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Diapause in Stingless Bees (Hymenoptera: Apidae)

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Abstract

Extreme environmental conditions may negatively affect the development of animals. Insects show a wide range of adaptive behaviors that have allowed them to respond successfully to adverse climatic conditions by temporarily interrupting some of their activities or development. One such behavior is diapause. Diapause can be defined as a gradual and progressive interruption in development or ontogeny of an organism in any phase of its lifecycle in order to survive cyclic unfavorable environmental conditions. This review presents an overview of the current knowledge of diapause in stingless bees and describes the various studies on this subject. It focuses on *Plebeia* species, the most studied genus in this regard. In this group of bees, provisioning and oviposition behavior ceases in autumn/winter, a so-called reproductive diapause. Besides the cessation of brood-rearing activity, other behaviors, such as foraging, are also modified. The mechanisms that induce the reproductive diapause are still unclear, but evidence points to temperature and photoperiodism as the main drivers of this behavior.

Diapause - a strategy for surviving unfavourable environmental conditions

Extreme environmental conditions may negatively affect the development of certain animals and many have evolved behavioral strategies in order survive. Insects present a wide range of adaptive behaviors that have allowed them to respond successfully to adverse climatic conditions by temporarily interrupting some of their activities or development in the face of, for example, extreme temperatures (cold or warm) (Tauber & Tauber, 1976; Denlinger, 1986; Kort, 1990; Tatar & Yin, 2001; Koštál, 2006). Such behavioral responses have variously been termed dormancy, quiescence, hibernation, aestivation and diapause (Tauber & Tauber, 1976; Denlinger, 1986). However, conceptual differences exist among these terms relating to the kind of environmental variable and whether the response is merely immediate (non-programmed) or rhythmical (endogenously and genetically programmed) (Tauber & Tauber, 1976; Denlinger, 1986, 2002; Koštál, 2006).

In a broader sense, dormancy is a term embracing several others (e.g., quiescence, hibernation, aestivation and diapause) and refers to any temporary arrest in behavior performed by an insect in response to any unfavorable climatic condition (Tauber & Tauber, 1976; Denlinger, 1986). However, the terms quiescence and diapause have also frequently been used as synonyms of dormancy, although both are actually different kinds of dormancy (Tauber & Tauber, 1976; Denlinger, 1986). Basically, both quiescence and diapause are temporary interruptions in the developmental cycle or activity of an organism in response to environmental adversity. Quiescence can be defined as any sudden, non-cyclic response, whereas diapause is a strictly cyclic or rhythmical response, i.e., genetically pre-programmed (Tauber & Tauber, 1976; Denlinger, 1986).

A concise and clear definition of diapause is a gradual and

progressive interruption in development or ontogeny of any organism in some phase of its life cycle in order to survive unfavorable environmental conditions that occur cyclically (Tauber & Tauber, 1976; Denlinger, 1986; Kort, 1990; Tatar & Yin, 2001; Koštál, 2006). Among the major insect orders, including hemimetabolous and holometabolous ones, diapause behavior has been best observed in Orthoptera, Diptera, Coleoptera, Lepidoptera, Hemiptera and Hymenoptera (Tauber & Tauber, 1976; Herman, 1981; Denlinger, 1986; Lefevere et al., 1989; Kort, 1990; Greenfield & Pener, 1992; van Benthem et al., 1995; Kipyatkov et al., 1997; Pick & Blochtein, 2002b). Diapause behavior may be divided into at least three stages: (1) pre-diapause, (2) diapause, and (3) post-diapause, during which insects exhibit a series of behavioral, biochemical and morphological changes (Denlinger, 2002; Koštál, 2006). Furthermore, diapause behavior may occur at any stage of the insect life cycle: egg, larval, pupal (or nymph) or adult (Tauber & Tauber, 1976; Herman, 1981; Denlinger, 1986; Lefevere et al., 1989; Kort, 1990; Greenfield & Pener, 1992; van Benthem et al., 1995; Kipyatkov et al., 1997; Pick & Blochtein, 2002b). When diapause occurs in sexually active insects, breeding is usually compromised due to the interruption of certain physiological processes related to: (1) female oogenesis, (2) the activity of male accessory glands, or (3) the reproductive behavior of both sexes (Kimura, 1988; Tatar & Yin, 2001). Diapause in sexually active insects has, therefore, been termed reproductive diapause, a period during which insects may temporarily cease egg-laying and searching for sexual partners (Kimura, 1988; Tatar & Yin, 2001).

