (1999) indicated that *Melipona* sp. and *Trigona* sp. were occasionally used in the midwestern region of Nepal. However, advances in the phylogenetics of stingless bees (e.g., Rasmussen and Cameron 2007a, Rasmussen and Cameron 2007b) make Partap's records difficult to evaluate. The genus name *Melipona* Illiger was applied to some species now placed in the genera *Lisotrigona* Moure, *Homotrigona* Moure, *Lepidotrigona* Schwarz, *Tetragonula* Moure and *Tetrigona* Moure, while *Trigona* Jurine was applied to virtually all Indo-Malaysian and Australasian stingless bees, which are now placed in 15 genera (Rasmussen 2008b). Currently, the genera *Melipona* and *Trigona* are each now recognized as strictly Neotropical (Rasmussen and Cameron 2007a).

The purpose of this work is to document for the first time a species with in the *Tetragonula iridipennis* Smith (Hymenoptera: Apidae, Meliponini) species group in Nepal, and to provide information

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on its nesting biology as well as ethno-entomological information on indigenous knowledge, uses and management practices. This dissertation chapter offers a quantitative assessment of the relative cultural importance of this *T. iridipennis* for the ethnic communities that participated in this study. In addition, using species distribution modelling (SDM), the potential distribution of this species in Nepal was also estimated and future directions for the conservation and sustainable use of this species in the country were discussed.

Methods

Bee surveys

During the summer of 2016, I conducted field surveys across the lowlands and mid-hills regions in 21 districts and 12 zones of Nepal (Table 4-1, Supplementary materials table S1, and Figure 4-1). Bee nests were located with the assistance of local people (Figure 4-2). Whenever a nest was found, I recorded the geographical coordinates using a hand-held GPS device (Garmin eTrex 10) and recorded the following features: type of nesting substrate (wood, wall, or tree cavity); height above ground of the nest entrance; maximum length and diameter of the nest entrance tube; number of bees guarding the nest entrance; and defensive behavior (aggressive or docile) when approached by an observer. If nests were in a tree, we recorded the maximum diameter of the branch or trunk. Tree species were determined with the help of local people and experienced forestry staff, as well as with the assistance of a plant taxonomist, Mr. Kuber Jung Malla, and the reference collection at the National Herbarium Center, Godawari, Nepal.

I documented the internal architecture of three nests, one located inside the adobe wall of a house and two that people cut from trees and brought to the village (Table 4-2). I opened nests using axes, chisels, and pocket knives. I estimated the volume of the cavity occupied by the nest, the volume of the nest structure built by the bees, and the population size of the brood and adult bees. As in Roubik (1979), I estimated the total number of brood cells by counting the numbers of brood cells in one brood cluster of known volume for a measure of cells per unit volume and multiplied this value by the total volume

occupied by all brood cells. I estimated the adult bees' population by counting the number of bees captured inside a mosquito net after we opened the nest. On the night prior to opening the nest, I closed the nest entrance after all foragers had returned and placed the nest inside a large mosquito net. In the morning, I opened the nest and counted adults in the net.

I used calipers to measure internal nest features, including height and diameter pollen and honey pots and brood cells. I also noted the presence or absence of typical stingless bee nest structures, including batumen layer, involucrum layer, and supporting pillars (Michener 2007), as well as the general layout of the brood, pollen and honey stores. Terms used in the description of the nest architecture follow those of (Michener 2007). For each nest found, I collected five to ten worker bees and placed them in ethanol (90%).

Bee identification and voucher specimens

Bee specimens from each nest were examined and identified by using the keys of (Sakagami 1978) and (Rasmussen 2013) and by comparing them with specimens of *Tetragonula* spp. deposited in the Snow Entomological Collection, University of Kansas Natural History Museum (SEMC), Lawrence, Kansas, USA. Specimens matched most closely the descriptions of *T. iridipennis* in the *T. iridipennis* species group. However, species in this group are notoriously difficult to identify (Sakagami 1978), species limits are uncertain (Rasmussen 2008a, Engel et al. 2017), and cryptic species may remain to be discovered. Thus, final identification of specimens may require a molecular survey and revision of the genus. Voucher specimens are deposited in the Snow Entomological Collection, University of Kansas Natural History Museum, Lawrence, Kansas, USA.

Traditional knowledge

Using semi-structured interviews, I obtained data on the common names, nesting substrates, management practices, and local uses of stingless bees. I sought cultural consultants using the snowball-

sampling method (Bailey 1987, Bhatta and Bardecki 2014), in which a network of experts is built on the recommendation of informants themselves. I interviewed nine male local villagers ranging in age from 43 to 73 (58.6 ± 10.9, $n = 9$) in places where bees were observed and sampled. I interviewed one consultant from each district, except for Banke, where we were able to work with two informants. I was not able to interview anyone in Dang district. Among informants, two were from the far-western development region (FWDR), three from the mid-western development region (MWDR), two from the western development region (WDR), and one each from the central (CDR) and eastern development regions (EDR). Informants are from four different ethnic communities: Chhetri, Brahmin, Tharu, and Kirat. Three of the informants interviewed in Kailali, Bardiya, and Kapilvastu districts are Tharu, an indigenous ethnic people living in the lowlands of Nepal (Meyer and Deuel 1998). I recorded interviews using a Sony IC Recorder (ICD-BX112) and gathered ethnographic information while using the "walk-in-the-woods" method (Phillips and Gentry 1993), in which informants were actively involved in looking for known locations of nests. Whenever informants found a nest, I asked them for the local name, local uses, and other information about the bee behavior. In addition, I used participant observation (DeWalt and DeWalt 2002, Bhatta and Bardecki 2013) to facilitate the interviews process. As I am a native of Nepal and have experience working with Community Based Organizations in the country, informal conversations with the informants helped to understand both 'explicit' and 'tactic' details about the research in questions (Bhatta and Bardecki 2013)

Relative cultural importance

To analyze the cultural use of stingless bees by the different communities, I grouped all mentioned uses into several categories (#C) and specific uses (#U) and employed a "uses totaled" or "researcher tally" method (Phillips 1996). I divided all recorded specific uses (18 total) into four categories: food, medicine, crafts, and others/beliefs (Table 4-6). To calculate the relative cultural importance (RCI), I modified an index developed by (Bennett and Prance 2000) to calculate the relative importance of medicinal plants, which is calculated on the sum of the proportion of the number of use

categories (C) and the proportion of the number of specific uses (U) multiplied by 50 ([C + U] × 50). Thus, this index is expressed in a scale from 0 to 100 (Table 4-7). For example, the community in EDR used stingless bees in two of the four use categories, and it thus has a C of 2/4 = 0.5. They reported only 2 out of the 18 total uses recorded, thus it has a U of 2/18 = 0.11. Therefore, for the human community in EDR, stingless bees have a RCI of 30.5 ([0.5 + 0.11] × 50). I chose this index because it is simple to calculate and requires the least amount of data collection (i.e., small number of informants and short field surveys) in comparison with other indices. It is worth noting that this index does not distinguish relative degrees of importance for different uses or between current and previous uses (for a discussion see, (Hoffman and Gallaher 2007).

Species distribution modelling

I obtained occurrence data for *T. iridipennis* in the narrow sense from Global Biodiversity Information Facility (GBIF; www.gbif.org; accessed 20 November 2017), Snow Entomological Collection, Division of Entomology, University of Kansas Natural History Museum, Lawrence, Kansas, USA, and the field collections conducted by the first author in Nepal (see results below). I assembled 74 unique records, 39 of them corresponding to localities in Nepal (Supplementary materials table S3). Remaining records are from localities in India, Sri Lanka, Myanmar, Malaysia, and Indonesia (Supplementary materials table S2).

I obtained environmental data from WorldClim (version 1.3, http://www.worldclim.org; (Hijmans et al. 2005), which contains climate data (i.e., monthly precipitation and monthly mean, minimum and maximum temperatures) at spatial resolution of 0.1° (ca. 10 × 10 km resolution) that is obtained by interpolation among climate-station records from 1950 to 2000. I excluded 4 of 19 available bioclimatic variables in WorldClim (mean temperature of wettest quarter, mean temperature of driest quarter, precipitation of warmest quarter and precipitation of coldest quarter) because of known spatial artifacts. To avoid overfitting and inflating model accuracy with overly dimensional environmental spaces and

colinearity among variables, I performed a principal component analysis (PCA) on the correlation matrix of the remaining 15 environmental variables using the *PCARaster* function in the package 'ENMGadgets' (Barve and Barve 2013) in R 3.3.1 software. I retained the first four components, which explained cumulatively >95% of the total variance in the dataset, for model calibration.

To estimate the potential distribution, we used MaxEnt version 3.3.3.k (Phillips et al. 2006), as implemented in the R package 'dismo'. I used default settings in model calibration and used 20 replicate bootstrap runs using 20% of calibration data (i.e., total number of occurrence records). Because of the total number of training sites was low, the bootstrap replication technique, which involves sampling from the original occurrence locations with replacement, helps to avoid losing valuable training data for model development (Phillips 2017).

