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Social Bees (Bombini, Apini, Meliponini)

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CONTENTS

11.1 Introduction

In this chapter, we will address the role of food in the organization of social bees' colonies from foraging activity to its use on offspring feeding, emphasizing the characteristics of the two main resources collected and processed by them, pollen and honey.

In bees, eusociality emerged in Apinae and is present in the tribes Bombini, Apini, and Meliponini. One of the most obvious social conflicts is expressed in sexual production and in the existence of a specialized reproductive caste. Food has a strong influence on caste differentiation, and control of its amount or quality is one of the central mechanisms in social life. Foraging activity on flowers and food processing inside the colonies directly influence the social life of the colony. More emphasis will be given to the mechanisms described for Meliponini species or stingless bees.

Stingless bees are floral generalists, but they present a selective foraging activity, and as a rule, the annual economic budget of the colony depends on intensive foraging on few available pollen and nectar floral sources in each habitat. The choice of food sources is mediated by morphofunctional characteristics of the foragers, foraging strategies (solitary or collective), and social interactions in the colony and in the field. In communities, food overlap among species is a rule, but the role of competition in the organization of local assemblies is

still quite controversial. Relationships among reproductive strategies of the colonies, diversity, and abundance distribution in the communities have just begun to be explored in recent studies.

11.2 Resources Acquisition

Although there are exceptions, social bees feed basically on pollen (protein source) and nectar (carbohydrate source) collected from flowers. Some species of stingless bees (Meliponini) are scavengers, feeding on decomposing organic matter (as *Trigona hypogea* Silvestri, *Trigona necrophaga* Camargo & Roubik, and *Trigona crassipes* (F.)); others also feed on honeydew, a sugar solution produced by membracids; and finally, some specialize in stealing food from other bees' nests (cleptobiotic bees, *Lestrimelitta* spp. in the Americas, and *Cleptotrigona* spp. in Africa).

11.2.1 Physical Factors and Temporal Partitioning of Foraging Activity

Stingless bees are found in tropical and subtropical regions. The most likely cause of this geographic distribution pattern is the sensitivity of both individuals and colonies to low temperatures. Although there are interspecific differences in relation to the ability of hive thermoregulation, bees of this tribe seem to depend more on structural characteristics of their nests than on physiological and behavioral responses for heat conservation (Sakagami 1982).

The main abiotic factors that singly or in combination influence stingless bees' flight activity are temperature, relative humidity, light intensity, and wind speed. According to Fowler (1979), extreme values would directly act on the bees, while moderate values would affect flight activity, as they reflect on food availability (for instance, nectar flow). Clearly, food availability only becomes important after bees meet favorable conditions for flight. Thus, species capable of flying in wider ranges of temperature, relative humidity, and so forth, eventually may have advantages over the others.

Temperature seems to be determinant to foragers, especially in small species, such as *Tetragonisca angustula* (Latreille) and *Plebeia* spp. that start foraging at temperatures above 16°C (Oliveira 1973; Iwama 1977; Kleinert-Giovannini 1982; Imperatriz-Fonseca et al. 1985). An exception, *Plebeia pugnax* Moure (in litt.) is capable to start foraging from 14°C (Hilário et al. 2001). All these species reduce flight activity at temperature below 20°C.

Larger species of Meliponini, with size between 8 and 12 mm, such as *Melipona*, begin flight activity in lower temperatures, starting from 11°C in *Melipona bicolor* Lepeletier (Hilário et al. 2000), and 13°C to 14°C in *M. quadrifasciata* and *M. marginata* Lepeletier (Guibu and Imperatriz-Fonseca 1984; Kleinert-Giovannini and Imperatriz-Fonseca 1986). Most species present optimal foraging activity between 20°C and 30°C, except *M. quadrifasciata* and *M. bicolor*, which preferentially collect food at 14°C to 16°C and 16°C to 26°C, respectively. Body biomass is the main variable in this relationship, since larger social bees such as *Apis mellifera* L. and *Bombus* spp. start foraging at lower temperatures, often well below 10°C (Gary 1967; Heinrich 1979). However, in Meliponini, relatively small species such as *Partamona helleri* (Friese) present an optimal foraging activity between 15°C to 24°C, similar to the range of large species such as *M. bicolor* (Azevedo 1997).

Optimum values of relative humidity (RH) for foraging range between 30% and 70 % for most species (Oliveira 1973; Iwama 1977; Kleinert-Giovannini 1982; Kleinert-Giovannini and Imperatriz-Fonseca 1986; Hilário et al. 2001). *Plebeia remota* (Holmberg), *Schwarziana quadripunctata* (Lepeletier), and *M. bicolor* present higher flight activity in higher ranges between 60% and 90% (Imperatriz-Fonseca et al. 1985; Imperatriz-Fonseca and Darakjian 1994; Hilário et al. 2000). *Plebeia emerina* (Friese) workers do not leave their nests when RH is above 70% (Kleinert-Giovannini 1982). With an optimal flight activity between 40% to 45% RH, *M. marginata* shows behavioral plasticity in relation to environmental conditions (Kleinert-Giovannini and Imperatriz-Fonseca 1986), intensifying foraging under extreme conditions (e.g., values above 80% RH), after prolonged periods of rainfall.

Light intensity seems to be important just for the start and the end of external activity. In other periods, it is difficult to dissociate it from changes in temperature, and therefore, its effects become secondary, or they are at least masked. Even so, some authors reported lower flight activity on cloudy days,

when compared with sunny days with the same temperatures (Oliveira 1973; Kleinert-Giovannini 1982; Kleinert-Giovannini and Imperatriz-Fonseca 1986).

Wind speed between 2 and 3 m/s causes a decrease in the number of small foragers of *P. emerina* leaving the nest, leading to the interruption of foraging when it reaches 4 m/s (Kleinert-Giovannini 1982). In the same conditions, foragers of *T. angustula* continue to collect food (Iwama 1977), while other species such as *Plebeia droryana* (Friese), *P. saiqui* (Friese), and *M. marginata* are only slightly affected (Oliveira 1973; Kleinert-Giovannini and Imperatriz-Fonseca 1986).

The state of the colony also influences flight activity of stingless bees. Usually, weak colonies are more susceptible to variations in temperature (Kleinert-Giovannini and Imperatriz-Fonseca 1986) and have their activity shifted to later hours of the day, when compared to other colonies of the same species (Hilário et al. 2001). Nunes-Silva et al. (2010) also recorded distinct foraging patterns during two different reproductive phases in colonies of *P. remota*: during reproductive diapause, bees collected primarily nectar, while during the reproductive phase, they collected predominantly pollen.

Although many species present similar or overlapping optimum ranges of temperature and relative humidity, periods of increased flight activity tend to be different, allowing temporal partitioning of floral resources. Several species of the genus *Melipona* present higher flight activity at different times (e.g., *M. bicolor* and *M. quadrifasciata* are more active in the early morning hours, while *M. marginata* is more active between 11:00 a.m.–1:00 p.m.). Among the small species of *Plebeia*, *P. saiqui*, *P. remota,* and *P. pugnax* have higher activity concentrated between midmorning and midafternoon (Oliveira 1973; Imperatriz-Fonseca et al. 1985; Hilário et al. 2001); *P. emerina* and *Plebeia droryana* forage mainly in the afternoon.

Foragers leave the nest mainly to collect food (pollen and nectar), resin, water, and mud (for nest building), and these resources are sought at different times of the day. For instance, *P. pugnax* collects pollen preferentially in the morning until noon, while resin is collected throughout the day (Hilário et al. 2001). Although active all day, foragers of *M. rufiventris* and *Melipona bicolor* Lepeletier preferentially collect pollen in the early hours of the morning. While *M. bicolor* collects resin and mud preferably in late afternoon, foragers of *M. rufiventris* present, besides this peak, an additional peak of resin collection coincident with the morning pollen peak (Hilário et al. 2000; Fidalgo and Kleinert 2007). This temporal partitioning of resource gathering was also observed in other neotropical regions for other *Melipona* species (Bruijn and Sommeijer 1997) and increases the chances of coexistence of different species of stingless bees in a same place.

11.2.2 Niche Width and Floral Resources Allocation

Except for rare species that specialize in nest robbing (*Lestrimellita*) and in the use of animal protein from carcasses (*T. hypogea*), stingless bees feed on pollen and nectar. In this case, they are generalists foraging on a wide spectrum of floral types. However, few floral sources are heavily exploited in local communities. This foraging pattern was conceived from studies on pollen analysis of bee colonies and bee censuses on flowers (Imperatriz-Fonseca et al. 1984, 1987; Ramalho et al. 1985, 1989, 1990, 1991, 2007; Kleinert-Giovannini and Imperatriz-Fonseca 1987; Cortopassi-Laurino and Ramalho 1988; Ramalho 1990, 1995, 2004; Wilms et al. 1996, 1997). Both techniques generate extensive and independent data that depict the expression of this pattern in two hierarchical levels: colonies and local populations (Figure 11.1).

Although differences in the speed with which different species are able to muster a large number of individuals for a given food source (Lindauer and Kerr 1960; Hubbell and Johnson 1978; Johnson 1982; Johnson et al. 1987), there is no relationship between efficiency of communication and a colony's ability to concentrate foraging activity in few floral resources.

In local stingless bee communities, measures of niche width vary widely with temporal changes in the availability of floral sources and with the specific responses to the distribution of resources relative abundance (Ramalho et al. 1991). However, on average, niche width appears to be similar between groups of species with different foraging strategies (Biesmeijer and Slaa 2006). In general, this analysis confirms the pattern of concentrated use of floral sources, detected in local communities. The index often used as a measure of niche width (H', Shannon-Wiener) is extremely sensitive to the evenness of

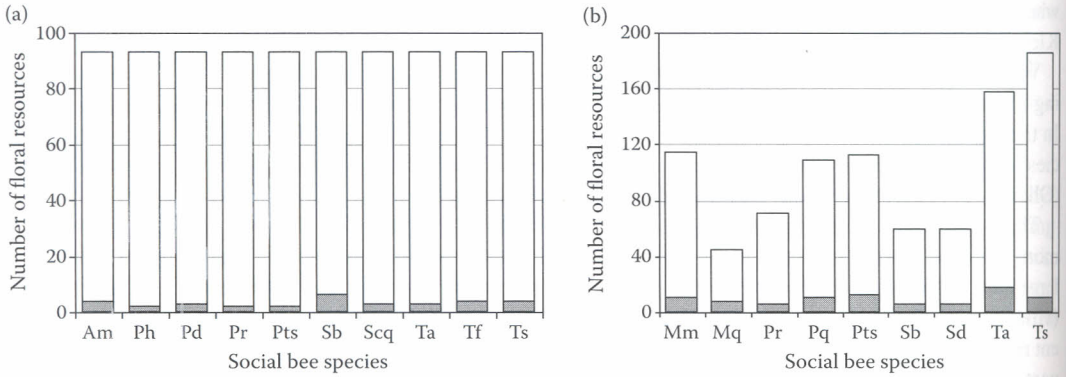

FIGURE 11.1 Floral sources allocation by stingless bees and Africanized honeybees (Am) in the Atlantic Forest domain: (a) Floral sources where at least 10% of foragers (black) were sampled in relation to all sources (white) visited by this bee group in the Atlantic Forest (State Park Cantareira, SP). (b) Floral sources with representation above 10% (black) in Meliponini diet—estimates by counting pollen grains in food stored in colonies at Universidade de São Paulo campus, SP. Meliponini: Ph = *Partamona helleri*; Pd = *Plebeia droryana*; Pq = *Plebeia saiqui*; Pr = *Plebeia remota*; Pts = *Paratrigona subnuda*; Sb = *Scaptotrigona bipunctata*; Sd = *Scaptotrigona depilis*; Scq = *Schwarziana quadripunctata*; Ta = *Tetragonisca angustula*, Ts = *Trigona spinipes*; Mm = *Melipona marginata*, Mq = *Melipona quadrifasciata*. (From Ramalho, M., A. Kleinert-Giovannni, and V. L. Imperatriz-Fonseca, In *Ecologia Nutricional de Insetos e Suas Implicações no Manejo de Pragas*, ed. A. R. Panizzi and J. R. P. Parra, 225–52, Editora Manole, São Paulo, Brazil, 1991; and Ramalho, M., Diversidade de abelhas (Apoidea, Hymenoptera) em um remanescente de Floresta Atlântica, em São Paulo, Ph.D. thesis, Universidade de São Paulo, Brazil, 1995. With permission.)

the most common sources in the diet, and only when foraging concentration becomes extreme, as for *Scaptotrigona* (Ramalho 1990), there is a marked reduction in its value.