Predicting the arrival of adverse environmental conditions

Since diapause is a rhythmical biological strategy to survive unfavorable environmental conditions, it requires mechanisms that anticipate the arrival and ending of such conditions. Indeed, many insects have been found to effectively decode certain environmental cues in order to enter and/or terminate diapause behavior (Tauber & Tauber, 1976; Denlinger, 1986; Kort, 1990; Tatar & Yin, 2001; Koštál, 2006). Such cues include the amount of food available in the environment, the relative humidity, the parasite load, and the physiological state of host plants (e.g., solute concentration, age, limb senescence) (Tauber & Tauber, 1976; Derr, 1980; Denlinger, 1986; Greenfield & Pener, 1992; Takagi & Miyashita, 2008; Togashi, 2014).

However, the most important environmental cues used by insects to prepare for the arrival or ending of adverse environmental conditions are photoperiodism and temperature (Tauber & Tauber, 1976; Tauber & Kyriacou, 2001; Saunders, 2012, 2014). Photoperiod, i.e., daylength, is a pivotal cue because of its seasonality and invariability (Tauber & Tauber, 1976; Tauber & Kyriacou, 2001; Saunders, 2012, 2014). Temperature is also a significant environmental cue, although it may fluctuate seasonally from year to year (Tauber & Tauber, 1976; Tauber & Kyriacou, 2001; Saunders, 2012, 2014). Nevertheless, both photoperiod and temperature often exert significant synergistic power influencing an insect's response (Kimura, 1988; Vaz Nunes & Saunders, 1989; Chen et al., 2014; Saunders, 2014).

Why study diapause in bees?

Diapause has been observed amongst insects which play diverse ecological roles, e.g., herbivorous agricultural pests (Adedokun & Denlinger, 1985) and pollinators (e.g., van Benthem et al., 1995; Goulson, 2010). These insects may exert social and economic impacts in many regions of the world. However, beneficial insects acting as pollinators currently add high economic value to agriculture globally (€153 billion; Gallai et al., 2009), in addition to their contribution to the reproduction of wild plants (Gallai et al. 2009; Ollerton et al. 2011).

It is known that many bee species undergo diapause at some point in their life cycle, including solitary bee species, such as *Megachile rotundata* (Fabricius), *Nomia melanderi* (Cockerell), *Osmia rufa* (Linnaeus), and *Osmia lignaria* Say (Hsiao & Hsiao, 1969; Bosch et al., 2010; Fliszkiewicz et al., 2012; Wasielewski et al., 2013). Amongst the social bees, honey bees (*Apis mellifera* Linnaeus) show certain behavioral adaptations for surviving low temperatures, e.g., the storage of honey, the decrease or complete absence of broods during autumn and winter, increased longevity, and reduced metabolism of the workers (Seeley, 1985). Bumblebees (*Bombus* Latreille) have queens that survive the winter in diapause, and are certainly the most commonly investigated in this respect (Plowright & Laverty, 1984; Beekman et al., 1998; Goulson, 2010). While there are many aspects of diapause in bees that could be dealt with here, our focus is to review diapause in stingless bees, a subject that has still been little studied.

Stingless bees and the diapause

Stingless bees are found in tropical and subtropical regions all around the world (Sakagami, 1982). They usually nest in holes in trees, in the soil, and even in cavities in human constructions, but some also construct aerial nests (Nogueira-Neto, 1997). Their nests are perennial and they are active throughout the year (Sakagami, 1982; Nogueira-Neto, 1997). However, some species may show quite a remarkable change in activities at the colony as a diapausal response to adverse climatic conditions.