To characterize the distribution of *T. iridipennis* in Nepal, I used two models. In the first (model 1), I predicted *T. iridipennis*'s potential distribution based on the occurrence records outside of Nepal (35 records), while in the second (model 2) I used all 74 available records, including Nepal (See supplemental material tables S2, S3). I chose to do this to determine if available records would predict *T. iridipennis*'s presence in Nepal and to estimate the gain in environmental suitability that occurrence points within Nepal would add to the model. I used individual calibration areas to both models, corresponding to areas likely to have been accessible to the species when occurring in Nepal (Barve et al. 2011). I processed and visualized model results using the GIS software and ArcGIS 10.4. I used partial receiver operating characteristic (partial ROC) approaches (Peterson et al. 2008), which considers only omission error and proportional area predicted as suitable, and only over a range of omission error deemed acceptable considering error characteristics of the input data. I used a Visual Basic routine developed by N. Barve (University of Kansas, Lawrence, KS; http://hdl.handle.net/1808/10059), using an omission error threshold of 5% (Peterson et al. 2008)). I performed 100 replicate analyses, each based on resampling 50% of test points with replacement, which is the customary proportion to maximize replicate-to-replicate variation and while still maintaining relatively high sample sizes (Peterson et al. 2008).

Results

Nesting biology

A single species of stingless bees, *T. iridipennis*, was identified and 38 nests were recorded from nine of the 21 districts and seven of 12 zones we surveyed (Table 4-1, Figure 4-1, and Supplementary materials table S1). Elevations of nesting sites ranged from 112 m at Gorusinghe, Kapilvastu to 965 m at Baddanda, Argankanchi. I did not find any stingless bee nest above 1000 m of elevation, although we searched for them at many locations from mid-hills up to 2000 m in similar nesting substrates (Table 4-1). River basin plains (19 of 38 nests) were the most common landforms where we found nests, followed by valleys and a few hillslopes at mid-hill elevations (Table 4-3). Vegetation at nesting sites included tropical and subtropical forest followed by crop and pasture lands. The climate of the locations where we found nests ranges from warm humid plains with summer precipitation of 800–1700 mm, to sub-humid mid-hills characterized by summer precipitation of 2000–2500 mm (GoN 2016).

I found nests at heights, from 0.5– 3.0 m above ground (Table 4-5). The majority of nests (31out of 38 nests, 81.6%) were in hollow cavities inside tree trunks, with an entrance tube emerging from openings in the tree trunk. A few nest entrances (4 out of 38, 10.5%) were spotted in cracks in the house walls, with or without long entrance tubes. A few nests (3 out of 48, 7.9%) were found in wall hives designed for catching swarms of Asian honey bees, *Apis cerana* (Fabricius) (Table 4-5, Figure 4-4C). The maximum circumference of tree trunks occupied by stingless bees ranged from 54 to 259 cm. While I was not able to measure the volume of nests built inside the cracks and openings of house walls, the volume of wall hives occupied by stingless bees ranged from 5668 to 7380 cm^3 (Table 4-4). Most trees used by *T. iridipennis* were medium to very large canopy trees, with *Shorea robusta* (Roth.) (Family Dipterocarpeaceae) being the most common (14 out of 38 nests, 36.8%), followed by *Dalbergia sissoo* (Roxb.) (7 out of 38, 18.4%), *Terminalia elliptica* (Wild.) (4 out of 38, 10.5%), *Senegalia catechu* (L.f.) Hurter & Mabb. (2 out of 38, 5.2%) (all Leguminoseae); *Bombax ceiba* (L.) (Malvaceae), (2 out of 38,

5.2%); *Ficus religiosa* (L.) (Moraceae) (1 out of 38, 2.6%); and *Haldina cordifolia* (Roxb.) (Rubiaceae) (*n* = 1, 2.6%) (Table 4-4). Among the 31 nests built in tree trunks, four had been collected in the form of a log hive by local beekeepers, who kept them in their backyards. Three nests (9.7%) were observed in dead trees, while remaining nests (77.4%) were in living trees.

Nest entrance

The nest entrance tubes observed were light brown to dark brown in color, made of resins mixed with granular foreign materials, such as soil or sand particles. The entrance tubes exhibited substantial variation in size and shape: circular, semi-circular, elliptical, and irregular (Figure 4-3; Table 4-5). Most nests had an entrance tube, which ranged from 5.0 to 15.0 mm in length and 4.5 to 13.5 mm in diameter. However, nests built in house walls had a short to absent entrance tube. The number of bees guarding the nest entrance range from 6 to 17.

Internal architecture

I observed architectural differences between nests built in tree trunks of trees and nests built in adobe walls of houses. Nests inside tree trunks had a batumen layer (mixture of resins and cerumen) lining the tree cavity but did not have an involucrum layer (a sheath made of a mixture of resin and cerumen) around the nest cells. In contrast, the nest inside the adobe wall did not have a batumen layer lining the cavity but did have a distinct involucrum (Figure 4-4C). Furthermore, nests in tree trunks had many stranded pillars (made up of resins and cerumen) of various sizes, most of them extending from the brood clusters to the wall of the nest cavity. I did not observe such pillars in the nest built inside the adobe wall.

The volume of the nests varied according to the size of the cavity in the tree or wall. The first log nest, opened at Mahendranagar, Kanchanpur, was in a cavity of approximately 6.5 L in volume; walls of the cavity were approximately 5 cm thick and the diameter of the log was 68 cm. The second log nest,

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opened at Ashrukot, Arghakhachi, was in a cavity of approximately 3.4 L with walls 3 cm thick and a log diameter of 35. The nest located in the adobe wall, opened at Naubasta, Banke, was in a wall cavity of 5.6 L, with a house wall 15 cm thick. A layer of plywood, about 1 cm thick, closed this wall cavity from the inside of the house.

Brood was arranged in tightly packed clusters of brood cells, not organized into layers or sheets of cells; each oval brood cell is about 3–4 mm long and yellowish brown in color (Figure 4-4B). The cocoon spun by a mature larva is visible after clearing out the waxes from the brood cell. There was no separate, inner involucrum covering the brood cells. Food resources are stored in separate clusters of waxen pollen and honey pots. Pollen pots (Figure 4-4B) are noticeably bigger than honey pots (Figure 4-4B). Pollen pots are 4.0–6.0 mm (4.8 mm ± 0.8 mm, n =16) in diameter and 8.0–11.0 mm (9.3 mm ± 1.9 mm, n = 16) in length, while honey pots are 3.0–5.0 mm (3.9 mm ± 1.6 mm, n = 16) in diameter and 7.0–9.0 mm (8.2 mm ± 1.6 mm, n = 16) in length. Clusters of pollen pots are located closer to the brood clusters than the honey pots (Figure 4-4B).

Rough estimates of the total number of brood cells ranged from 1600 to 3500 (2633 ± 961, n = 3), while the number of adult bees from 1100 to 1800 (1367± 379, n = 3). Unfortunately, I was not able to find queen and drone cells in any of the dissected nests.

Defensive behavior

When the nest entrance was blocked, and the researcher was collecting the sample specimens, returning foragers did not show any aggressive behavior. However, when I opened the three nests, many bees landed on hands, hairs, and eyes and used their mandibles to deliver light bites.

Common names

The ethnic communities in Nepal have given unique common names to *T. iridipennis* (Table 4-7). Chhetri people of FWDR and Brahmin people of CDR called them *puttka* while Brahmin people of WDR

and Chhetri people of MWDR called them *puttko* and *puttiko* respectively. Tharu people in all development regions called them *mangrasha* and Rai (Kirat) people from EDR recognized them as *dammer* bees. Our informants were unable to provide the meaning of the associated common names, except for *dammer*, which refers to the resins produced by dipterocarps trees. In fact, the Kirat people called them "*dammer* bees", as they collect resins from dipterocarp trees.

Traditional uses

I recorded 18 specific uses of *T. iridipennis* by different ethnic groups in Nepal and divided them into four categories of use (Table 4-6). The first category is "food," where informants mentioned that they consumed the honey, pollen and brood. Tharu people consume bee broods along with honey and pollen. Brahmin and Kirat people only use honey as a food, while Chhetri people use both honey and pollen as a food source. Some informants reported that every season, the skilled villagers might collect up to two liters of honey per household.

In the "medicine" category, most of the informants reported that they consumed raw honey immediately after extraction, while 25% said they drank honey mixed with lemon juice when needed to recover from bronchitis, flu, and other respiratory illness (Table 4-7). One of the informants at Mahendranagar, Kanchanpur said: "*Puttka mauri (stingless bees) is the gift of God. Its honey is useful for many things such as healing the wounds for cattle, to recover from eye infection and many more. It is really a holy creation of God*". Informants use the stingless bee honey to treat eye infections, toothaches, and sore throat/flu. They believe that stingless bee honey expedites wound healing and maintains strong overall immunity. Honey is also used to control vomiting due to excess of alcoholic consumption. In addition, honey is used for facial treatment and hair therapy. Pollen is used to cleanse the digestive system and to expedite the overall digestion process. Tharu people consume bee broods to increase libido in men. An informant from Tharu ethnic group, interviewed at Ghodaghodi Tal, Kailali said: "*Mangrasha (stingless bee) broods are very powerful sources to increase sexual desires and performances in men. We*

have been taught by our parents and grandparents that it should be consumed by newly married man or the man who have had trouble to have a child".