Measures for niche width of these generalist consumers most likely reflect the effect and not the cause of ecological dominance. For instance, in local communities, as the number of foragers sampled on flowers increases, the number of sources visited by a species also increases (Ramalho 1995 and Figure 11.2). Similarly, greater colonies tend to use broader spectrum of floral sources (Cortopassi-Laurino and Ramalho 1988; Ramalho et al. 1991). Even species considered primitive and specialized in habitat types or nesting sites, such as *Mourella caerulea* (Friese) (Camargo and Wittmann 1989), are extremely generalist consumers of floral resources. Therefore, one should not expect a correlation between niche width (realized) and relative abundance of stingless bee species in ecological communities. For instance,

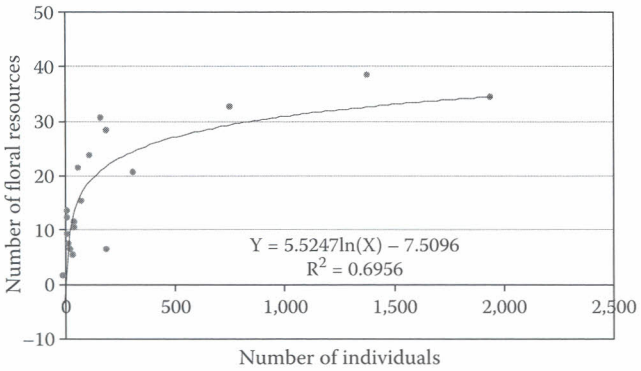

FIGURE 11.2 Relationship between number of individuals sampled on flowers (relative population size) and number of floral sources visited by 17 species of stingless bees, *Apis mellifera* and two species of *Bombus*. Linear correlation: $r = 0.72$, $p < 0.05$. Data regression curve is indicated. (From Ramalho, M., Diversidade de abelhas (Apoidea, Hymenoptera) em um remanescente de Floresta Atlântica, em São Paulo, Ph.D. thesis, Universidade de São Paulo, Brazil, 1995. With permission.)

Scaptotrigona bipunctata (Lepeletier) is dominant in the Atlantic forest at Serra da Cantareira, but has one of the smallest realized niche width (Ramalho 1990, 1995, 2004).

The premise of specific choices by modifying foraging pattern led to the hypothesis of floral preference in *Melipona* (Ramalho et al. 1989). The high relative pollen frequency from Solanaceae, Melastomataceae, and Myrtaceae in the diet of *Melipona* is related to pollen extraction capacity by vibration, especially from flowers with poricidal anthers, a skill that differentiates the group in relation to other Meliponini. Studies on feeding habits in different habitats as Cerrado, Atlantic forest, and Colombia Llanos, have been gradually giving empirical support to this hypothesis, with some reservations about preferred plant families (Silva and Schlindwein 2003; Antonini et al. 2006; Nates-Parra 2006).

Ramalho et al. (2007) showed that floral choices of *Melipona scutellaris* Latreille are not random. Using Africanized *A. mellifera* as control, they demonstrated that the diversity of floral sources in a colony's diet was dependent on species. In other words, independent of habitat type, colonies of *M. scutellaris* remained more similar to each other, forming clusters (Figure 11.3) significantly narrower than with *A. mellifera* colonies. The pattern also remains in line with more similar responses among colonies of *M. scutellaris* to local variations in blossoms supply.

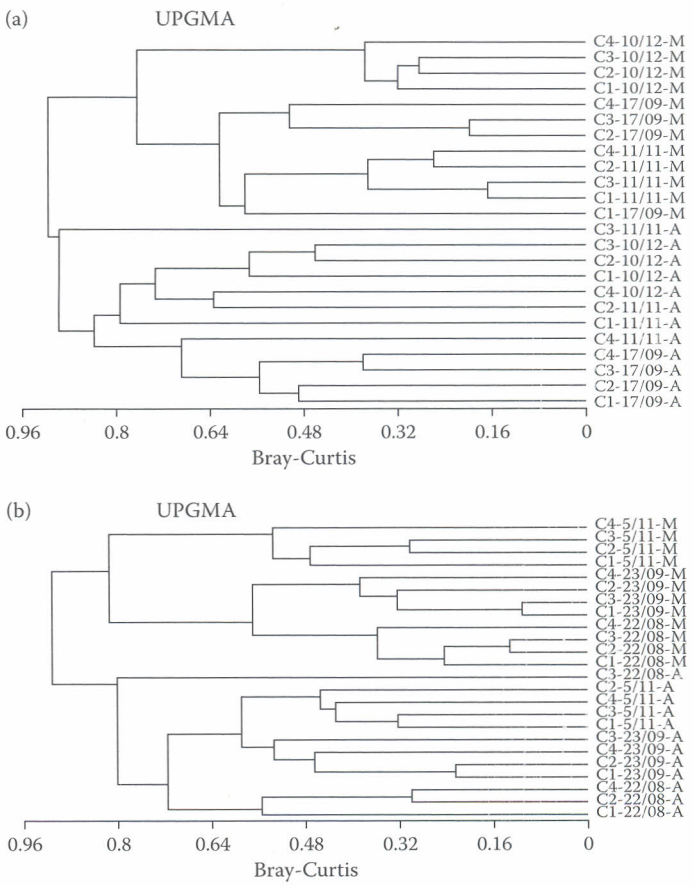

FIGURE 11.3 Analysis of trophic similarity between colonies of *Melipona scutellaris* (M1 to M4), with paired samples control from Africanized Apis mellifera colonies (A1 to A4) at two localities of the tropical Atlantic forest, in Bahia. Dissimilarity results (Bray-Curtis index, UPGMA method) of paired samples at two locations during three periods (months): (a) Alagoinhas, (b) Cruz das Almas. The significance test of similarity analysis (ANOSIM) supports the hypothesis that clusters are not random. (From Ramalho, M., M. D. Silva, and C. A. L. Carvalho, *Neotrop. Entomol.*, 36, 38–45, 2007. With permission.)

Secondary data analysis of 28 communities in several habitat types in Eastern Brazil indicates that the apparent competitive structure of Meliponini communities (Biesmeijer and Slaa 2006) is expressed in three trends: (1) retraction of niche width, with increasing number of species in communities, (2) with increasing number of plant species in communities, the ratio between number of stingless bee species and number of plant species decreases, that is, smaller niche packaging ("species packing"), and (3) local communities tend to be formed by species of distinct genera.

As more floral sources are explored, the number of sources shared among pairs of stingless bee species increases (Figure 11.4). Food overlap among these potentially generalist consumers tends to be very diffuse and extensive, and this is reflected mainly on measures based on presence–absence (e.g., Sorensen and Cody indexes). Clusters derived from these measures (Biesmeijer and Slaa 2006) poorly reflect the trophic distance among consumers and conceal the real community structures. But when measuring the intensity of use of shared floral sources (percentage of similarity, e.g., Schoener's index) (Ramalho 1995), we obtain the more functional ecological expression of species overlap (Figure 11.3), and clusters differ greatly. Comparing the percentage of food similarity in two nearby communities in the Atlantic forest from data generated with two independent techniques (pollen analysis and bee census on flowers), Ramalho et al. (1991) and Ramalho (1995) found that diets of *P. droryana* and *T. angustula* were closer to each other and to *Melipona* and *Scaptotrigona,* deflecting too much from clusters based on meta-analysis of presence-absence measures of species on flowers mentioned above.

Measures of diffuse exploitation of floral resources eventually shared (presence–absence) are certainly less informative than measures of Meliponini dependence in relation to a few productive flower sources in environment, for instance, mass flowering in the Atlantic forest (Ramalho 2004). An extreme case was observed in three species of *Scaptotrigona*, whose colonies concentrate their annual protein demands in one or few food sources, storing hundreds of surplus pollen grams for future offspring production (Ramalho 1990).

The increase in the average quality of a slightly higher number of floral sources in communities with more diverse flora would be enough to change sharing opportunities and to reduce niche width. For instance, in a local community in the Atlantic forest, less than two dozen trees with mass flowering attracted more than 70% of individuals sampled on flowers, and 100% of stingless bee species (Ramalho 1995, 2004). From an ecological point of view, that is, common in space and time, a recent

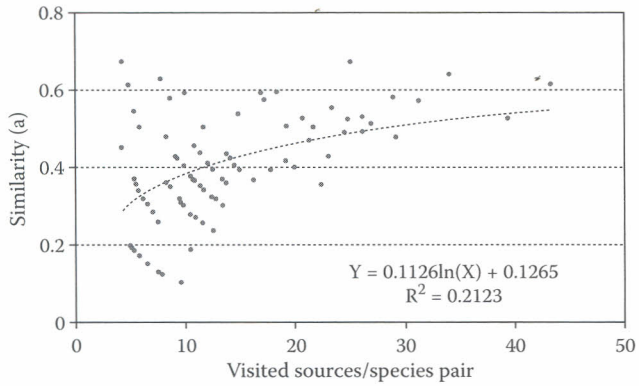

FIGURE 11.4 Variation in similarity between pairs of bee species (Apoidea) according to the number of foragers sampled on flowers (relative population size) in the Atlantic Forest (State Park Cantareira, SP). Comparison between species with proper number of individuals ($N \geq 50$) sampled over 18 months: 11 stingless bee species, *Apis mellifera,* and one species of each genus *Ceratina*, *Paratetrapedia,* and *Megachile.* Several points are overlapped: total number of pairs is equal to 105 (15!). Linear correlation: $r = 0.51$, $p < 0.05$. Data regression curve is indicated. Despite the consistent trend, there is a wide dispersion because most species forage with high intensity on few floral sources—when these few sources are also shared, overlapping (a = Cody index) is also high. (From Ramalho, M., Diversidade de abelhas (Apoidea, Hymenoptera) em um remanescente de Floresta Atlântica, em São Paulo, Ph.D. thesis, Universidade de São Paulo, Brazil, 1995. With permission.)

study supports the premise that there is a close (or predictable) relationship between Meliponini and mass flowering canopies (Monteiro and Ramalho 2010).

High overlap in abundant floral sources is not translated into higher competition. For instance, in the Atlantic forest of Cantareira state park, São Paulo, the vast majority of stingless bee species concentrated foraging activity in the canopy, where there are also more commonly canopies with huge mass flowering (Ramalho 2004). In these flowerings, spatial resources partitioning is easier and high values of similarity (above 50%) were observed among 22 species pairs that concentrated their foraging in the canopy, while only three pairs of species showed high similarity in the lower strata (Ramalho 1995). In a still more incisive way, *S. bipunctata* and *Paratrigona subnuda* Moure were extremely locally dominant and concentrated their visits in less than a dozen productive blossoms, with more than 50% overlap between them.

The mechanisms for floral sources sharing also need to be better contextualized in terms of costs and benefits. For instance, with their large relative size, species of *Melipona* should avoid floral resources whose supply is being depressed by exploitation; conversely, smaller bees such as *T. angustula* and some *Plebeia* and *Friesella* with larger pollen load capacity (Ramalho et al. 1994) could continue exploring floral resources in the process of local pollen depression, because they get profitable return rates with lower supply levels.