Most descriptions in the literature of diapause in stingless bees have concerned the genus *Plebeia* Schwarz, e.g., *Plebeia droryana* (Friese), *Plebeia emerina* (Friese), *Plebeia wittmanni* Moure & Camargo (Friese), *Plebeia nigriceps* (Friese), *Plebeia remota* (Holmberg), *Plebeia julianii* Moure, and *Plebeia saiqui* (Friese) (Juliani, 1967; Terada et al., 1975; Imperatriz-Fonseca & Oliveira, 1976; Kleinert-Giovannini, 1982; Wittmann, 1989; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Witter et al. 2007; Alves et al., 2009; Nunes-Silva

Table 1. Biological and ecological features presented by stingless bees (Hymenoptera: Apidae: Meliponini) during the reproductive diapause in autumn and/or winter in southern South America. Locality: PR=Paraná, Brazil.

Species	Overwintering strategy	Nest architecture	Worker size [3]	Number of workers per nest	Months in diapause	Maximal worker age (days)	Temperature (°C)	Locality	References
Plebeia droryana	Obligatory	Involucrum	3.5 - 4.7	2,000 – 3,000	3	–	11.7[7], 9.8[7]	São Paulo Rio Grande do Sul	Terada et al. (1975); Blochtein (pers. obs.)
Plebeia emerina	Obligatory	Involucrum	4.0 - 4.5	–	3	107	11.7[7], 12.16[8]	São Paulo Rio Grande do Sul	Kleinert-Giovannini, (1982); Santos et al. (2009)
Plebeia julianii	Obligatory	Pillars	3.0	300[4]	3 – 4	–	11.1[7], 9.8[7]	Paraná Rio Grande do Sul	Juliani (1967); Witter et al. (2007)
Plebeia witmanni	Obligatory	Pillars	4.5	–	3	274	9.8[7]	Rio Grande do Sul	Wittmann (1989)
Plebeia saiqui	Obligatory	Involucrum	4.0 - 5.0	7000[5]	2.5 – 6	174	11.0[8]	Rio Grande do Sul	Pick and Blochtein (2002 a) Pick and Blochtein (2002 b)
Plebeia nigriceps	Obligatory	Pillars	3.5	200[4]	3 – 4	–	9.8[7]	Rio Grande do Sul	Witter et al. (2007)
Plebeia remota	Obligatory	Pillars	2.75 - 4.0	2,000 – 5,000[6]	2 – 5	–	11.3[8]	São Paulo	van Benthem et al. (1995); Ribeiro et al. (2003); Nunes-Silva et al. (2010)
Melipona obscurior	Facultative	Involucrum	7.1	–	2 – 6	120	9.8[7]	Rio Grande do Sul	Borges & Blochtein (2006); Blochtein et al. (2008)
Melipona quadrifasciata	Facultative	Involucrum	8.6 – 9.6	300 – 400	2 – 3 (?)	–	11.7[7]	São Paulo	Nogueira-Neto (1997)
Melipona bicolor shencki	–[2]	Involucrum	8.9 – 9.1	–	3	–	9.8[7]	Rio Grande do Sul	Blochtein et al. (2008)
Trigona ventralis hoozana [1]	Facultative (?)	Involucrum	–	10,000	–	–	5.0	Chhiayi County, Taiwan	Sung et al. (2008)

(1)East Asian (may reach mountainous regions up to 2,500 m above sea level);

(2) Reproductive diapause not detected, but marked decrease in egg-laying in winter;

(3)Measures (in mm) made in a reference bee from the collection of the Science and Technology Museum of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS);

(4)Drumond et al. (1998); (5)Witter 2007;(6)van Benthem et al., 1995;

(7)Average minimum temperature (Instituto Nacional de Metereologia [INMET], 2014);

(8) Minimum temperature registered during diapause according to mentioned reference.

et al., 2010). However, diapause has also occasionally been observed or suspected in *Melipona quadrifasciata* Lepeletier, *Melipona obscurior* Moure and *Trigona ventralis hoozana* Strand (Kleinert-Giovannini & Imperatriz-Fonseca, 1986; Nogueira-Neto, 1997; Borges & Blochtein, 2006; Sung et al., 2008) (Table 1). Due to the more frequent occurrence of diapause in *Plebeia* species compared to the other genera of stingless bees, this paper mainly describes the diapause of *Plebeia* species in southern Brazil, with reference to diapause in other stingless bees whenever possible.