"Crafts" is the third category of use. Tharu people from all developmental regions use both wax and resins. They use wax to polish furniture, metal containers, and even doors and windows in their houses. Resins (propolis) are used as a sealing glue. One of the Tharu informants, interviewed in Gorusinge, Kapilvastu, mentioned that every summer, between April and May, they have a campaign to collect wax and resins nests, which they heat and mix together to make a big ball known as *pattharkhatta,* meaning "*patthar*: stone" and "*khatta*: very strong." The *pattharkhatta* is used locally in potteries and carpentries year around to rub on the finished pots and wooden structures. Extraction of bee products often results in the loss of the entire colony.

In the last category, "other/beliefs", all miscellaneous uses that do not fall under the previous categories are included. Some of the specific uses included here illustrate the diverse and intricate historical relationships between people and stingless bees in Nepal. For example, Tharu people feel lucky if the *mangrasha* nests on their property. They also believe that consuming bee broods and honey increases fertility in men. Likewise, Chhetri people use stingless bees honey to make a holy drink [*panchamrit*] during special occasions, such as births and deaths. One Chhetri informant from Kohalpur, Banke, remembers his grandfather singing the following song when he was a child: "*डाँडै माथि टोड्को साल तेइ माथि पूत्तिको, मायाँमा परानी दिने को होला हूतिको*" [*There are stingless bees nesting on hollow trunks of Sal tree on the top of the hills, when I will find a true love that will love me lifetime with all means*].

Relative cultural importance

The Relative cultural importance index (RCI) ranged from 30.5 in the Brahmin and Kirat communities in CDR and EDR to 91.5 in the Tharu community at FWDR. Chhetri people have moderate uses of stingless bees, although according to the informants, Chhetri people of the Far Western

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Development Region have a closer relationship with stingless bees than those of the Mid-Western Development Region. A Kruskal-Wallis H test showed that there was a statistically significant difference in the relative cultural importance index among the Chhetri, Tharu, and Brahmin (H [3] = 6.25, p = 0.011). Because a single community represented the Kirat people, I excluded it from the analysis. Dunn's pairwise tests showed significant differences only between the Tharu and Brahmin (p = 0.042, adjusted using the Bonferroni correction) (Figure 4-5).

Potential distribution in Nepal

The potential distribution maps obtained for *T. iridipennis* in both models were largely similar in their predictions (Figure 4-6). Both predicted high suitability of habitats across the Terai regions in southern Nepal, although the model using all records also shows high values of habitat suitability in mountainous regions in northwestern Nepal. Model evaluation showed high scores of performances for both models (Model 1: Partial ROC $\bar{x}$ = 0.965, 0.958–0.996; Model 2: Partial ROC: $\bar{x}$ = 0.965, 0.958–0.996).

Discussion

I found a single species of stingless bee in Nepal belonging to *Tetragonula* Moure, the most diverse, common, widely distributed, and economically important group of stingless bees in the Eastern Hemisphere. The genus consists of more than 30 species of small to very small bees (2.5 to 4.5 mm in length), many of them similar to one another and often difficult to recognize, found in the Indian subcontinent, Southeast Asia, and Australia (Michener 2007, Engel et al. 2017). The species occurring in Nepal belongs to the *T. iridipennis* species group, which currently consists of four species [*T. bengalensis* (Cameron), *T. praeterita* (Walker), *T. ruficornis* (Smith), and *T. iridipennis*] and has long been suspected to contain multiple cryptic species because of their exceedingly broad distributions as well as morphological variation over their ranges (Sakagami 1978, Rasmussen 2008a, 2013). For example, *T. iridipennis* was first described from Sri Lanka but there are specimens in collections standing under that name from several locations in India, Myanmar, Malaysia, and Indonesia. Further taxonomic studies are

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needed to clarify the species limits of this and other species of *Tetragonula*. Although I only found a single species of stingless bees, other species might also occur in Nepal. This could be the case for *Lepidotrigona arcifera* (Cockerell), which has been collected in Sikkim, India, near the eastern border of Nepal (Rasmussen 2013).

Nesting biology

Species of *Tetragonula* nest inside pre-existing cavities in living or dead trees, rocks, stone walls, and soil. Nesting habits, as well as nest defense behavior, differ significantly among species. For example, some are highly aggressive while others are docile; some build their brood cells in spiral combs while others in clusters (Michener 1961, Dollin and Dollin 1997, Engel et al. 2017). Thus, in general, my observations on the nesting biology of *T. iridipennis* in Nepal fall within the range of previous observations on other species of the genus in terms of the nesting substrate, brood arrangement, and defensive behavior. Nest entrances of *Tetragonula* species are often composed of a stiff, pliable, aromatic mixture that appears to be made of wax and plant resins. It is possible that the differences in the shape and dimensions of the nest tube entrance is related to the type of opening present on the surface of the tree trunk or house wall. The very short to nearly absent nest tube entrance found in nests within walls might be related to newly established nests or damage caused by humans, as reported in other docile, synanthropic species of stingless bees (Velez-Ruiz et al. 2013). Overall, the defensive behavior of *T. iridipennis* in Nepal was weak compared with some other species of *Tetragonula*, which add an irritating substance to their bites [e.g., *Tetragonula biroi* (Friese) in the Philippines] or bite so firmly that the bees cannot be removed without pulling their heads off [e.g., *T. fuscobalteata* (Cameron) in Thailand] (D. R. Smith, personal observations, January 7-8, 2013 in Ratchaburi, Thailand; (Roubik 2006).

The presence of an involucrum in nests built inside adobe walls might serve as a defense against nest invaders, and to maintain the optimum temperature brood development (Rasmussen 2008a). Intra-

specific variations in internal, as well as external nest features, are common in stingless bees, and they are often related to constraints imposed by nest site limitations (Roubik 2006).

Traditional knowledge

Informants demonstrated extensive knowledge of the ecology and natural history of the bees. They knew with great precision the nesting sites, trees, and habitats preferred by the bees and quickly found their nests. The number and kind of uses for *T. iridipennis* in Nepal fall within those known for other species of *Tetragonula*, as well as for other species of stingless bees in other regions of the world (Quezada-Euán et al. 2001, Ayala et al. 2013, Reyes-González et al. 2014, Vit et al. 2015). In India, *T. iridipennis* is also known by some local people as *puttka* or *dammer* bees, and its honey is also used for medicinal purposes, such as in the treatment treating burns, eye infection, diarrhea, ulcer, etc. (Singh 2016). *Tetragonula iridipennis* is also culturally important for some indigenous tribes of India, such as the Lepcha or Rong of Sikkim Himalaya, as their traditional hat contains layers of plant fibers that represent the eyes of this bee species (Lepcha et al. 2012).

The traditional uses documented here for Nepal is a clear evidence of the cultural importance of *T. iridipennis* to the local communities. This is particularly true of the Tharu people, as indicated by the high values of RCI (81.1–91.5). These high values may reflect their heavy reliance to the natural resources in their everyday life, as the Tharu people are underprivileged and economically less stable than other communities in Nepal (Dahal 2003). In fact, Tharu people not only consume honey and bee broods as a source of protein and carbohydrates, but also depend on hunting small mammals and snails (Parajuli et al. 2012). Although our sample sizes are small, our analysis of the RCI among communities only revealed significant differences between the Tharu and Brahmin, thus suggesting an overall shared body of knowledge. The low RCI values of the Brahmin are not surprising, as these people are less reliant on the local natural resources because of their higher economic stability when compared with other

communities in Nepal (Thoms 2008). Further studies with greater samples sizes are necessary to draw stronger conclusions.

Potential distribution in Nepal

Both models, using previously known localities for *T. iridipennis* and all localities including the new records from Nepal, largely predicted high suitability of habitats across low elevations in the Terai region of Nepal (Figure 4-6). Although we confirmed the presence of *T. iridipennis* in some areas, we were not able to find this bee in the places we visited in the Morang, Sunsari, Siraha, Mahottari, and Dhanusa districts, in southeastern Nepal. Thus, either I failed to detect this bee species during our surveys, or this species is locally extinct due to current extractive management practices. However, some informants claimed to see bees during the flowering period of lychee (*Litchi chinensis* Sonn., Sapindaceae), an economically important and widely cultivated tree in the Terai region. Lychee flowers are mainly visited by honey bees in India (Kumar and Kumar 2014), and at least in Australia, also by *Tetragonula* sp. (King et al. 1989). Thus, there exists the possibility that *T. iridipennis* occurs in those districts.

Both models also predicted low to medium values of habitat suitability for *T. iridipennis* in Jhapa, the easternmost district of Nepal bordering India. However, I found this species in all localities I surveyed in this district. Although such a discrepancy in the results was unexpected in retrospect, it is not surprising. As documented here, given the cultural importance of *T. iridipennis* to several human populations in Nepal, it is reasonable to assume that people could have easily transported nests to new localities. Yurrita et al. (2017) reported a similar case for *Melipona beecheii* Bennett, a culturally and economically important stingless bee species in Central America. This species has widely been reared in the Yucatán Peninsula since Pre-Columbian times (Villanueva-Gutiérrez et al. 2013), yet SDMs suggest low habitat suitability in this region.