Considering random projections of taxonomic organization of 28 communities in Eastern Brazil, there is an overrepresentation in the number of genera in some cases (Biesmeijer and Slaa 2006). This situation seems consistent with the concept of limiting similarity. However, taxonomic proximity does not well represent functional similarity: there are common foraging strategies to several genera and cogeneric species with different strategies. Both pollen analysis from colonies (Ramalho et al. 1989, 1991; Ramalho 1990) and bee census on flowers (Ramalho 1995; Martins et al. 2003) have generated high percentage values of diet similarity between species of the same genus as *Melipona*, *Plebeia,* and *Scaptotrigona*.

In summary, the number of floral sources shared among stingless bee species depends on encounter chances and basically on the size of foragers' populations. However, the percentage of food similarity (i.e., the intensity of use of shared resources) is not related to niche width. Therefore, measures of food niche should be taken with caution in the analysis of Meliponini community structure because they cannot be translated into potential measures of competition between these generalist consumers.

11.2.3 Floral Constancy, Load Capacity, and Foraging Strategies

Under the adaptive logic, animals must be modeled to optimize their diet, and this means making the best possible choices in face of fluctuations in food supply, with appropriate adjustments in foraging (Pyke 1984). Models of foraging strategies explore the relationship between the total time of food intake and net energy gained (Schoener 1971). At the extremes, there are foragers that minimize food intake time and those that maximize energy gain. Animals with stable reproductive rates best fit to the first case, and those with variable offspring number to the second. Choices also depend on intrinsic factors such as body size, metabolic rate, and niche width. Furthermore, a set of environmental factors such as distribution, abundance, and risk exposure modify the access to food.

A significant part of experimental research on the economic foraging decisions in bees is included in the prediction analysis of optimal foraging theory and associated hypotheses (Pyke 1984). The basic premise is that the way an animal equates food acquisition, in terms of costs and benefits, determines its adaptive value, that is, the number of viable offspring it will leave for next generation (fitness). In the case of social bees with huge perennial colonies, this issue demands a solution and integration of decisions at different moments and in two hierarchical levels: in the field by forager, and in the colony, through social interactions that influence the long-term reproductive success. For these bees, there are two intrinsic constraints on foraging: displacement from a central point (i.e., the colony) and the short life of foragers. The need to return to the colony (central point) turns critical the equation of foraging cost and floral source distance. For these very small foragers with high-energy consumption during flight, autonomy is low and ultimately depends on the storage capacity of nectar in the honey pouch. Hence there is some common sense about the relationship between body size (honey pouch volume) and flight range in Meliponini (van Nieuwstadt and Iraheta 1996; Araújo et al. 2004).

Besides being short, forager longevity also has an inverse relationship with work intensity. This puts foraging decisions under the following perspective: forager should obtain and carry the largest possible load for each foraging trip or would have been modeled for maximizing net energy over lifetime, for the benefit of overall colony efficiency. Should it forage to maximize its own longevity, and thus indirectly increase long-term net returns for colonies? Studies with *A. mellifera* support this second alternative.

Foraging experimental studies have multiplied in recent decades, and in general confirm the expectations that foraging decisions are constrained by body size, metabolism, foragers' longevity, and social interactions. For instance, the selection process known as *majoring–minoring* seems to be typical of large foragers of the genus *Bombus* (Heinrich 1979). In this case, foragers modulate the intensity of use of several floral sources simultaneously in a single foraging trip. On successive trips they can make continuous adjustments intensifying visits to more profitable flowers (majoring), gradually reducing foraging on those that are depreciated (minoring). A *minor* source at a time becomes *major* in the other and vice versa.

Surveys on *A. mellifera* put into perspective foragers' ability to discriminate between net and gross foraging incomes (Seeley 1995). In the second case, foragers should focus on sources with a larger food supply, despite acquisition costs (collection and transport). In the case of net incomes, sources that offer a higher food return rate per unit of foraging time preferred to sources with a lower food return rate.

Foragers also carry information about foraging conditions to the colony. Their role as an information channel function on the frequency of food collecting trips (Nuñez 2000): the more times they get information in the field and delivery to the colony the higher their value. This would explain why foragers do not always completely fill the honey pouch. For instance, there is a gradual reduction of honey pouch load when nectar flow rate decreases. With this response, foragers reduce travel time and increase their value as information channel, contributing to speed collective colony responsiveness to changes in the relative value of floral sources.

Nuñez (2000) argues that the informational capacity of *M. quadrifasciata* is higher than that of the Africanized honeybee (*Apis mellifera scutellata* Lepeletier hybrid), which in turn is higher than that of European *A. mellifera*. When exposed to higher floral diversity, visits shorten and increased frequency of collecting trips explains higher foraging efficiency of Africanized bees. Stingless bees and Africanized honeybees are faster at choosing alternative sources, an ability correlated with higher floral diversity in tropical forests.

Stingless bees' foraging decisions are influenced by social interactions within colonies. For instance, some species of *Trigona* and *Scaptotrigona* use odor trails to communicate the location of attractive food sources; the expression of collective group foraging depends on the direct perception of stimuli in the field. Surprisingly, foragers' reaction to the presence of a conspecific on flowers is not related to the communication system (Slaa et al. 2003).

Honeybees are at the maximum extreme of colonial influence. Foragers bring to the colony profitability "expectations" of foraging in different floral sources, and through continuous exchange of information individuals compare sources' profitability and make joint decisions so the colony continuously redirects foraging effort to the most productive sources. Seeley (1985) named this process colonial thought to emphasize the emerging properties of the efficient integration of information.

The way a forager responds to the presence of individual conspecifics and heterospecific on flowers affects the spatial distribution pattern in food sources. Johnson and Hubbell (1974, 1975), Hubbell and Johnson (1978), Johnson (1983), Johnson et al. (1987) analyzed these responses in several species and proposed three forager categories: grouped, facultatively grouped, and opportunistic solitary. Considering the differences in aggressiveness among species, they recognized the existence of monopolistic and aggressive groups, nonaggressive group, and peaceful foragers. The strategy of aggressive groups also characterizes a "syndrome" called high-density floral specialists.

When a forager approaches a flower, its response to the presence of another individual of another species may be of repulsion or attraction. The answer is species specific but depends on the characteristics of the individual who is already on the flower: for instance, foragers avoid landing in the vicinity of individuals of larger or more aggressive species (Slaa et al. 2003).

Conspecific social interactions have an effect on spatial distribution and therefore affect foragers' activity (Slaa et al. 2003). On the approach to flowers, foragers of some stingless bee species react

positively to the presence of a conspecific, while in others the reaction is negative. In the first case, there is a tendency for the distribution of foragers in groups. Also, there seem to be rules for individual decision making: inexperienced and experienced foragers react quite differently to the presence of a conspecific in *Trigona amalthea* Olivier, while the response is always positive in *Oxytrigona mellicolor* Packard. This difference explains satisfactorily why groups of foragers are less compact or more dispersed in the first species.

Foraging decisions and the role of communication in social bees were subjects of numerous experimental studies, especially in the last three decades. Even a brief review of this topic would be beyond the scope of this chapter. However, the reference is necessary to put into perspective the complexity and peculiarities of the economic functioning of large perennial colonies and also to better contextualize seemingly simple behaviors such as floral constancy of foragers.

When a worker visits just one floral source on each foraging trip, it displays floral constancy or fidelity. Foragers of stingless bees may also present fidelity to the same source during multiple trips for days (White et al. 2001). Bee floral fidelity has three basic causes: need, innate restriction, or preference (Faegri and van der Pijl 1979). The first two have no true relationship with floral constancy, because in the first case, environment offers no opportunity for choice, and in the second, choice is limited by morphophysiological constraints. In generalist species, as those of Meliponini, individuals have physical, physiological, and behavioral skills to visit several types of flowers, so that fidelity is expressed as preference (i.e., true floral constancy). Two nonmutually exclusive hypotheses were proposed for these learned responses: foraging efficiency (Levin 1978; Heinrich 1979) and memory constraints (Waser 1983).

Foraging efficiency hypothesis is based on the use of search images by bees: foragers discriminate between floral types and use this information from a distance before landing on the flower. The alternative hypothesis assumes that bees (e.g., *Bombus*) are able to use more than one search image simultaneously by memory constraints.

The basic problem of stingless bee colonies relies on equating the high food demands with temporal variation in floral resources availability in a small range area, given the constraints of foraging from and to a central point. In tropical environments with high floristic diversity, a perennial colony should be generalist, and a forager's floral constancy should be regarded as "behavioral specialization." As stingless bee foragers have a very short life, the learning cost of handling several flower types must have become an ecological constraint. An experimental approach with *Plebeia tobagoensis* Melo indicates that foragers avoid the trade-off between resource types, unless there are changes in food supply, due to the embedded cost of learning time (Hofstede and Sommeijer 2006).

The most widespread evidence of floral constancy by stingless bees resulted from analysis of foragers' pollen loads. This behavior was found in all studied species (Ramalho et al. 1994, 1998; Slaa et al. 1997, 1998; White et al. 2001). However, there are variations when comparing different species or habitats. Ramalho et al. (1994.) reported very high levels of floral constancy in nine species of stingless bees (Figure 11.5) foraging in gardens with high diversity of tree species on the Brazilian Atlantic coast: nearly 95% of the pollen loads came from one plant species. In gardens from Queensland, Australia, a high proportion of *Trigona carbonaria* Smith foragers (88%) also presented a high level of floral constancy even during successive foraging trips (White et al. 2001). On the other hand, in the Amazonic region, *Melipona* foragers carrying mixed pollen loads have often been recorded (Absy and Kerr 1977). This is not a peculiar pattern for this genus, for in three *Melipona* species studied in the Atlantic coast (Figure 11.5), floral constancy was close to 100%.

Floral constancy should represent the compromise between rate of change in floral resources supply and species-specific capabilities. For instance, the informational capacity (Nuñez 2000) and foraging speed (Slaa et al. 2003) should change the expression frequency of this behavior simply because there are differences in species responsiveness to fluctuations in floral resources supply. Floral constancy is expressed even when stingless bee foragers have many available alternative sources (Ramalho et al. 1994; White et al. 2001) and therefore it should be interpreted as part of a set of foraging strategies to maximize individual efficiency. Through floral constancy, a generalist visitor can become an efficient pollinator. There is huge interest in measuring the expression of this behavior in Meliponini, given their numerical dominance in mellitophilous flowers in most tropical habitats and biomes of the Americas, especially in the Atlantic and the Amazonic forests. Analysis of pollen loads from workers could be

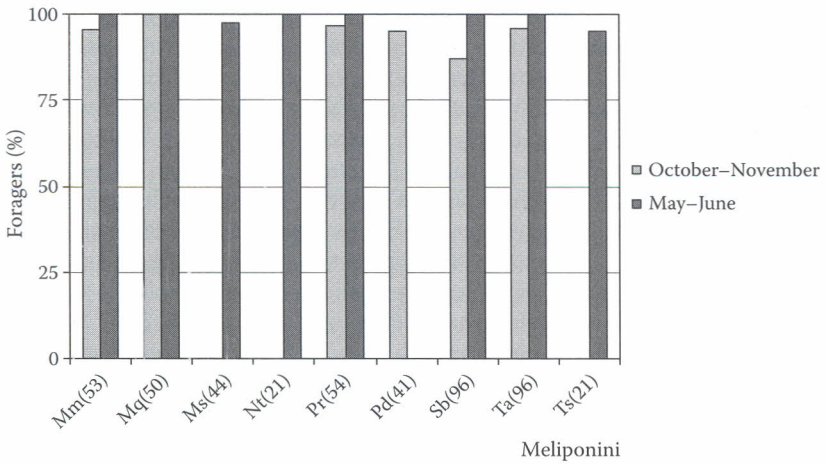

FIGURE 11.5 Floral constancy in stingless bee species. Percentage of foragers with unifloral pollen loads in two flowering periods: Mm = *Melipona marginata*; Mq = *M. quadrifasciata*; Ms = *M. scutellaris*; Nt = *Nannotrigona testaceicornis*; Pr = *Plebeia remota*; Pd = *P. droryana*; Sb = *Scaptotrigona bipunctata*; Ta = *Tetragonisca angustula*, Ts = *Trigona spinipes*. The numbers of sampled foragers are in parentheses. (From Ramalho, M., T. C. Giannini, K. S. Malagodi-Braga, and V. L. Imperatriz-Fonseca, *Grana*, 33, 239–244, 1994. With permission.)

widely used as an exploratory tool for choosing the more appropriate focal trees to analyze the effects of stingless bee foraging activity on plant reproduction (Ramalho 2004; Ramalho and Batista 2005).

Ramalho et al. (1994, 1998) focused on the relationship between pollen load capacity and workers size in Meliponini under standardized natural conditions, in the latter case comparing the transport of monofloral pollen (*Eucalyptus* pollen). They observed that pollen-carrying capacity/weight unit (load capacity) decreased as an exponential function of body weight or bee size (Figure 11.6a). Comparing pollen loads from different floral sources and pollen loads from *Eucalyptus* (Figure 11.6b), it was also evident that the fitting curve of body size becomes more accurate when comparing loads of the same pollen type.

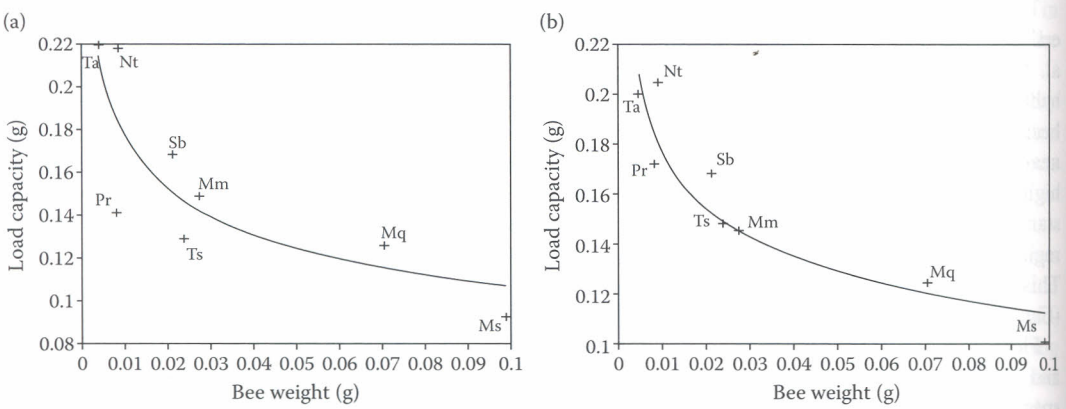

FIGURE 11.6 (a) Variation in pollen load per unit of body weight (load capacity) among stingless bee species. Pollen load capacity decreases with species size, independently of floral source ($N = 8$, $r = -0.77$, $p < 0.05$, and Y = aXb, and a = 0.065, b = –0.218). (b) Curve fits data better when comparing monofloral Eucalyptus sp pollen loads. ($N = 8$, $r = -0.90$, $p < 0.05$, Y = aXb and a = 0.073, b = –0.191). Mm = *Melipona marginata*, Mq = *Melipona quadrifasciata*; Ms = *Melipona scutellaris*; Nt = *Nannotrigona testaceicornis*; Pr = *Plebeia remota*; Sb = *Scaptotrigona bipunctata*; Ta = *Tetragonisca angustula*, Ts = *Trigona spinipes*. (From Ramalho, M., T. C. Giannini, K. S. Malagodi-Braga, and V. L. Imperatriz-Fonseca, *Grana*, 33, 239–244, 1994. With permission.)

There are variations in the weight of foragers' loads that rely on their own pollen source and/or pollen type. Huge variations between individuals of a same species and same size category were also observed.

Load capacity decay is higher in the transition from small Meliponini, such as *T. angustula*, *P. remota*, *Nannotrigona testaceicornis* (Lepeletier), to those of medium size, such as *S. bipunctatata* and *Trigona spinipes* (F.). From one category to another, general differences were also observed concerning foraging strategies: from solitary opportunist, that avoids antagonistic interactions, to group foragers, sometimes aggressive and monopolists.

The variation pattern in workers' load capacity refers to theoretical questions about ecological constraints of body size, and considering hypotheses about foraging (Schoener 1971), two basic predictions arise: (1) it is expected that large bees are able to meet their energy needs more quickly than small bees when food is abundant, and more slowly when scarce, and (2) if competitors reduce the abundance of floral resources in a uniform way in several blossoms, size convergence should be favored, while differential depletion would promote divergence among species size. The first hypothesis leads to the following prediction: as the average floral resources supply changes, larger bees must answer to localized reduction, moving quickly to another site or another floral source. An experimental study with *M. quadrifasciata* (Nuñez 2000) suggests that foragers can behave according to this general prediction. In contrast, in ecological communities, the largest *Melipona* species would often avoid overlapping and antagonistic interactions with small Meliponini species. Both bee censuses on flowers as comparative analysis of pollen sources from colonies point in that direction. Among small stingless bees, there are extreme opportunistic strategies, such as presented by *Paratrigona subnuda* Moure, that often collect pollen remains on floral parts resulting from other visitors' activity. The second hypothesis serves as a starting point for a reflection about interactions among several midsized Meliponini. In particular, species that have more or less regular nest spacing (Hubbell and Johnson 1977; Breed et al. 1999) tend to homogenize spatial resources offered in the nearby habitat. These species would then present greater convergence of body size, as seems to be the case for a number of *Trigona* species.

Also common is the variation in worker size within the same colony and among colonies of the same species. Laboratory data suggested a slight trend toward smaller worker production by weak colonies. From the standpoint of foraging efficiency, reduction in size has survival colonial value (Ramalho et al. 1998). *M. quadrifasciata* small workers carried little more pollen/unit of body weight (Figure 11.7)

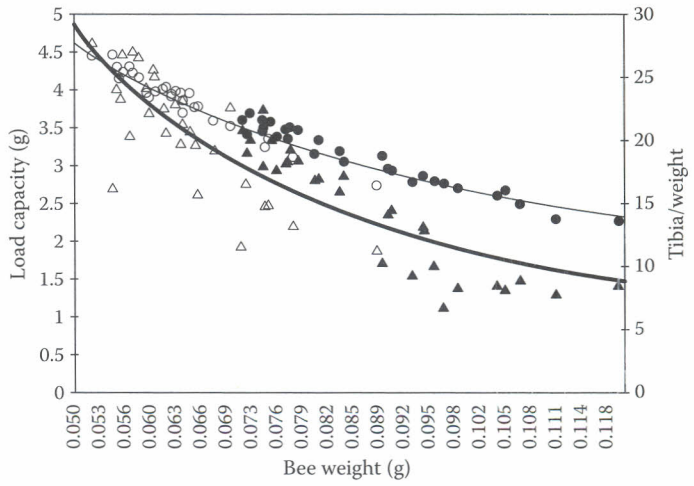

FIGURE 11.7 Relationship between pollen load capacity and forager body weight of *Melipona quadrifasciata*. Full and empty symbols represent workers of strong and weak colonies, respectively. Adjustment curve: Y = aXb. Triangles = relationship between load capacity and body weight (a = 0.08, b = –1.37, $r = -0.88$, $p < 0.05$). Circles = relationship between tibia surface (pollen-carrying structure) and body weight (a = 2.61, b = 0.79, $r = -0.97$, $p < 0.05$). Workers carry a little more pollen per unit of body weight (higher load capacity) and tibia allometric development explains most of observed variation. (From Ramalho, M., V. L. Imperatriz-Fonseca, and T. C. Giannini, *Apidologie*, 29, 221–8, 1998. With permission.)

(Ramalho et al. 1998). As pollen is essential for offspring production and smaller workers were also associated with weak colonies, the adaptability argument seemed to be supported. However, the primary cause of worker size variation is one of the basic problems of this apparently circular argument. When there are less floral resources in the environment, colonies need to reduce offspring production. There are fewer workers to forage, build, and provision the cells and thus a brood receives less food, and emerging bees are smaller. But why does the colony produce smaller bees rather than less offspring?

With the decrease in floral resource availability in a restricted range area, a colony has three options: reduce the amount of offspring, the size of offspring, or both. If the stability threshold of social functions in large perennial colonies were sensitive to the number of workers, colony survival would be less committed to the decrease of worker size: population fall is smaller and there is some efficiency gain in collecting pollen for production of the future offspring.

The inverse relationship between body size and pollen load capacity (Ramalho et al. 1994) means that food balance of colonies from different species can be achieved through different investment levels in foraging activity, with effects on life history. For instance, species with very small workers (*Plebeia, Tetragonisca, Paratrigona*) achieve a larger return of pollen biomass/foraging effort per capita and address more energy (and time) for offspring production. Variation in nests density and colonies longevity of *T. angustula* in disturbed forest habitats (Batista et al. 2003; Slaa 2006) supports this prediction. The opposite argument applies in very general lines to the large species of *Melipona*, whose foragers have lower pollen load capacity (Ramalho et al. 1994) and colonies invest more in longevity (Roubik 1989; Slaa 2006).

In summary, foraging economic decisions lead to floral constancy of stingless bee foragers. Allied to morphofunctional body size constraints, social interactions, and so forth, it can also be expressed as floral preference or realized niche narrowing, as has been observed in *Melipona* and *Scaptotrigona* (Ramalho 1990; Ramalho et al. 1989, 2007).

11.3 Resource Utilization by Colonies

In adult bees, food changes promote development of endocrine glands, determining workers skills. Bees in early adulthood participate in brood cell provisioning, producing larval food in their hypopharyngeal glands, which develop due to the consumption of large amounts of pollen. At a later age, the ingestion of pollen may stimulate ovarian development.

The queen receives a food rich in proteins that allows her to lay eggs continuously. In stingless bees, a queen may receive protein food through trophallaxis with workers or through ingestion of trophic eggs placed by workers. The queen occasionally feeds directly in the food pots or eats larval food from brood cells before oviposition. Studies with *P. remota* indicated that colony condition and food received by the queen determine egg size (M.F. Ribeiro, personal communication). During the larval period, food plays a key role in caste determination and/or differentiation.

11.3.1 Caste Determination and Differentiation in Bombini

Bombini are primitively eusocial bees (i.e., queens establish their nests and work at all tasks until the emergence of the first workers). Larval feeding is progressive or massal depending on the groups, which therefore are named pollen storers when larvae are fed slowly (*Bombus terrestris* (L.), *Bombus hypocrita* Perez) or pocket makers (present a food bag) when larvae obtain their food directly from a pollen mass (a bowl of wax with pollen, where eggs are laid by the queen) (Sladen 1912; Michener 1974).

In *Bombus,* mechanisms of caste determination differ among species. In *Bombus perplexus* Cresson, the size of female larvae is related to the amount of food in the colony, which is more abundant as the number of workers becomes larger in relation to the larvae (Plowright and Jay 1968). In *Bombus terricola* Kirby and *B. ternarius* Say, other mechanisms affect feeding rate and development of larvae into queens (Brian 1957; Plowright and Jay 1968). In some *Bombus hypnorum* (L.), *B. diversus* Smith, *B. ignitus*, and *B. hypocrita*, caste differentiation occurs later and larvae with longer developmental time

eat more and become queens (Katayma 1966, 1973, 1975; Röseler 1970). A high feeding rate in the last phase of development in *B. rufocinctus* Cresson influences larval destiny, causing changes in growth rate and silk production. Larvae that are fed less often begin to produce silk earlier and soon weave their cocoons, becoming workers. Others, who receive food more frequently, spend less time in silk production, delaying pupation and achieving larger size to become queens (Plowright and Jay 1977). In *B. terricola*, the larval development period differs, although there are no differences in growth rates of queens and workers larvae (Pendrel and Plowright 1977). In pocket-maker species, reproductives and workers are distinctively fed. During most of their development, workers feed from pollen pockets. From an early age, males and larvae destined to be queens are fed regurgitated food by adult workers (Alford 1975).