Another unexpected result is the high habitat suitability in the mountainous regions of north western Nepal predicted by the model that combines all occurrence data (Figure 4-6B). While several species of stingless bees live exclusively at high altitudes, others have broad altitudinal ranges, occurring from sea level up to more than 2500 meters of altitude (Gonzalez and Engel 2004). *T. iridipennis* seems to be restricted to low elevations, as I did not find this species at locations above 1000 m nor did the people, I interviewed at these sites know about the local occurrence of this bee. Thus, such a prediction for the mountainous regions of north-western Nepal appears to be an overestimation of the distribution area of *T. iridipennis*.

Conservation and future directions

Many species of *Tetragonula* are recognized locally by several indigenous names and are often exploited for honey or wax, and several species [e.g., *T. carbonaria* (Smith) in Australia, *T. biroi* in the Philippines] are already being used for commercial pollination (Thummajitsakul et al. 2008, Rasmussen 2013). In the Indo-Malayan region, meliponiculture is still in its infant stage (Cortopassi-Laurino et al. 2006) and such activity is unknown in Nepal. As documented herein, only extractive management of wild populations of *T. iridipennis* is practice in Nepal. Even when people cut logs containing the natural bee colony and transport them to their home area, they do not propagate or manage them for honey or pollen because they lack this knowledge. The latter can also explain why colonies of *T. iridipennis* that occasionally occupy cavities in house walls, which are designed to catch swarms of native honey bees in the summer, are never managed (P. Basnet, personal communication, June 26, 2016 in Kohalpur, Banke, Nepal).

Most informants (89%) expressed concerns about the future of bees. They believed that the number of colonies is quickly declining due to the current extractive practice (100%), forest fires (89%), use of modern housing materials such as cements and its products (33%), heavy droughts (22%), and the arrival of European honey bees (11%). During the surveys, I had the opportunity to observe forest fires as

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well as logging activities impacting several trees containing live colonies of *T. iridipennis*. The destruction of the natural nesting sites of *T. iridipennis* was almost inevitable, as this bee species tends to nest in the medium to very large canopy trees used as timber (*S. robusta* and *D. sissoo*) or animal feed (*T. elliptica* and *H. cordifolia*) (Joshi and Singh 2008). This is alarming because stingless bees often nest in clusters within a particular habitat (Nagamitsu and Inoue 1997), and nest density is positively correlated with the density of large trees (Samejima et al. 2004).

T. iridipennis also used tree species that are protected under religious beliefs, such as *F. religiosa*, a Buddha tree (Ingles 1995), *S. catechu*, a threatened aromatic plant (Sharma et al. 2004), and *B. ceiba*, a species protected as the nesting tree of vultures (Baral et al. 2004). Thus, the level of exploitation of natural populations of *T. iridipennis* might be different depending on the status of the tree species used as nesting site.

The cultural significance of this bee species to the participant communities in southern Nepal contrasts with the current extractive management practices, which might negatively affect some populations. To develop a sustainable use of *T. iridipennis* in Nepal, researchers need to explore other aspects of its biology, distribution, and taxonomy. For example, future researchers might focus their attention in understanding the role of *T. iridipennis* in pollination, its reproductive behavior, local nest density, host plants for nectar and pollen, and natural predators. Additional fieldworks to develop local collections and molecular works are needed to gain a better understanding of the species diversity and distribution in Nepal. Finally, future researchers also need to begin assessing productive aspects of the colonies (honey, pollen, and wax) for developing new management techniques and a sustainable commercialization.

Figures and figure legends

Figure 4-1. Map of Nepal showing locations where we conducted field surveys and ethnographic research to determine the presence of stingless bees. Green circles indicate locations where we found *Tetragonula iridipennis* (Smith) (upper right), red circles indicate locations where we searched but did not find this species. The numbers followed by red and green dots are Map # indicated in Table 1 to represent the localities where this species is sampled in Nepal.
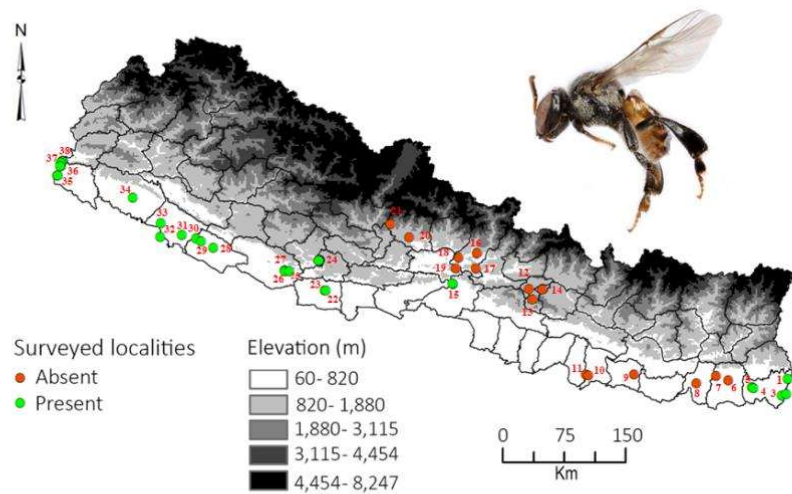
Figure 4-1

Figure 4-2. Natural and extracted nests of *Tetragonula iridipennis* (Smith) observed in different regions of Nepal. A, author collecting samples of bees surrounded by local people. The nest was found inside the trunk of a living Sal tree (*Shorea robusta*) at Kusum, Banke; B, author (right) and Mr. Banjade with log hives collected from nearby Sal forest and kept in his balcony, Ashrukot, Argankanchi; C, Mr. Thagunna and his grandson with a log hive at Mahendranagar, Kanchanpur; D, author pointing the nest entrance on a log hive placed in a backyard of the beekeeper at Ashrukot, Arghakanchi.

Figure 4-2

Figure 4-3. Variation in shape and dimensions of the nest entrance tube of *Tetragonula iridipennis* (Smith) observed in different substrates in Nepal. A–D, inside hollow tree trunks of Peepal (*Ficus religiosa*) (Kohalpur, Banke), Sisam (*Dalbergia sissoo*) (Gorusinghe, Kapilvastu), Sal (*Shorea robusta*) (Pipelneta, Argankanchi), and Simal (*Bombax ceiba*) (Mahendranagar, Kanchanpur), respectively; E, nest entrance inside wall of a mud and stone house wall at Suryapatewa, Bardiya; F, nest entrance on crack of cemented house wall at Badepur, Mahendranagar.
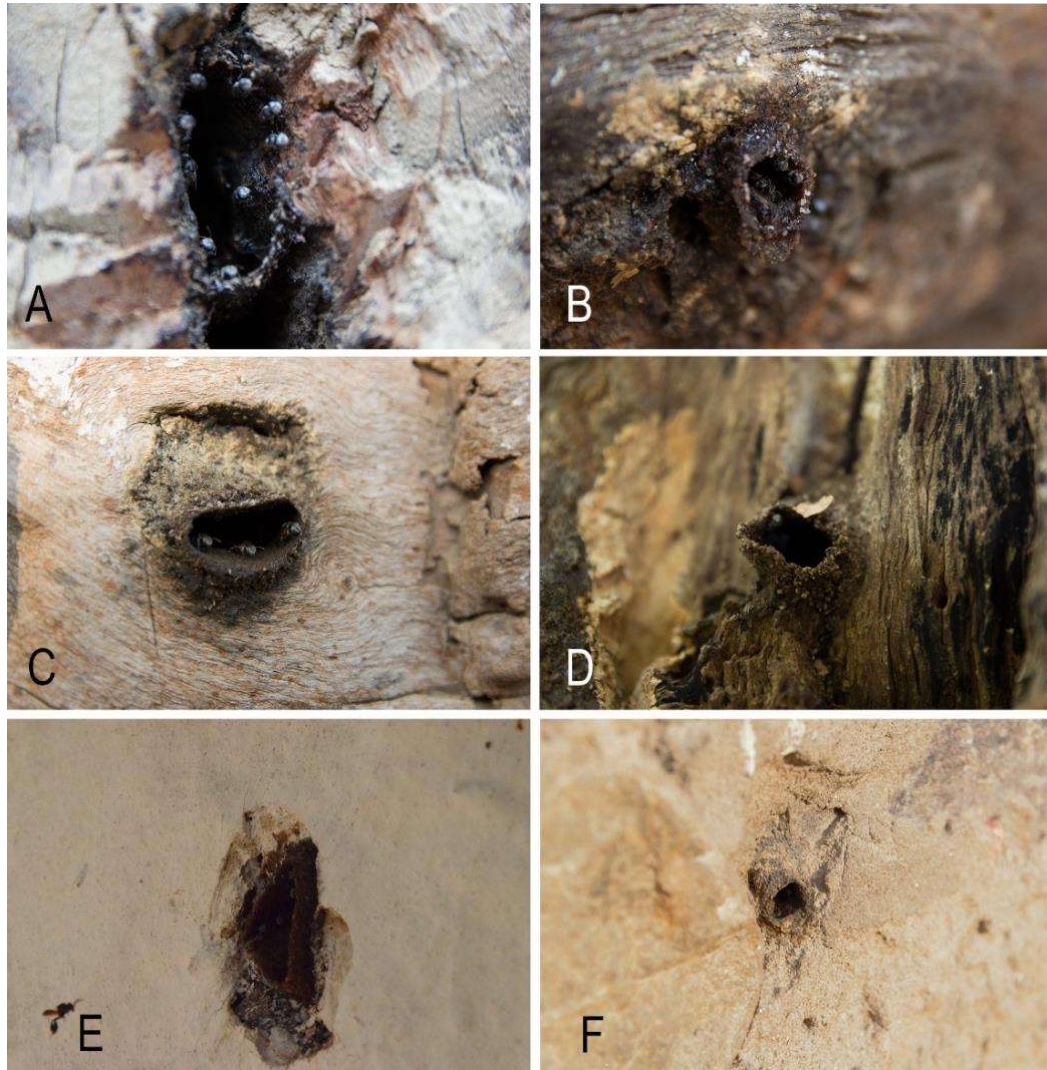
Figure 4-3