Finally, in *B. terrestris*, the mother queen produces a pheromone that suppresses the endocrine system of female larvae, preventing them from becoming queens. With aging and the likely decrease in pheromone production by the queen and/or increase in colony size, some larvae escape this control and have their endocrine system activated and become queens (Röseler and Röseler 1974; Röseler 1976, 1991). The pheromone involved has not yet been identified but apparently acts in suppression of juvenile hormone production, leading larvae to suffer the first molt earlier and therefore become smaller.

Caste differentiation is expressed by the ingestion of different amounts of food. In turn, food availability in the colony depends on the ratio between workers that collect food and larvae that consume it. Efficiency of individual workers in foraging and offspring care is also important.

Another relevant aspect is food quality. Enzymes produced by workers' hypopharyngeal glands added to the food given to larvae apparently help in digestion (Free and Butler 1959; Röseler 1974). A protein source besides pollen was found in the food of *B. terrestris* larvae, not exclusively on the food of larvae that developed into queens (Pereboom and Shivashankar 1994; Pereboom 1996).

Growth rate of queen larvae is different from that of workers. Queens ingest proportionally less pollen than expected and probably accumulate more fat. This suggests that queen larvae make better use of ingested pollen, or receive an extra source of protein in their diet (Ribeiro 1994). During the second development period, feeding frequency is also higher in queen larvae (Ribeiro et al. 1999). As in *A. mellifera*, each feeding time is probably related to the presence of glandular material added to larval food (Browers et al. 1987). This is important in the final developmental phase of queen larvae, as they receive larger amounts of these nutritive substances, which promote higher growth even in the absence of adequate pollen supply to the colony (Ribeiro 1999).

Bombus species, like honeybees (Free et al. 1989; Huang and Otis 1991; Le Comte et al. 1995), signal a hunger state with pheromones modulating the larvae feeding pattern by workers. Comparing larvae that experienced food deprivation with a control group, Pereboom (1996, 1997) found that the former were fed before and with a higher initial rate than control larvae. Larval food composition induces females' caste development in *Bombus* (Pereboom 2000).

Approximately one million colonies of *B. terrestris* are sold each year for pollination in agriculture. This successful rearing trade, especially in Holland and Belgium (Velthuis and van Doorn 2006), brought contributions to the knowledge of food quality influence on nest development. For *Bombus*, nest development demands lots of pollen, obtained from colonies of *A. mellifera*. Ribeiro et al. (1996) found that pollen quality influences queens' production. Queens reared with dry pollen (which loses nutritional value in the drying process) in greenhouses were smaller, had higher mortality rates, and produced smaller colonies than those supplied fresh pollen. Qualitative and quantitative pollen variations influence colony development and reproductive success (Génissel et al. 2002).

11.3.2 Caste Determination and Differentiation in Apini

In honeybees, *A. mellifera*, caste determination occurs early in larval development. From the third day of life, food provided to worker and queen larvae changes quantitatively and qualitatively. Food for queen larvae, royal jelly, contains larger amounts of mandibular glands secretions than food for worker larvae. Three components were described in larval food: white (mandibular gland secretions), clear (hypopharyngeal gland secretions), and yellow (pollen) one. Worker larvae receive these components in the following proportions: 2:9:3, respectively, while a queen would receive 1:1 mainly from the first two components (Jung-Hoffman 1966).

Larvae feeding frequency varies. Queen larvae are fed >1,600 times while workers only 143 times during their development (Lindauer 1952). The amount of food seems to be relatively less important than quality. Larvae that were fed a queen's artificial diet *ad libitum* develop into queens, while those fed a worker's diet *ad libitum* do not (Moritz 1994). Queen larvae gain weight twice as fast than those of workers, weight gain 30 mg to >300 mg in just 2 days (Moritz 1994). Therefore, queen larvae are reared in larger cells, called royal cells.

Queen larvae have phagostimulant sugar present in 34% of royal jelly, while phagostimulant sugar is present in only 12% of workers' food (Beetsma 1979; Winston 2003). The type of sugar also differs in larval food: queens receive mainly glucose, whereas workers receive glucose in the first larval phases and fructose in the last ones (Browers 1984).

Finally, juvenile hormone (JH) produced by *corpora allata* exerts influence in caste differentiation. Queen larvae with 72 hours of age have JH levels 10× higher than worker larvae of the same age (Wirtz 1973). JH levels remain high throughout the remaining larval phases in queens (Beetsma 1979).

11.3.3 Caste Determination and Differentiation in Meliponini

In Meliponini, brood cells receive all food before egg laying by the queen, a feeding behavior known as mass provisioning. Individual brood cells are built by workers, following complex behavioral sequences (Sakagami and Zucchi 1963; Sakagami 1982). Workers provision cells with liquid larval food, and may place trophic eggs on this larval food that are consumed by the queen and more rarely by workers (Silva-Matos et al. 2000). The queen lays her egg in this cell, which is then closed by workers. This sequence of events called provisioning and oviposition process (POP) is variable (Sakagami and Zucchi 1963).

A basic question is whether the provisioned food differs in quality between cells that give rise to queens than in those of workers. In *Melipona* caste differentiation is genetic, although environmental influence may be also important (Kerr 1950b). Queens and workers are reared in identical cells; queen larvae are double heterozygous (AaBb) while worker larvae are homozygous; the amount of food is also important (Kerr 1950b, 1969; Kerr et al. 1966; Velthuis and Sommeijer 1991). Queens have four nodes in the ventral nerve cord, while workers have five (Kerr and Nielsen 1966). Another peculiarity of *Melipona* species is the large number of queens produced in the colonies, which can reach up to 25% of the offspring (Kerr 1946, 1948, 1950a,b; Santos-Filho et al. 2006).

A second hypothesis about *Melipona* caste determination was based on self-determination (Ratnieks 2001; Wenseleers et al. 2003). They consider that larvae "decide" their fate by choosing whether to be queens. Their model forecasts 14% of queens in the offspring, close to the 25% model suggested by Kerr (Santos-Filho et al. 2006). Caste determination in *Melipona* using molecular markers (Judice et al. 2004; Makert et al. 2006) provide a complete list of genes differently expressed in queens and workers of *M. quadrifasciata,* available at *http://www.lge.ibi.unicamp.br/abelha*. Hartfelder et al. (2006) put together a comprehensive review on caste determination in Meliponini.

In other genera of stingless bees, caste determination is essentially trophic, although several strategies have been developed for queen rearing in large cells, known as royal cells. Thus, consuming more food, female larvae become queens rather than workers (Engels and Imperatriz-Fonseca 1990). In *Frieseomelitta* and *Leurotrigona* species, where royal cells are not built by workers, one larva may consume all food of its neighboring cell and become a queen (Terada 1974; Faustino et al. 2002). This also happens in *Plebeia lucii* Moure, a species that builds bunch brood cells. In queenless colonies, these bees build cells for queen production (Teixeira and Campos 2005), and the larger amount of food determines the differentiation of larvae into queens.

Other studies suggest a greater complexity in trophic determination process. Giant workers can emerge from royal cells (a single observation in *P. remota*, Imperatriz-Fonseca 1975) and dwarf queens can arise from cells of equal size to those of workers (Ribeiro et al. 2006a), indicating that food amount alone is not enough to explain caste determination in royal cell builders. The emergence of dwarf queens from "normal" sized cells occurs in several genera on a regular *(Schwarziana, Cephalotrigona)* or occasional (*Plebeia, Nannotrigona*) basis. In general, some dwarf queens are viable, mate, and lay eggs normally, surviving for long time (Ribeiro and Alves 2001; Ribeiro et al. 2003; Wenseleers et al. 2005; Ribeiro et al. 2006a,b). Explanations for the existence of dwarf queens and their production mechanisms vary

depending on the genus and on circumstances. Some larvae may escape the fate of becoming workers, using their ability of "self-determination" to become dwarf queens (Wenseleers et al. 2005; Ribeiro et al. 2006a). In this case, dwarf queen development is under the control of genetic mechanism (Wenseleers et al. 2004). Another possibility is the presence of larval food of better quality or in larger amounts in some cells. Castilho-Hyodo (2002) studied the quality of larval food in *S. quadripunctata*, showing the high variability in protein content of brood cells from the same comb.

In *Melipona beecheii* Bennet, colonies with reduced amount of food produced fewer queens than those that receive extra food. However, the latter did not produce a significantly higher number of queens, when compared with control colonies (Moo-Valle et al. 2001). In *P. remota*, however, there is no relationship between variation in the number of produced queens and colony food storage (Ribeiro et al. 2003).

11.4 Larval Food in Meliponini

In stingless bees, larval food seems to be species specific. Darchen and Delage-Darchen (1971) reared queens even with larval food of different species. Silva (1977) obtained queens in mixed colonies, made up of queens and workers from related species. Hartfelder and Engels (1989) studied the composition of larval food in stingless bees. They analyzed the water soluble constituents in larval food of seven species, and found that the variation in larval food proteins was consistent with phylogenetic trees. They also suggested that nurse workers of stingless bees would not control queen development, for instance, provisioning certain cells with a special diet. Instead, they would just place larger amounts of the same food type given to any other cell inside royal cells.

In stingless bees, the protein content of larval food is about 10 times lower than in *Apis* (Takenaka and Takahashi 1980), and that is the main difference between the two groups. The proportion of sugars and of free amino acids in larval food is similar in both (Shuel and Dixon 1959; Rembold and Lackner 1978).

Bionomic knowledge of the necrophagous bees (*T. crassipes*, *T. necrophaga,* and *T. hypogea*, Roubik 1982; Camargo and Roubik 1991) brought forth important issues on the quality of stingless bee larval food. These bees replaced pollen with animal protein. There is no pollen in their nests, but there are sugar solutions storages, probably obtained from extrafloral nectaries. Among the basic adaptations of these species to these new feeding habits are jaws with five teeth (maximum number found among Meliponini) and reduced corbicula on the third pair of legs (as they do not carry pollen).

Gilliam et al. (1985) studied the microbiology of larval food of *T. hypogea*, considered at that time an obligatory necrophagous. They mentioned these bees gathered food in a wide variety of freshly killed animals (frogs, toads, lizards, fish, birds, even monkeys). Later, Mateus and Noll (2004) found that this species fed on live wasp offspring caught in abandoned or unprotected nests. Once they find their food source, bees quickly recruit their nestmates, who monopolize the food source, excluding other insects. Workers place secretions on the organic matter for a predigestion (see review in Noll et al. 1997), then they ingest it and carry the thick liquefied material to the nest. There, that food is processed by other workers, probably adding large amounts of enzymes from hypopharyngeal glands. In *T. hypogea*, these secretory units are multicellular, while in Meliponini species that feed on pollen, they are unicellular (Cavasin-Oliveira and Cruz-Landim 1991). After being processed, the resulting viscous liquid has a pH between 3.0 and 4.0, very similar to *Apis* royal jelly, and it is stored in food pots. Several microorganisms transform and probably play an important role in the conservation of this protein food of animal origin. Gilliam et al. (1985) found in samples of larval food of this species *Bacillus pumilus, B. meggaterium, B. subtilis, B. circulans,* and *B. licheniformis*, which produce several enzymes. These microorganisms are responsible or have an important role in converting these reserves into a nutritious and metabolizable food for larvae and young bees. The same *Bacillus* species were found in pollen stored by *A. mellifera* (Gilliam and Morton 1978). Machado (1971) verified an association of a species similar to *B. pumilus* with pollen of *M. quadrifasciata*, which seemed to predigest pollen. It appeared in large amounts just in the glandular secretion placed between layers of pollen and nectar in brood cells. Machado (1971) also found *Bacillus* in larval food of 13 species of stingless bees: four species of Melipona, two of *Plebeia* and *Trigona*, and one of *Partamona*, *Frieseomelitta, Leurotrigona, Tetragona,* and *Nannotrigona*. Gilliam et al. (1985) argue that bees can add to larval food beneficial microorganisms, responsible for conversion,

fermentation, and preservation of larval provisions, which also inhibit proliferation of other undesirable microorganisms, for instance, producing antibiotics and fat acids.

11.5 Pollen

Since plants cannot move to find reproductive partners, flowering plants developed a series of traits to overcome this difficulty: they attract insects or other animals to their flowers, favoring crossing among them. In flowers, plants provide food, nectar, and pollen, and use several features, such as vibrant colors, perfumes, and petals that serve as landing platforms, to attract floral visitors that carry pollen (male part) from one flower to the stigma (female part) of another, a phenomenon called pollination.

Pollen-collecting bees favor effective pollination of plants more than nectar gathers (Free 1966). Unlike nectar, which is available throughout the day, pollen from plants is a resource offered all at once. It is the main protein source for most bees and it is used for offspring development. Pollen is part of the diet of other insects and supplements the diets of bats, birds, and marsupials, and these animals, as well as bees, are pollinator agents.

11.5.1 Protein Value

Protein content of pollen grains varies from 2.5% up to 61% (Buchmann 1986). Pollen grain nutrients are found in their cytoplasm and are recovered after a digestive process. The grains' outer layers are not digested, because they are made of cellulose and sporopollenin, which are hard to decompose. As they retain their external structure, grains can be identified after passing through animals' digestive tract, which allows paleoecologists to reconstruct the original flora and climate of regions where they occurred.

Protein of pollen grains consists mainly of enzymes that act during pollen tube growth (Stanley and Linskens 1974). Roulston et al. (2000) showed that the protein content of pollen grains of 377 plants species is highly conservative within genera and families, with the exception of Cactaceae and Fabaceae. Plants taxa with buzz pollination are rich in proteins (x = 47.8%), despite minute pollen grain size.

Anemophilous pollen grains have lower protein content than zoophiles, although anemophilous grains, such as those of Poaceae (maize) and Moraceae (*Cecropia*) are frequently collected by *Apis mellifera* and stingless bee species (Cortopassi-Laurino and Ramalho 1988). Protein content of pollen grains from corbiculae of some Meliponini of the Amazon region presented values between 15.7% and 23.8% (Souza et al. 2004).

11.6 Food Rich in Sugars Produced by Bees: Honey

Honey is still the main product of commercial rearing of honeybees and stingless bees. Flower nectar is the raw material used to create honey, which is produced and stored in large amounts inside the nests. As alternative food sources used by bees (Figure 11.8), there are plant secretions such as those from sugar cane, or excretions of insects that suck living parts of plant originating honeydew honey. In stingless bees, honey is stored in large oval pots, which vary in size according to species, whereas in *Apis* it is stored in hexagonal cells similar to those used to rear the brood.

11.6.1 Honey Microscopy

Bees visit flower nectaries mainly to collect nectar. In some cases, nectar gets contaminated with flower pollen. When observing honey under the microscope, pollen grains from flowers that were visited for nectar collection are identified (Figure 11.9). As a rule, most represented pollen grains indicate floral origin (i.e., the nectar that contributed most to the honey composition). Some pollen grains are considered geographical indicators, for they are only found at certain places.

FIGURE 11.8 Africanized honey bee (*Apis mellifera*) and *Nannotrigona testaceicornis* sucking secretions of scale insects. Wasps and ants also collect these secretions.

Melissopalynology, the study of honey pollen grains, depends mainly on data accumulation and knowledge of grain morphology. Pollen grains show typical shapes for each species, with different openings and ornamentation, and sizes ranging from 5 to 300 μm. Only the smallest grains are collected mostly by *Apis* and stingless bees (Barth 1989; Ramalho et al. 1990; Pirani and Cortopassi-Laurino 1993; Moreti et al. 2002).

Pollen analysis of food carried to nests has been used as an indirect method of assessment of bee visits to flowers. This has advantages and disadvantages in relation to field observations, which depend on several aspects, such as collection time, tree height, and "plant apparency." For beekeeping, pollen analysis allows identification of poorly known wild flora, supports planning honey annual production by migratory beekeeping, and allows control of floral and geographical origin of honey, information increasingly important for product credibility and for adoption of appropriate processing measures.

11.6.2 Honey in Apini

The most productive bees in Brazil are *A. mellifera*, or Africanized honeybees (Figure 11.10) as they are better known, frequently observed in urban centers. Africanized honeybees are not native to Brazil; they are a crossbreed between *A. mellifera*, brought from Portugal to Rio de Janeiro in 1839 by Father Antonio Carneiro and others (Nogueira-Neto 1997), and African *A. mellifera*, introduced in 1956, in order to increase honey production through selective breeding. Currently, it is estimated that domestic honey consumption in Brazil is around 40 to 60 thousand tons/year (C. Zara, personal communication).

Honeybee honey is composed mostly of water and sugars (99%). The remaining (1%) contains substances present in tiny amounts, but which are important in honey characterization, such as enzymes,

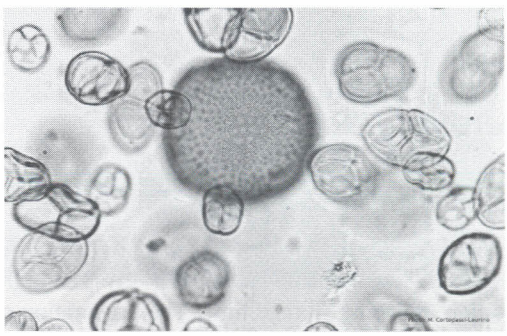

FIGURE 11.9 Pollen grains found in honey slides. The isolated central grain belongs to the family Euphorbiaceae, identified by the croton ornamentation pattern. The other grains are from Mimosaceae (*Mimosa bimucronata* and *M. taimbensis*).

FIGURE 11.10 Africanized honey bee (*Apis mellifera*) sucking nectar from *Citrus* sp. flower.

amino acids, and minerals. Its humidity is about 20% and it has approximately 80% of sugars such as glucose, fructose, and sucrose. Glucose is relatively insoluble and its amount determines honey crystallization tendency. Fructose is very sweet and hygroscopic that absorbs air humidity (Crane 1987). Color patterns, scent, and flavor vary with floral origin, geographical regions, and climatic conditions. Floral honeys can be separated from honeydew by morphological elements and physicochemical analyses (Barth 1989; Campos et al. 2003).

11.6.3 Honey in Meliponini

Honey production can reach just a few liters per hive per year. Nevertheless, the high market value turns stingless bee rearing into a profitable activity, at least in small scale. These bees' rearing, or meliponiculture, is based mainly on bees of the genus *Melipona*, which are large (15 mm) and store honey in big pots, which facilitates extraction. Since pre-Hispanic times in Mexico, rearing of *M. beecheii* testifies this long tradition; species of *Tetragonisca* and *Scaptotrigona* have also been widely reared. Traditionally, medicinal value is attributed to honey from the former genus, while the second are good producers because their colonies are very populous.

In Brazil, honey production from *Melipona* species is more expressive in the Northeast, where the product can be found in labeled packages, with details of the producer, origin, and collection date (Figure 11.11).

T. angustula (Figure 11.12) is the most popular stingless bee species, widely distributed throughout Latin America. Although having a small nest production, around one liter/year, its honey is considered medicinal and used in treating eye diseases by rural populations. Easiness of recognition and management have contributed to its popularity. Species of *Scaptotrigona* also have a wide distribution in Latin America. Usually, they have populous nests, are aggressive, and produce large amounts of honey. In Mexico and in Central and South America, several different species are reared for this purpose, as *Scapotrigona mexicana* (Guérin-Méneville), *S. depilis* (Moure), *S. nigrohirta* Moure, *S. polysticta* Moure, and *S. postica* (Latreille) (Cortopassi- Laurino et al. 2006).

11.6.3.1 How to Exploit Meliponini Honey

Compared with *A. mellifera* honey, stingless bee honey frequently has higher water percentage, higher acidity, and lower pH values (Cortopassi-Laurino and Gelli 1991). The high water percentage makes it more susceptible to fermentation, reducing storage time. The preparation of the Technical Regulation of Identity and Quality of Stingless Bee Honey faces two major basic problems: the lack of results of physicochemical analyses and the wide variety of bees.

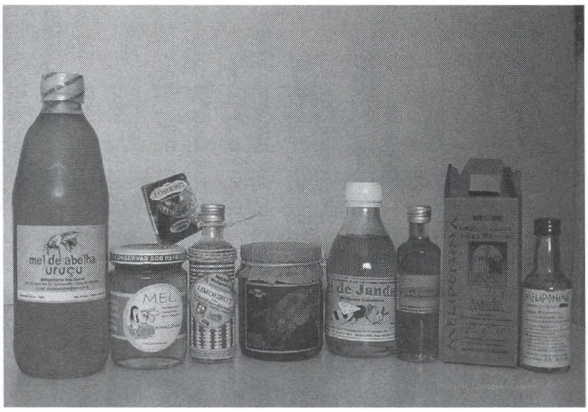

FIGURE 11.11 Several honey packages of stingless bee honey. From left to right: honey from *M. scutellaris*; *Melipona* honey from the Amazon region; honey from *M. fasciculata* with glass coated with buriti fibers, which adds value to the product; honey from *M. rufiventris*; honey from *M. subnitida*, a unique honey with an annual registration label at the Agriculture Secretary from Rio Grande do Norte state; honey from *Scaptotrigona* sp, Belterra, PA; package and honey glass from *M. fasciculata* provided by the nongovernmental organization AMAVIDA from Maranhão state.

Technical studies of honey have focused on a few dozen species, especially *Melipona*. It has been suggested as a protocol for honey control from *Melipona, Trigona,* and *Scaptotrigona* (Vit et al. 2004). There is a technical foundation for a preliminary proposal on Meliponini honey legislation, considering that more than 1,100 samples of 18 species were already examined. Of these, there are a higher number of results for humidity, pH, acidity (free and total), ash, and HMF (hydroxy-methyl-furfural) parameters. However, as these physicochemical characteristics vary widely, there is need to enlarge the number of samples to obtain a consistent honey profile from most genera and species studied up to now (Bazlen 2000; Souza et al. 2004, 2006, 2009; Almeida and Marchini 2006; Carvalho et al. 2006; Cavalcante et al. 2006; Oliveira et al. 2006; Persano-Oddo et al. 2008, Anacleto et al. 2009, Rodrigués-Malaver et al. 2009). Table 11.1 summarizes the results of stingless bee honey analyses with at least five samples.

Until now, of the specified tests in the Technical Regulation for Identity and Quality of *A. mellifera* Honey, eight have been used in stingless bee honey analyses. This physicochemical test is applied with

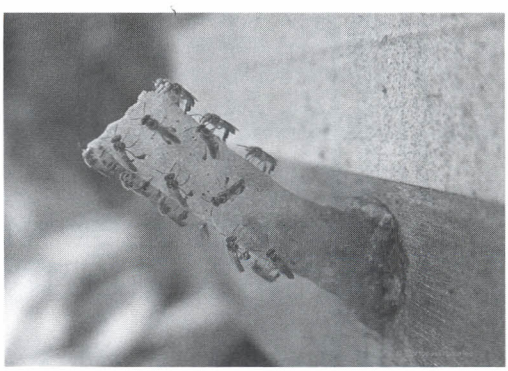

FIGURE 11.12 Nest entrance of *Tetragonisca angustula* in a rational wooden box. This is one of the best-known stingless bee species that presents a wide geographical distribution, from Mexico to Misiones, Argentina. (From Nogueira-Neto, P., *Vida e Criação de Abelhas Indígenas sem Ferrão*, Editora Nogueirapis, São Paulo, Brazil, 1997. With permission.)