Figure 4-4. Dissected nests of *Tetragonula iridipennis* (Smith). A, nest inside a hollow trunk of a Sal tree (*Shorea robusta*) collected from Ashrukot village, Arghakanchi district; B, internal architecture of log nest; C, colony of *T. iridipennis* that occupied a wall cavity designed to capture *Apis cerana* in Naubasta, Banke; the brown involucrum was removed in the center to expose brood cells; D, detail of clusters of honey pots in the wall cavity nest of Banke (C). Abbreviations: bc = brood cells, hp = honey pots, pp = pollen pots, and rd = resin deposits.
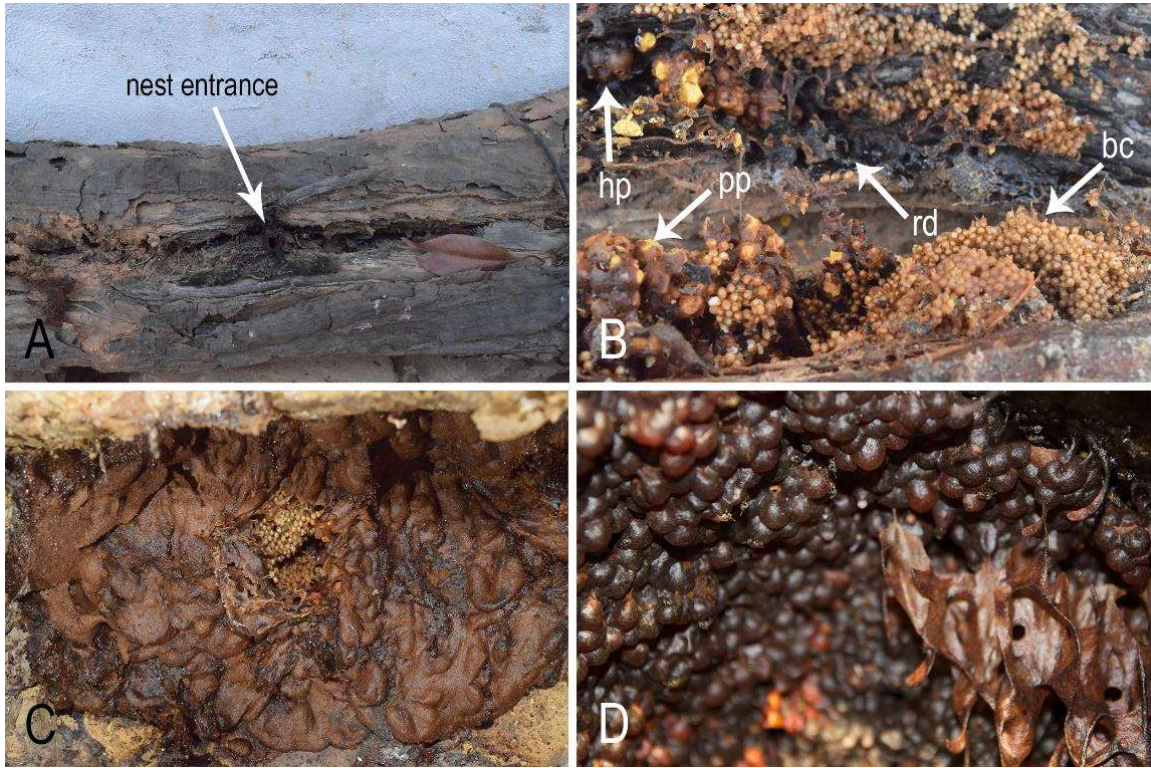
Figure 4-4

Figure 4-5. Relative cultural importance index (RCI) calculated for each ethnic community that participated in the study. Each color bar represents the ethnic community. Dunn's pairwise tests showed significant differences only between the Tharu and Brahmin (p = 0.042, adjusted using the Bonferroni correction).
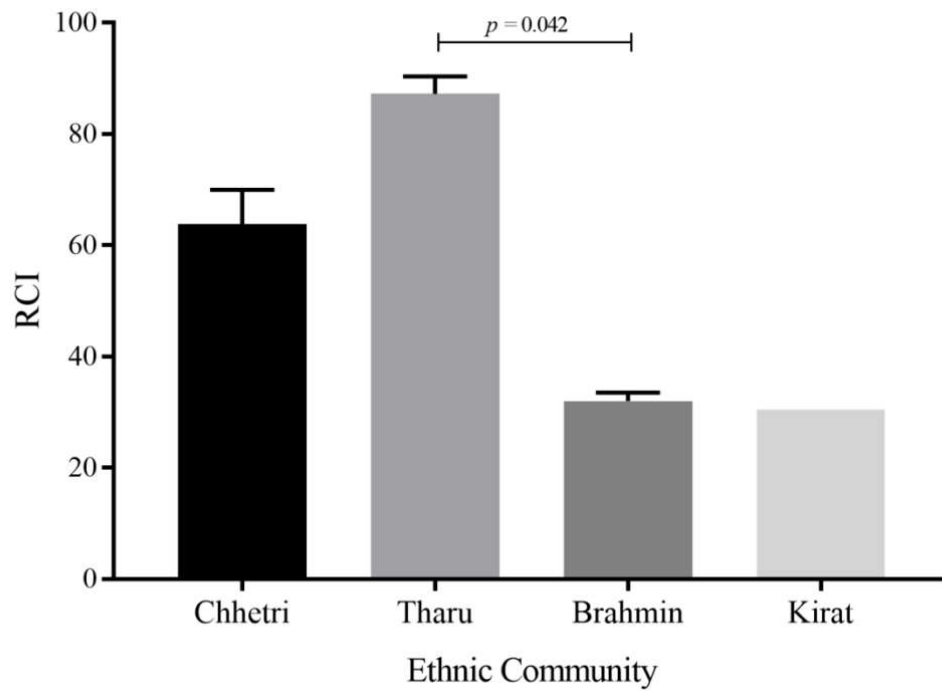
Figure 4-5

Figure 4-6. Species distribution models showing habitat suitability of *Tetragonula iridipennis* (Smith) in Nepal. Higher numbers (warmer colors) indicate greater suitability. A, potential distribution using previously known records for *T. iridipennis* outside Nepal (Model 1, n = 35 occurrence records). Known occurrence records are provided in Supplementary Materials Table S2; B, potential distribution using all available occurrence data, including localities for Nepal recorded in this study (Model 2, n = 74 occurrence records). Detail occurrence records are provided in Supplementary Materials Table S3 Black circles indicate locations where we found *T. iridipennis*, white circles locations where we did not find this species.

Figure 4-6

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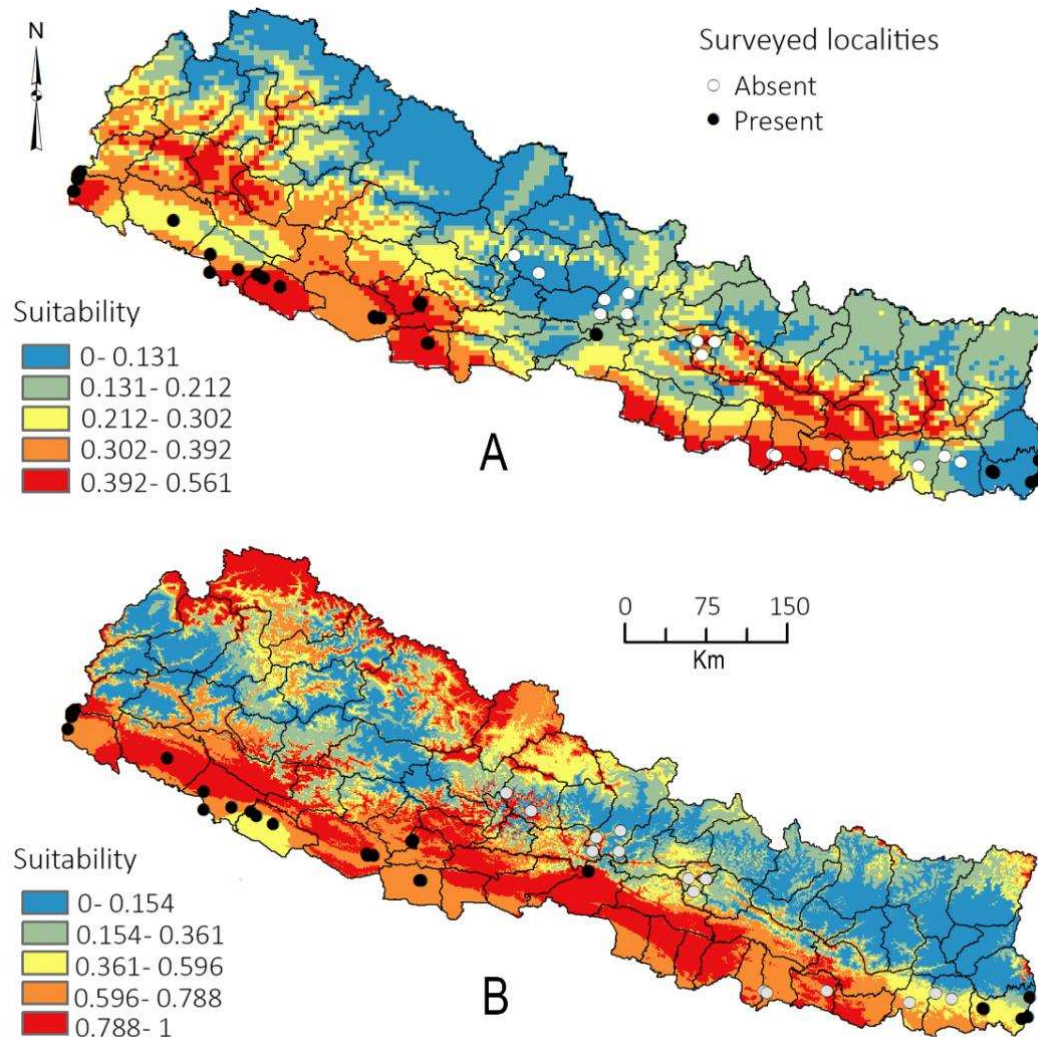
Tables and table legends