TABLE 11.1

Stingless Bee Honey: Physicochemical Characteristics

Species	Number	pH	Total Acidity	Humidity	HMF	Diastase Index	Invertase Index	Ashes	Locality	Reference
M. asilvai	11	3.3	41.6*	29.5	2.44				BA	Souza et al. 2004
M. asilvai	7	3.6	54.2	37.5	30.9			0.09	BR-BA	Souza et al. 2004
M. beecheii	5	4.2	59.4	27.0	5.4				Mexico	Santiesteban 1994
M. beecheii	7	3.7	23.2*	17.3	0.1	21.3		0.07	Guatemala	Dardón and Enriquez 2008
M.compressipes	35	3.3	91.1	25.6					MA	Oliveira et al. 2006
M. compressipes	5		48.4	23.4	1.0	1.1		0.3	Venezuela	Vit et al. 1994
M. favosa	511			31.2					Trinidad/ Tobago	Bijlsma et al. 2006
M. favosa	14		62.9*	25.5	1.2	0.9		0.3	Venezuela	Vit et al. 1994
M. favosa favosa	6		36.8	24.2	17.1	2.9	90.1	0.2	Venezuela	Vit et al. 1994
M. grandis	5			27.5					Peru	Rodrigués-Malaver et al. 2009
M. mandacaia	20	3.3	43.5	28.8	5.8				BA	Alves et al. 2005
M. quadrifasciata	8	3.5	132.6*	32.2				0.4	SP	Cortopassi-Laurino 1997
M. quadrifasciata	6	4.0	38.5*	25.5	3.8	1.8		0.1	BA	Oliveira et al. 2006
M. quadrifasciata	6					1.2–2.2			BA	Fonseca et al. 2006
M.quadrifasciata anthidioides	9	4.0	40.6	32.1	16.0			0.1	BA	Souza et al. 2004
M. scutellaris	20	4.1	31.1	28.6	2.7	4.7	201.9		BA	Bazlem 2000
M. scutellaris	7	3.6	39.8*	26.9	3.3	4.0		0.04	BA	Cavalcante et al. 2006
M. scutellaris	7					0.7–19.8			BA	Fonseca et al. 2006

M. scutellaris	15	4.4	19.9	29.1	2.0			0.19	BA	Souza et al. 2004
M.subnitida	47		2.4*	24.0	8.7			0.5	PI	Camargo et al. 2006
M.trinitalis	62			33.0					Trinidad	Bijlsma et al. 2006
Plebeia wittimani	10	3.3	117.5					0.2	Argentina	Spariglia et al. 2010
Scaptotrigona pachysoma	7	3.9	66.6	26.9	1.0				Mexico	Santiesteban 1994
Tetragonisca angustula	12	4.3	71.9					0.07	Argentina	Spariglia et al. 2010
Tetragonisca angustula	261	4.2		27.7					SP	Iwama 1977
Tetragonisca angustula	20	4.0	54.1	27.9	5.7	22.0	38.9		SP/BA	Bazlem 2000
Tetragonisca angustula	10	4.4	20.6*	23.9	7.5	30.0		0.4	SP	Almeida and Marchini 2006
Tetragonisca angustula	14			24.9					Costa Rica	Demera and Angert 2004
Tetragonisca angustula	7	4.2	74.7	25.0				0.3	SP	Cortopassi-Laurino 1997
Tetragonisca angustula	20	4.1	45.2	24.4	9.4	7.2–54.1		0.39	SP	Anacleto et al. 2009
Trigona carbonaria	8	4.0	124.2*	26.5	1.2			0.48	Australia	Persano-Oddo et al. 2008

Note: Number of samples > 5. Most data from Brazil (BA = Bahia; MA = Maranhão; SP = São Paulo; PI = 0020 Piauí); other countries are identified.
* Free acidity.

reservations in the proposed legislation of stingless bee honey. Techniques adopted by the European Honey Commission (Bogdanov et al. 1997) can be adjusted to enhance technical control, and Souza et al. (2006) emphasized the need of obtaining additional data, such as sugar types, electric conductivity, and pollen analysis. Stingless bee honey collected in areas with different rainfall levels or from different nests in the same place shows variation in water content for the same species (Bijlsma et al. 2006).

In the Amazon region, the recent production of about three honey tons from *Melipona compressipes* F. and *M. seminigra* Friese (Villas-Boas and Malaspina 2004) shows that there is an underutilized potential production. Paradoxically, this "surplus" honey production is facing distribution and quality certificate problems. While this situation remains unsolved, stingless bee honey will continue to be sold as a natural product without official record, becoming more subject to adulteration. Table 11.2 presents a summary of physicochemical parameters that can be used for the Technical Regulations for the Quality of Stingless Bee Honey. They were compiled from analyses of 332 honey samples from *T. angustula* and 813 samples from *Melipona* spp.

11.6.3.2 Antibacterial Activities

Since ancient times, honey has been used as an antibacterial agent for wound and burn treatment. Initially it was thought that honey's antibacterial property was associated with high sugar concentration (± 80% to *Apis*) and low pH. However, some organisms that survive at low pH, such as *Staphylococcus aureus*, did not survive in honey, indicating that other substances were active against bacteria. This "inhibin" was later identified as hydrogen peroxide. This compound is produced by the action of a bee enzyme (glucoseoxidase) in honey sugar (glucose), resulting in gluconic acid plus hydrogen peroxide. The presence of H_2O_2 is higher in diluted honey.

Stingless bee honey still presents antibacterial activity even when hydrogen peroxide production is inhibited by catalase addition. Therefore, there are still other compounds that need to be chemically identified. Recently, antioxidant compounds such as polyphenols and flavonoids have been quantified in honey because they have bioactive properties that may be responsible for their biological and therapeutic properties (Vit and Tomaz-Barbéram 1998; Guerrini et al. 2009; Persanno-Oddo et al. 2009; Pitombeira et al. 2009; Rodrigués-Malaver et al. 2009).

Water activity available in honey has been quantified as it contributes to the development/inactivation of microorganisms. These values are between 0.59 and 0.82 (0.66) for *T. angustula* (Anacleto et al. 2009), 0.79 for *M. asilvai*, 0.75 for *M. mandaçaia*, 0.76 for *M. quadrifasciata anthidioides*, 0.71 for *M. scutellaris* (Souza et al. 2009), 0.74 for *T. carbonaria* (Persanno-Oddo et al. 2008). In honeybees, honey has lower humidity, with values between 0.48 and 0.65 (Schroeder et al. 2005).

Minimal inhibitory concentration (MIC) is another way to evaluate honey antibacterial value; this parameter identifies the minimum amount of honey with activity against certain bacteria strains. Rodrigués-Malaver et al. (2009) found in native bees of Peru an MIC of 50% (w/v) to inhibit *E. coli* and 12.5% to 50% to inhibit *S. aureus*. According to Boorn et al. (2009), *T. carbonaria* honey presented an

TABLE 11.2

Suggestions of Physical and Chemical Parameters for Stingless Bee Honey

Parameters	*Melipona*	*Tetragonisca angustula*
pH	3.3–4.4	4.0–4.4
Free acidity	<132.6	<71.9
Humidity	<37.5	<27.9
Ashes	<0.5	<0.4
HMF	<30.9	<9.4
Diastase	0.7–21.3	7.2–54.1
Invertase	90.1–201.9	38.9

MIC between 4% and >10% to inhibit Gram-positive bacteria, between 6% and >16% to inhibit those Gram-negative, and between 6% and >10% to inhibit *Candida* spp. Of nine species of stingless bees in Guatemala, except for *M. solani*, all presented an MIC between 2.5% and 10% against microorganisms, especially *N. perilampoides* with values between 2.5% and 5% (Dardón and Enriquéz 2008). Likewise, ethanolic fractions of honey from native bees of Ecuador presented inhibitory values against bacteria (Guerrini et al. 2009).

In relation to topical honey applications, Vit and Jacob (2008) found significant inhibition of induced cataracts in sheep when treated with flavonoids present in ethanolic fractions of honey, such as luteolin and orientin. Alves et al. (2008) verified that the application of *Melipona subnitida* honey in infected wounds of rats' skin stimulated immune response and reduced infection and healing time.

Unprocessed honeybee honey has been recommended as a topical agent in infected wounds, chronic ulcers, and burns, with excellent results in reducing infection and healing time (Tostes and Leite 1997). Similarly, honey from stingless bees has also been used as a topical agent in insect and snake bites and ocular inflammations in several Latin America countries. In the laboratory, stingless bee honey has shown bacteriostatic and bactericidal capacity equal to or greater than that of *A. mellifera*, against several bacteria strains, both Gram-positive and Gram-negative; however with less action against fungi and yeasts (Cortopassi-Laurino and Gelli 1991; Martins et al. 1997; Grajales-C. et al. 2001; Demera and Angert 2004; Gonçalves et al. 2005; Oliveira et al. 2005).

Tables 11.3 and 11.4 summarize current knowledge of stingless bee honey inhibiting power as compared to Africanized honeybee honey. In these tests, two methods have been used: dilution and application in a Petri dish (Anonymous 1977) and agar diffusion (Bauer et al. 1966). The most tested honeys were those of more productive bees such as *Melipona* and *T. angustula*. The most tested bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa* as they are the major infectious agents of wounds and burns.

11.6.4 Honey Microorganisms

There is great interest in the characterization of microorganisms in honey, because it can be used as food or as a component of drugs and cosmetics. The microbial content of honey affects its "shelf life" and its validity for human use. Microorganisms associated with honey are fungi and spore-forming bacteria. Spores are present everywhere, even inside bee nests. They may come from external sources such as

TABLE 11.3

Antibiosis Values of Stingless Bees and Honeybees Honey by Dilution Methods[a] and Application on Petri Dishes

	Stingless Bees						Honeybees
Microorganisms	**Msc = 5**	**Ms = 2**	**Pl = 1**	**Ta = 3**	**Mq = 2**	**Tc = 1**	**Am = 20**
Bacillus subtilis	3.0	4.13	5.0	3.7	4.0	4.8	2.8
Bacillus subtilis Caron	3.3	3.9	5.0	3.7	4.0	4.0	2.7
Staphilococcus aureus	2.9	3.9	4.8	3.9	4.4	4.0	3.2
Klebisiella pneumoniae	3.1	4.3	5.0	3.3	5.0	5.0	3.0
Pseudomonas aeruginosa	3.0	3.8	5.0	3.8	4.6	5.0	3.1
Escherichia coli	1.7	3.8	5.0	3.3	4.3	4.8	2.0
Bacillus stearothermofilus	4.5	4.5	5.0	5.0	5.0	5.0	4.1

Source: Cortopassi-Laurino, M., and D. S. Gelli, *Apidologie*, 22, 61–73, 1991.

Methodology source: Anonymous, J. *Officiel de la République Française*, 22 avril, 3485–514, 1977.

Note: Species of bees: Msc = *Melipona scutellaris*; Ms = *M. subnitida*; Pl = *Plebeia pugnax*; Mq = *M. quadrifasciata*; Tc = *Tetragona clavipes*.

[a] 5%, 10%, 15%, 20%, and 25%, which correspond to notes 5, 4, 3, 2, 1, respectively.