Table 4-1. Locality data for sites in Nepal that were searched for presence of stingless bees. Map numbers correspond to numbered localities in Figure 1. A total of 38 nests were found at 23 of the 38 numbered sites. Latitude and longitude (in decimal degrees) are averaged over all nests or sites searched within a district. Altitude is given as the range for all nests or sites searched. More detailed locality data are given in Supplementary Materials, Table S1.

Development Region	Zone	District	Locality #	# of nests	Latitude	Longitude	Altitude (m)
Eastern	Mechi	Jhapa	1–5	5	26.6	88.0	105–205
	Koshi	Morang	6–7	0	26.7	87.4	106–259
		Sunsari	8	0	26.6	87.1	95
	Sagarmatha	Siraha	9	0	26.7	86.4	119–125
Central	Janakpur	Dhanusa	10	0	26.7	85.9	117
		Mahottari	11	0	26.7	85.9	132
	Bagmati	Kathmandu	12	0	27.7	85.3	1403
		Lalitpur	13	0	27.6	85.3	1512
		Bhaktapur	14	0	27.7	85.4	1332
	Narayani	Chitwan	15	2	27.7	84.4	191–197
Western	Gandaki	Gorkha	16–17	0	28.3	84.7	871–1189
		Lamjung	18	0	28.0	84.5	471

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			19	0	27.9	84.5	760
		Tanahu	19	0	27.9	84.5	760
		Kaski	20–21	0	28.3	83.8	1468–1971
	Lumbini	Kapilbastu	22–23	2	27.6	83.0	112–132
		Arghakhanchi	24	9	28.0	83.0	547–965
Mid-western	Rapti	Dang	25–27	3	27.9	82.6	289–465
	Bheri	Banke	28–30	3	28.3	81.4	144–195
		Bardiya	31–33	3	28.3	81.3	165–212
Far-western	Seti	Kailali	34	1	28.2	81.7	207
	Mahakali	Kanchanpur	35–38	10	29.0	80.1	220–378

Table 4-1

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Table 4-2. Location, nesting substrate, and type of hive of the three nests of *Tetragonula iridipennis* (Smith) from Nepal dissected to document internal architecture.

Locality: District, collection site	Map #	Latitude (N), Longitude (E)	Substrate	Hive Type	Collection date
Kanchanpur, Mahendranagar	38	29.0836, 80.1552	Trunk cavity of *Shorea robusta* (Sal tree)	Log	23–Apr-16
Arghakhachi, Ashrukot	24	27.9954, 82.9872	Trunk cavity of *Shorea robusta* (Sal tree)	Log	27–Apr-16
Banke, Naubasta	29	28.2322, 81.6285	Adobe house wall	Wall	25–Apr-16

Table 4-2

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Table 4-3. Physical attributes and vegetation type of the locations where we found nests of *Tetragonula iridipennis* (Smith) in Nepal.

Zone/District	Map #	# of nests observed	Nesting substrates	Landform	Vegetation type
Mechi/Jhapa	1-5	5	Tree trunks	Plains	Tropical forest/crop-land
Narayani/Chitwan	15	2	Tree trunks	Inner plain/ river basin	Tropical forests/crop-land
Lumbini/Kapilvastu	22-23	2	Tree trunks	Plain	Tropical forest/ crop-land
Lumbini/Argankhanchi	24	9	Tree trunks	Mid-hill/hillslope	Sub-tropical forest/ crop-land
Rapti/Dang	25-27	3	Tree trunks	Valley	Sub-tropical forest/ crop-land
Bheri/Banke	28-30	3	Tree trunks/ mud-wall/ brick wall	Plains/river basin	Tropical forest/ crop-land
Bheri/Bardiya	31-33	3	Tree trunks/ mud-wall	Plains/river basin	Tropical forest/ crop-land
Seti/Kailai	34	1	Tree trunk	Plains/river basin	Tropical forest/ crop-land
Mahakali/Kanchanpur	35-38	10	Tree trunks/ cemented /mud-stonewall	Plains/river basin	Tropical forests/ crop-land

Table 4-3

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Table 4-4. Summary of nesting substrates used by *Tetragonula iridipenis* (Smith) in Nepal. Ranges, means and standard deviations (for 3 or more observations) of tree circumference or cavity volume. —, not applicable.

Nesting substrate	Nest trees common name	Trunk circumference, cm or volume of wall cavity, cm3		Number Nests (% of total)
		Range	mean ± s.d.	
Shorea robusta (Roth.)	Sal	73–235	110±45	14 (36.8)
Dalbergia sissoo (Roxb.)	Sisam	56–149	75.4±35.2	7 (18.4)
Senegalia catechu (L.f.) Hurter & Mobb.	Khayer	91–101	---	2 (5.2)
Terminalia elliptica (Wild.)	Saj	54–98	81.8±19.7	4 (10.5)
Ficus religiosa (L.)	Peepal	254	---	1 (2.6)
Bombax ceiba (L.)	Simal	186–259	---	2 (5.2)
Haldina cordifolia (Roxb.)	Haldu	167	---	1 (2.6)
Wall cavity designed for *Apis cerana* Fabricius	---	5668–7380	5954.7±600.3	3 (7.9)
Cracks in house wall (mud and stone wall)	---	---	---	4 (10.5)

Table 4-4

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Table 4-5. Characteristics of *Tetragonula iridipenis* (Smith) nests in Nepal. Ranges are followed by mean values and standard deviations. –––, not applicable or data not collected. For entrance tube shape, the number of nests exhibiting a given feature are provided in a parenthesis.

Nest substrate	# nests	Entrance- Height above ground, m; range, mean, s.d.	Entrance tube length, mm; range, mean, s.d.	Shape of entrance opening (# nests)	Entrance tube diameter, mm; range, mean, s.d.	Number of guard bees at entrance; range, mean, s.d.
Shorea robusta (Roth.)	14	0.5–2.5, 1.6 ± 0.6	0–15, 9.5 ± 4.7	circular (1) semicircular (3) irregular (2) roughly circular (2) elliptical (3)	7.5–13.5; 9.4 ± 4.4	5–16; 6 ± 6
Dalbergia sissoo (Roxb.)	7	0.6–1.9, 1.1 ± 0.4	0–16, 9.9 ± 5.3	irregular (1) roughly circular (3) elliptical (3)	6.5–11; 7.9 ± 4.0	6–17; 11 ± 6
Senegalia catechu (L.f.)				roughly circular (1)		
Hurter & Mobb.	2	1.23, 2.30	11, 11	elliptical (1)	8, 9.5	---
Terminalia elliptica (Wild.)	4	1.8–2.5, 2.1 ± 0.4	8–12, 10.4 ± 1.7	roughly circular (4)	9–12.5, 11.1 ± 1.5	8–19, 13 ± 5
Ficus religiosa (L.)	1	1.2	10.5	elliptical (1)	12	12
Bombax ceiba (L.)	2	1.89, 2.45	12, 12	roughly circular (2)	11, 11.5	---

Haldina cordifolia (Roxb.)	1	1.98	10	irregular (1)	11.5	---
Wall cavity designed for *Apis cerana* Fabricius	3	2.7–3.0, 2.9 ± 0.1	5–12.5, 8.2 ±3.4	circular (3) circular (2)	4.5-11, 7.3 ± 3.3	10-12, 11 ±1
Cracks in house wall (mud and stone wall)	4	0.5–2.7, 1.8 ± 1.1	0	irregular (2)	---	9-17, 13 ± 4

Table 4-5

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Table 4-6. Categories of use (C) and specific uses (U) documented for *Tetragonula iridipennis* (Smith) by four ethnic communities (Tharu, Brahmin, Chhetri, and Kirat) in Nepal. For each medicinal use, the type of bee product employed is provided in parentheses Abbreviations: H, honey; P pollen; L, larvae.