TABLE 11.4

Antibiosis Value of Meliponini and *Apis* Honey by Agar Diffusion Method[a]

	Meliponini						Apis
Microorganisms	**Msc = 1[b]**	**Ms = 1[b]**	**S.bip = 1[b]**	**Nt = 1[c]**	**Ta = 5[d]**	**Tl[e]**	**Am = 3[b]**
B. subtilis	10.0	14.5	10.0				13.3
S. aureus	13	22	15.0			3.8	23.5
E. coli	10	28	10.0			2.9	24.0
S. cholerasuis	21	12	13.0				14.8
E. coli				19.0			
Proteus sp				10.0			
Pseudomonas aeruginosa				11.0			
Staphylococcus spp (coag-)				15.0			
Staphylococcus pyogenes				14.0			
							Am = +
Bacillus cereus					7.5		10.0
Pseudomonas aeruginosa					6.8		8.0
Saccharomice cerevisae					15.5	2.7	18
Candida albicans					20.4	2.7	18.0

Source: Bauer, A.W., W. M. M. Kirby, J. C. Sherris, and M. Turk, *Am. J. Clin. Pathol.*, 45, 493–6, 1966.

Note: Meliponini species: Msc = *Melipona scutellaris*; Ms = *M. subnitida*; S.bip = *Scaptotrigona bipunctata*; Nt = *Nannotrigona testaceicornis*; Ta = *Tetragonisca angustula*; Tl = *Trigona laeviceps*; Am = *Apis mellifera*.

[a] Inhibition zone size in 24 hours.

[b] Martins, S. C. S., L. M. B. Albuquerque, J. H. G. Matos, G. C. Silva, and A. I. B. Pereira, *Higiene Alimentar*, 52, 50–3, 1997.

[c] Gonçalves, A. L., A. Alves Filho, and H. Menezes, *Arq. Inst. Biol.*, 72, 455–9, 2005.

[d] Demera, J. H., and E. R. Angert, *Apidologie*, 35, 411–7, 2004.

[e] Chanchao, C., *Pak. J. Med. Sci.*, 25, 364–9, 2009.

pollen, nectar, air, and digestive tract of bees, and can survive in honey (Snowdon and Clever 1996). Secondary sources are those that can be incorporated into honey at any time after it is taken from the nest, but good handling practices and hygiene control these contaminants.

The greatest problem related to the presence of molds and yeasts in honey is fermentation, which results from sugar consumption by yeast, producing byproducts that change the final taste and flavor. The presence of yeasts in stingless bee honey is easily verified, since often they have a characteristic fermentation scent, besides physical identification in pollen grain slides (Barth 1989). Bacteria do not reproduce in honey and a large number of vegetative forms indicate recent contamination of honey is from secondary sources. As honey has antibacterial properties, it is expected to contain a low number and limited diversity of microorganisms. Tables 11.5 and 11.6 show the analyses of microorganism amounts in stingless bee honey. As there are no parameters for this honey, the results only indicate the number of colony forming units (CFU/g or ml). Parameters for honeybee honey in Brazil accept up to 100 CFU/g for fungi and yeasts. In all tested honeys, with a single exception, yeast amounts were higher than that of mold. Results indicate that those honeys from more humid areas tend to have higher values than those from dry regions such as Caatinga and Cerrado (*M. subnitida* and *M. quinquefasciata*, respectively), suggesting environmental influence. Standard bacteria counting (Table 11.6) revealed the same amount in all samples, regardless of bee species and geographic region. The value found, 10^2, regardless of bacteria type, indicates that stingless bee honey is not a sterile product. However, the National Agency for Sanitary Surveillance (Anvisa 2001) accepts the same value in products such as sweeteners, brown sugar, and molasses. Over time, a single honey sample from *T. angustula* exposed to different conditions and time periods showed no significant change in bacteria amount.

From a microbiological point of view, presence of *Bacillus*, yeasts, and molds in honey is considered a common occurrence, since these microorganisms are found in the intestinal microflora of solitary and

TABLE 11.5

Microbiological Analysis of Stingless Bee Honey Collected Aseptically

	Mold	Yeast	Total Coliform	Fecal Streptococci
Species/Locality	**CFU/g**	**CFU/g**	**MPN/g**	**MPN/g**
M. fasciculata/MA[a]	1.5	<10.0	<0.18	<0.18[b]
M. fasciculata/PA[a]	2.5	23.5	<0.18	<0.18
M. quadrifasciata/SP	25	615	<0.18	<0.18
M. quinquefasciata/GO	1.5	55	<0.18	<0.18
M. rufiventris/SP	55.0	2.3×10^3	<0.18	<0.18
M. rufiventris/SP	70	255	<0.18	<0.18
M. rufiventris/SP	200	2.5×10^3	<0.18	<0.18
M. subnitida/RN	50	90	<0.18	<0.18
M. subnitida/RN	100	150	<0.18	<0.18
Tetragona clavipes/SP	<1	7.0×10^3	<0.18	<0.18
Tetragona clavipes/SP	50	3.3×10^3	<0.18	<0.18
Tetragona clavipes/SP	100	1.4×10^3	<0.54	<0.54
Tetragona clavipes/SP	<1	5.5	<0.18	<0.18
Melipona sp/AM[a]	2	3.0	<0.18	<0.18
S. depilis/Uruguay[a]	1.0×10^3	1.29×10^5	<0.18	<0.18
M. fuscopilosa/AC	<1.0	1.81×10^3	<0.1.8	<0.18
M. fuscopilosa/AC[c]	3	<1.0	<0.18	<0.18
M. crinita/AC	2×10^4	1.72×10^6	<0.18	<0.18

Note: CFU = colony forming unit according to Cetesb standard technique L5204; MPN = most probable number according to standard methods-APHA 2005; AC = Acre state; SP = São Paulo state; PA = Pará state; MA = Maranhão state; GO = Goiás state; RN = Rio Grande do Norte state; AM = Amazonas state. Technical advice: Elayse Maria Hachich from Microbiology and Parasitology Laboratory of Cetesb, São Paulo.

[a] Producer.

[b] <0.18 = absence of contamination within tests limits.

[c] Heated honey.

social bees, and its amount varies with bee age (function), seasons, food diets (deficient), and nest exposure to pesticides (Gilliam 1997). *Bacillus* species produce antimicrobial substances and enzymes, as do molds, and yeasts are the most important contributors of substances from a nutritional standpoint (Pain and Maugenet 1966). The questions here are which are those parameters limits and which are nonpathogenic and pathogenic microorganisms that can be found in stingless bee honey. Several species have already been studied in relation to pollen, honey, or larval food microflora: *Dactylurina staudingeri* (Gribodo), *T. hypogea*, *M. quadrifasciata*, *Melipona fasciata* Latreille, *T. angustula,* and *Frieseomelitta varia* (Lepeletier) (Machado 1971; Delage-Darchen and Darchen 1984; Gilliam et al. 1985, 1990; Rosa et al. 2003).

Of the 12 honey samples tested from Southeastern Brazil (Table 11.6), three indicated the presence of total coliforms (environmental), but not of fecal coliforms. More rigorous testing of presence/absence (P/A), which use samples ten times larger (10 g), showed one positive result for *E. coli* (fecal coliforms), three for *Enterococcus*, also of fecal origin, and six for *B. cereus*. *E. coli*, whose specific habitat is the gut of warm-blooded animals, does not multiply in nature and can be naturally found in honey if bees collect any material in creeping plants. In Table 11.5, samples analyzed with another method (NPM) also indicated the absence of contamination within the tests limits. Even in these samples, *Salmonella* sp., *S. aureus,* and *P. aeruginosa* were not found. These results open a perspective for the consumption of stingless bee honey, because some species have been observed visiting animal wastes and carcasses (Nogueira-Neto 1997), and therefore it was believed that their honey could contain large amount of fecal coliforms. If bees use this material in nests, it should be used in a restricted place, not in the food storage area, or honey eliminates these microorganisms with its antibacterial properties.

TABLE 11.6

Microbiological Analysis of Stingless Bee Honey Aseptically Collected from Southeastern Brazil

	Bacteria	Total Coliform	Fecal Coliform
Species	**CFU/ml**	**MPN**	**MPN**
Tetragonisca angustula	0.32×10^2	7.3×10^2	0
Tetragonisca angustula	0.51×10^2	39×10^2	0
Melipona bicolor	$>3 \times 10^2$	0	0
Melipona bicolor	$>3 \times 10^2$	0	0
Plebeia sp	0.2×10^2	0	0
Plebeia sp	$>3 \times 10^2$	0	0
Nannotrigona testaceicornis	$>3 \times 10^2$	0	0
Nannotrigona testaceicornis	$>3 \times 10^2$	0	0
Melipona subnitida	0.64×10^2	0	0
Melipona subnitida	0.18×10^2	0	0
Tetragonisca angustula	0.15×10^2	2.4×10^2	0
Tetragonisca angustula 1 day	5.6×10^2	—	—
Tetragonisca angustula 7 days in freezer	10×10^2	—	—
Tetragonisca angustula 7 days in environment	14×10^2	—	—

Note: CFU = colony forming unit according to Cetesb standard technique L5204; MPN = most probable number according to standard methods-ALPHA, 2005. Technical advice: Dilma S. Gelli and Harumi Sakuma from Microbiology Laboratory of Adolfo Lutz Institute, São Paulo, Brazil.

11.7 Final Considerations

Studies on the feeding habits of the social Apidae have contributed specifically to the understanding of energetics or foraging economy of these animals. Foragers of *Apis*, *Bombus*, and Meliponini are relatively easy to manage in field and laboratory, fitting well to the goals of controlled experiments where behaviors, benefits, and costs during foraging are analyzed. Information thus obtained refers to the discussion of an "optimal foraging theory," perhaps a controversy in itself (able to encompass the exceptions and dependent on them to explain the improvement of consumers in the evolutionary flow towards optimization) but without doubt, a biological paradigm.

Colonies of social Apidae are at the center of foraging economics both spatially (the fixed point for displacement) and behaviorally (changing foragers behavior). In a retrospective of the ecology of *A. mellifera*, Seeley (1985) notes that studies on colony functioning are well advanced, while investigations about historical conditions that favor emergence and establishment of specific responses (e.g., an elaborated communication system) began to appear only in the late 20th century. There is an intersection between physiological behavioral approaches (why a particular type of colony functions) and behavioral ecology (why a certain type of functioning was selected). In this new phase, intensification of studies in tropical regions is crucial, because these environments are the molds on which complex ecological mechanisms arose, and many geographic variants of *A. mellifera* and hundreds of species of stingless bees were differentiated.

When populations are isolated by any barriers, they start independent evolutionary histories. Among these barriers, genetic differentiation has often irreversible ecological and population effects. Thus, in Meliponini, hundreds of species with independent evolutionary histories share basic characteristics of

the common ancestor, have a wide geographical distribution, and often occupy the same habitat. Given these facts, there is one basic question: what mechanisms regulate this coexistence?

In terms of feeding ecology, each species of stingless bees brings more or less altered "solutions" already encountered by its ancestors and that overlap with its own acquisitions, so that each colonial system works and acts on the environment, repeating in part the need to maintain foraging efficiency in different habitats or food sources, and differentiation of food habits to escape interspecific pressures represented by ancestral characteristics. The apparent contradiction between these two ecological goals was probably settled by morphological and functional diversification, often subtle, but still feasible in economic terms, allowing specific strategies for use of floral food sources and occupation of different habitats. Nevertheless, comparisons among most local Meliponini communities show relatively moderate variations in the number of coexisting species, indicating that there are also narrower limits for generalist social bees packaging in ecosystems.

In recent years, information on stingless bees' feeding habits have accumulated, but still with many basic gaps in view of the large number of species. In addition, there were few attempts to relate the expression of morphofunctional characteristics to food availability conditions. Thus, tracing parallels on how to allocate resources between colonies of closely related (e.g., same genus) and unrelated species is an open field for research that undoubtedly will help to understand the behavioral and ecological mechanisms that made coexistence possible, and therefore were important for any differences in feeding habits (e.g., floral preferences) and for finding specific solutions in colonial functioning (e.g., type of communication system).

Solving basic questions on how stingless bee species manage to coexist in the same locality will also be relevant for stingless bee management and utilization on applied fields, in terms of their use on crop pollination, since they are already known as effective pollinators of dozens of plant species.

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