Categories of use (C)	Specific uses (U)
Foods	• Honey
	• Pollen
	• Larvae
Medicines	• Eye infection (H)
	• Wound healing (H)
	• Skin ointment (H)
	• Cleansing digestive tract (P)
	• Strong immunity (H)
	• Toothache (H)
	• Sore throat/flu (H)
	• Anti-vomiting (H)
	• Facial and hair treatment (H)
	• Libido in men (L)
Crafts	• Wax for polishing
	• Resin as a sealing glue
Others/beliefs	• Feel lucky owning colonies
	• Increase fertility
	• Sing a song about bees
C = 4	**U = 18**

Table 4-6

Table 4-7. Common names, categories of uses, and relative importance value of *Tetragonula iridipennis* (Smith) as provided by participants from different human communities across different regions of Nepal. Abbreviations: H = honey; P = pollen; L = larvae; — = non-applicable; #C = number of categories of use; #U = number of specific uses; C = proportion of the total number of categories of use; U = proportion of the total number of specific uses; RCI = relative cultural importance index ([C + U] × 50). See text for description of each specific use.

| Human Communities/ Informants | Ethnicity | Bee common name | Food | Categories of use | | | Relative cultural importance | | | | |
				Medicine	Crafts	Other/ Beliefs	#C	#U	C	U	RCI
Far-Western Development Region (FWDR 1)	Chhetri	Puttka	H P	Eye infection, wound healing, skin ointment, cleansing digestive tract, strong immunity	Wax for polishing	Makes holy drink	4	9	1.00	0.50	75
Far-Western Development Region (FWDR 2)	Tharu	Mangrasha	H P L	Toothache, eye infection, sore throat/flu, wound healing, anti-vomiting, facial and hair treatment, libido in men, and strong immunity	Wax for polishing, resin as a sealing glue	Feels lucky owning colonies, increase fertility	4	15	1.00	0.83	**91.5**
Mid-Western Development Region (MWDR 1)	Tharu	Mangrasha	H P L	Skin ointment, strong immunity, fever/headache, libido in men,	Wax for polishing, resin as a sealing glue	Feels lucky owning colonies, increase fertility	4	11	1.00	0.61	**81.1**
Mid-Western Development Region (MWDR 2)	Chhetri	Puttiko	H P	Headache/fever, eye infection, wound healing, skin ointment, strong immunity	—	Sings a song, makes holy drink	3	9	0.75	0.50	62.5
Mid-Western Development Region (MWDR 3)	Chhetri	Puttiko	H P	Sore throat/flu, wound healing, strong immunity	Resin as a sealing glue	—	3	6	0.75	0.33	54
Western Development Region (WDR 1)	Tharu	Mangrasha	H P L	Eye infection, sore throat/flu, wound healing, anti-vomiting/relief from alcohol, facial and hair treatment, libido in men, and strong immunity	Wax for polishing, resin as a sealing glue	Feels lucky owning colonies, increase fertility	4	14	1.00	0.78	**89**

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Western Development Region (WDR 2)	Brahmin	Puttko	H	Wound healing, strong immunity	—	2	3	0.50	0.17	33.5
Central Development Region (CDR 1)	Brahmin	Puttka	H	Strong immunity	—	2	2	0.50	0.11	30.5
Eastern Development Region (EDR 1)	Kirat	Dammer bees	H	Strong immunity	—	2	3	0.50	0.11	30.5

Table 4-7

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Appendix I: Supplementary material tables

Supplementary material table S1. Locality data for sites in Nepal that were searched for presence of stingless bees. Map numbers correspond to numbered localities in Figure 1. Nests were found at 23 of the 38 numbered sites.

Development Region	Zone	District	Collection Site	# of nests	Map #	Lat. (N)	Long. (E)	Altitude (m)
Eastern	Mechi	Jhapa	Budhabare	1	1	26.6839	88.1457	205
			Jalthal 1	1	2	26.5013	88.0790	105
			Jalthal 2	1	3	26.5167	88.1290	154
			Dharmapur 1	1	4	26.5913	87.7507	107
			Dharmapur 2	1	5	26.5846	87.7623	115
	Koshi	Morang	Bahunu	0	6	26.6665	87.4904	106
			Kerabari	0	7	26.7156	87.3558	259
		Sunsari	Dumraha	0	8	26.6354	87.1377	95
	Sagarmatha	Siraha	Lahan	0	9	26.7363	86.4505	125
						26.7328	86.4420	119
Central	Janakpur	Dhanusa	Janakpur	0	10	26.7208	85.9469	117
		Mahottari	Bharmapura	0	11	26.7363	85.9234	132
	Bagmati	Kathmandu	Chobar	0	12	27.6750	85.2917	1403
		Lalitpur	Lele	0	13	27.5635	85.3311	1512
		Bhaktapur	Bhaktapur	0	14	27.6677	85.4394	1332
	Narayani	Chitwan	Ramnagar	2	15	27.7304	84.4528	197
						27.7368	84.4525	191
Western	Gandaki	Gorkha	Masel/	0	16	29.0457	84.6711	871
			Pandrung			28.0714	84.7210	1189
			Namjung	0	17	27.9041	84.7108	1074
		Lamjung	Tinpiple	0	18	28.0189	84.5201	471
		Tanahu	Aanbu Khairini	0	19	27.9037	84.4891	760
		Kaski	Sarankot	0	20	28.2416	83.9750	1468
			Lumle	0	21	28.3910	83.7691	1971
	Lumbini	Kapilbastu	Gorusinge 1	1	22	27.6581	83.0427	112
			Gorusinge 2	1	23	27.6570	83.0543	132
		Arghakhanchi	Ashrukot	9	24	27.9929	82.9857	921
						27.9954	82.9872	965
						27.9929	82.9856	932
						27.9908	82.9835	745

Region	Zone	District	Site	No		Latitude	Longitude	Count
Mid-western	Rapti	Dang	Sisahaniya	25	1	27.9893	82.9789	611
			Sonpur 1	26	1	27.9874	82.9769	882
			Sonpur 2	27	1	27.9891	82.9817	726
						27.9899	82.9785	547
						27.9860	82.9752	588
						27.867	82.6564	289
						27.8799	82.6000	465
						27.8683	82.6076	453
	Bheri	Banke	Mahadevapuri	28	1	28.6804	80.9316	144
			Naubasta	29	1	28.2322	81.6285	189
			Kusum	30	1	28.1304	81.8164	195
		Bardiya	Suryapatewa	31	1	28.4000	81.2365	212
			Deudakala	32	1	28.2500	81.2346	165
			Deudakala	33	1	28.2682	81.4698	169
Far-western	Seti	Kailali	Ghodaghodi Tal	34	1	28.1984	81.6794	207
	Mahakali	Kanchanpur	Dus Gaja	35	1	29.0286	80.12885	222
			Badepur	36	1	29.0636	80.1319	297
			Community forest	37	1	29.0655	80.1349	237
						29.0836	80.1552	378
			Ward #8 of Mahendranagar	38	7	29.0836	80.1553	365
						29.0787	80.1526	363
						29.0269	80.1323	220
						29.0269	80.1324	222
						28.9215	80.0982	223
						28.9233	80.1002	225

Supplementary material table S1

Supplementary material table S2. Known records of distribution of *Tetragonula iridipennis* (Smith) outside Nepal (India, Sri Lanka, Myanmar, Malaysia, and Indonesia) used to predict its potential distribution in Nepal (model 1, n = 35 occurrence records).

Country of occurrence	Latitude (N)	Longitude (E)	Source
Sri Lanka	6.73333	81.1	GBIF
Sri Lanka	6.74277	79.84778	GBIF
Sri Lanka	6.76454	80.0166	GBIF
Sri Lanka	6.83008	79.84778	GBIF
Sri Lanka	7.08333	80.06667	GBIF
Sri Lanka	7.14430	81.2228	GBIF
Sri Lanka	7.490558	80.620812	GBIF
Sri Lanka	6.93194	79.84778	KU Snow Entomological Museum
Sri Lanka	6.74277	79.84778	KU Snow Entomological Museum
Sri Lanka	6.83008	79.84778	KU Snow Entomological Museum
Sri Lanka	6.76454	80.0166	KU Snow Entomological Museum
Sri Lanka	7.1443	81.2228	KU Snow Entomological Museum
Indonesia	-1.841667	121.491667	GBIF
Indonesia	-1.8416666667	121.4916666667	KU Snow Entomological Museum
Malaysia	2.866667	102.233333	GBIF
Malaysia	2.8666666667	102.2333333333	KU Snow Entomological Museum
Myanmar	26.359167	96.720001	GBIF
Thailand	18.79038	98.98468	KU Snow Entomological Museum
Thailand	18.79038	98.98468	GBIF
India	10.92156	79.83425	GBIF
India	12.282003	77.374432	GBIF
India	12.30623	76.64768	GBIF
India	12.97706	77.58711	GBIF
India	13.15311	80.02468	GBIF
India	18.51327	73.84985	GBIF
India	12.30623	76.64768	KU Snow Entomological Museum
India	12.97706	77.58711	KU Snow Entomological Museum
India	10.92721	79.6899	KU Snow Entomological Museum
India	13.15311	80.02468	KU Snow Entomological Museum
India	10.92156	79.83425	KU Snow Entomological Museum
India	11.00555	76.96612	KU Snow Entomological Museum
India	18.51327	73.84985	KU Snow Entomological Museum

Supplementary material table S2

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Supplementary material table S3. Distributional records of *Tetragonula iridipennis* (Smith) outside Nepal (India, Sri Lanka, Myanmar, Malaysia, and Indonesia) and inside Nepal used to predict its potential distribution in Nepal (model 2, n = 74 occurrence records).

Country of occurrence	Latitude (N)	Longitude (E)	Source
Sri Lanka	6.73333	81.1	GBIF
Sri Lanka	6.74277	79.84778	GBIF
Sri Lanka	6.76454	80.0166	GBIF
Sri Lanka	6.83008	79.84778	GBIF
Sri Lanka	7.08333	80.06667	GBIF
Sri Lanka	7.14430	81.2228	GBIF
Sri Lanka	7.490558	80.620812	GBIF
Sri Lanka	6.93194	79.84778	KU Snow Entomological Museum
Sri Lanka	6.74277	79.84778	KU Snow Entomological Museum
Sri Lanka	6.83008	79.84778	KU Snow Entomological Museum
Sri Lanka	6.76454	80.0166	KU Snow Entomological Museum
Sri Lanka	7.1443	81.2228	KU Snow Entomological Museum
Indonesia	-1.841667	121.491667	GBIF
Indonesia	-1.8416666667	121.4916666667	KU Snow Entomological Museum
Malaysia	2.866667	102.233333	GBIF
Malaysia	2.8666666667	102.2333333333	KU Snow Entomological Museum
Myanmar	26.359167	96.720001	GBIF
Thailand	18.79038	98.98468	KU Snow Entomological Museum
Thailand	18.79038	98.98468	GBIF
India	10.92156	79.83425	GBIF
India	12.282003	77.374432	GBIF
India	12.30623	76.64768	GBIF
India	12.97706	77.58711	GBIF
India	13.15311	80.02468	GBIF
India	18.51327	73.84985	GBIF
India	12.30623	76.64768	KU Snow Entomological Museum
India	12.97706	77.58711	KU Snow Entomological Museum
India	10.92721	79.6899	KU Snow Entomological Museum
India	13.15311	80.02468	KU Snow Entomological Museum
India	10.92156	79.83425	KU Snow Entomological Museum
India	11.00555	76.96612	KU Snow Entomological Museum
India	18.51327	73.84985	KU Snow Entomological Museum
Nepal	29.09	80.49	Rasmussen, (2013)
Nepal	26.6839	88.1457	Here in
Nepal	26.5013	88.0790	Here in

Nepal	26.5167	88.1290	Here in
Nepal	26.5913	87.7507	Here in
Nepal	26.5846	87.7623	Here in
Nepal	27.7304	84.4528	Here in
Nepal	27.7368	84.4525	Here in
Nepal	27.6581	83.0427	Here in
Nepal	27.6570	83.0543	Here in
Nepal	27.9929	82.9857	Here in
Nepal	27.9954	82.9872	Here in
Nepal	27.9929	82.9856	Here in
Nepal	27.9908	82.9835	Here in
Nepal	27.9893	82.9789	Here in
Nepal	27.9874	82.9769	Here in
Nepal	27.9891	82.9817	Here in
Nepal	27.9899	82.9785	Here in
Nepal	27.9860	82.9752	Here in
Nepal	27.867	82.6564	Here in
Nepal	27.8799	82.6000	Here in
Nepal	27.8683	82.6076	Here in
Nepal	28.6804	80.9316	Here in
Nepal	28.2322	81.6285	Here in
Nepal	28.1304	81.8164	Here in
Nepal	28.4000	81.2365	Here in
Nepal	28.2682	81.4698	Here in
Nepal	28.1984	81.6794	Here in
Nepal	29.0286	80.12885	Here in
Nepal	29.0636	80.1319	Here in
Nepal	29.0655	80.1349	Here in
Nepal	29.0836	80.1552	Here in
Nepal	29.0836	80.1553	Here in
Nepal	29.0787	80.1526	Here in
Nepal	29.0269	80.1323	Here in
Nepal	29.0269	80.1324	Here in
Nepal	28.9215	80.0982	Here in
Nepal	28.9233	80.1002	Here in

Supplementary material table S3

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Appendix II: Human subjects waiver

IRB OVERSIGHT NOT REQUIRED

June 29, 2016

Chet Bhatta

Dear Chet Bhatta:

On 6/29/2016, the IRB reviewed the following submission:

Type of Review:	Initial Study
Title of Study:	Ethno-entomology and development: Cerana beekeeping vs mellifera Beekeeping in Nepal: An influence of seasons and landscapes
Investigator:	Chet Bhatta
IRB ID:	STUDY00004310
Funding:	Name: University of Kansas
Grant ID:	
Documents Reviewed:	• Consent Agreement form (Chet Bhatta, EEB, KU).doc, • Illitrate Consent Agreement form (Chet Bhatta, EEB, KU).doc, • Interview guides, • HSCL New Submission Form V3 (Chet Bhatta).pdf, • Beekeepers recruitment Script (In person, Chet, EEB, KU).docx, • Email Script for NARC (Chet, EEB, KU).docx, • Beekeepers recruitment Phone Script (Chet, KU, EEB).docx, • Email Script Beekeeper Association Nepal (Chet, EEB, KU).docx, • Bhatta_Entom-award_ltr_Apr2016.docx, • Bhatta_Travel-ltr_March2016 (Entomology Endowment).pdf

After reviewing your application, the project described does not meet the criteria of activities subject to federal regulations at 45 CFR 46. Based on the materials submitted, it has been determined that IRB oversight is not required at this time. Though IRB oversight is not required, all activities proposed in the submission should be conducted in a responsible and ethical manner, and held to standards required by your field of study and your responsibilities as a researcher affiliated with the University of Kansas.

This determination applies only to the activities described in the IRB submission and does not apply should any changes be made. If changes are being considered and there are questions about whether IRB review is needed, please submit a study modification to the IRB for a determination. You can create a modification by clicking **Create Modification / CR** within the study.

Sincerely,

Stephanie Dyson Elms, MPA
IRB Administrator, KU Lawrence Campus

Human Subjects Committee Lawrence
Youngberg Hall | 2385 Irving Hill Road | Lawrence, KS 66045-7568 | (785) 864-7429 | www.research.ku.edu

Appendix III: Interview guide

This study is aimed to collect information about nesting biology, distribution, and traditional uses of stingless bees in Nepal. I am Mr. Chet Bhatta, a graduate student in the department of ecology and evolutionary biology at the University of Kansas. The information collected from this study will be solely used for dissertation and scientific purpose. Questions on this interview guide are focused on nesting sites, nesting trees, and traditional knowledge about stingless bees.

Informant demographics

- What is your age?
- What is your full address?
- What is your marital status?
- What is your primary occupation?
- What is your (wife's or Husband's) occupation?
- Do you have any children? How many? What ages and sex? What are they doing?

General knowledge about stingless bees

- Do you know about stingless bees?
- How long have you been seen this type of bees and where did you saw it for the first time?
- Did your parents or grandparents keep these bees?
- Are you interested to keep these bees at your house now or in future?

Nesting biology

Nesting sites, nest characteristics, and nesting trees

- Where do these bees' nest on (tree trunk, ground, house wall…)?
- How do you differentiate sting less bees and honey bees?
- Have you seen any of these bees' nests on ground?
- Do they survive year long? Or you see them in summer only.
- Do they prefer certain nesting sites over other such as elevation, cliffs, trees around river banks, or trees of certain species, or near water source?
- Could you please tell me what variety of trees you have been seen these bees' nest on?
- In general, at what height from the ground, nest entrances are found at the most?
- What types of tree trunk they are using to nest on (healthy tree trunks or hollow tree trunks)?
- How many types of nest entrances have you seen so far in terms of shape and size?
- Any idea on types of materials used on building nest entrance?

Connection to beekeeping and its importance

- How many years have you been practicing beekeeping or honey hunting?
- Did your parents or grandparents keep bees?
- How about your children? Are they interested on bees and beekeeping?
- Do you ever think about beekeeping using stingless bees?
- Have you ever multiplied colonies of stingless bees?
- What do you know about pollination services?
- Do you know the importance of honeybees and stingless bees in pollination services?
- If you get opportunity to participate in training that involves modern meliponiculture, will you be interested to participate?

Honey harvesting/hunting

- How many times per year do you harvest honey from stingless bees?
- Is there particular season or time of the year which is most suitable for honey hunting?
- How do you harvest the honey from the traditional log hive at home on from log in forest?
- Do you destroy the entire colony while harvesting honey?
- What are the precautions to be used while harvesting honey?
- What products do you harvest from stingless bees' hive? and how?

Traditional uses

- How do you use honey of stingless bees?
- Is there any medicinal, religious, or any other use of it?
- Do you use wax or resins from stingless bees' hive? How and when?
- What do you do with young brood while harvesting honey?

Follow-up questions

- Do you know any other information of stingless bees which I didn't ask you so far?
- Do you know anybody who keeps stingless bees in near by village or other districts of country?
- Do you know anybody who is knowledgeable about wild honey harvesting from stingless bees?