In this sense, we know little about whether other stingless bee species in subtropical regions of South America also show any facultative or obligatory reproductive diapause. Many stingless bee species occur in this region, such as *Melipona quinquefasciata* Lepeletier, *Melipona quadrifasciata* Lepeletier, *Mourella caerulea* Friese, *Paratrigona subnuda* Moure, *Scaptotrigona bipunctata* (Lepeletier), *Scaptotrigona depilis* (Moure), *Schwarziana quadripunctata* (Lepeletier), *Tetragonisca fiebrigi* (Schwarz) and *Trigona spinipes* (Fabricius) (Camargo & Pedro, 2013). Not all of these species are managed in these areas due to their aggressiveness, rarity or fragility when kept in hives, making observations of the provisioning and oviposition process (POP) difficult.

The colony reproductive phase

Plebeia spp. occurs from the northernmost parts of Mexico to the southern parts of South America (Camargo & Pedro, 2013). In Brazil, this genus is represented by at least 19 species (Camargo & Pedro, 2013), although many species remain to be identified and described (Silveira et al., 2002; Camargo & Pedro, 2013). Nine of these 19 *Plebeia* species occur in the southern parts of Brazil (Silveira et al., 2002; Camargo & Pedro, 2013), and seven of them are known to undergo diapause (see above). Within the tribe Meliponini, *Plebeia* is considered a phylogenetically basal group among the Neotropical stingless bees (Camargo & Pedro, 1992). A remarkable feature of this group is the aggressive and physically intense queen-worker interactions (Sakagami, 1982; Zucchi, 1993). These interactions occur predominantly during the POP, which is highly ritualized and complex in *Plebeia* spp. (Sakagami, 1982; Zucchi, 1993).

Briefly, the POP can be defined as the construction of brood cells and provisioning of larval food by nurse workers, followed by queen oviposition and the subsequent sealing of the brood cells by nurse workers (Sakagami, 1982; Zucchi, 1993). In *Plebeia*, POP is characterized by a large number of brood cells being built simultaneously until they reach the collar stage, a phase that may last from 3.5 to 7 hours (occasionally less than 30 minutes) (van Benthem et al., 1995; Drumond et al., 1996, 1997, 1998, 2000). During this stage, some workers may lay trophic and/or reproductive eggs on the comb or in the periphery of a brood cell, and such eggs are often eaten by the queen (van Benthem et al., 1995; Drumond et al., 1996, 1997,

1998, 2000). While some brood cells are still in the collar stage, workers on the comb display intense agitation, apparently stimulated by the presence of the queen (van Benthem et al., 1995; Drumond et al., 1996, 1997, 1998, 2000). Subsequently, many workers start to load larval food into the new brood cells, and after a certain volume has been reached, the queen quickly lays her eggs onto the food and the cells are sealed by workers (van Benthem et al., 1995; Drumond et al., 1996, 1997, 2000, 1998).

Hence, in the colony reproductive phase (outside of diapause) there is a clear sequence of brood cell building, provisioning and egg-laying, which differs according to species. Furthermore, there is usually very little, if any, involucrum cover over the brood cell area (van Benthem et al., 1995; Drumond et al., 1996, 1997, 1998, 2000).

It is usually thought that stingless bees have a rather low ability to effectively thermoregulate their nests. In general, these bees build lamellae of wax and propolis, often called cerumen involucra, which partially or entirely cover the brood combs during the cold season (Nogueira-Neto, 1997). Such nest architecture provides thermal insulation that passively protects brood combs and the adult bee population during unfavorable climatic conditions, such as unduly low temperatures (Jones & Oldroyd, 2006). However, such passive thermoregulation in stingless bees is not nearly as effective as the active thermoregulation provided by honey bees, for example, which use contractions of their thoracic muscles to produce heat inside their nests (Jones & Oldroyd, 2006).

The addition of wax and propolis lamellae by stingless bees may be directly related to their need for facultative or obligatory reproductive diapause. It has also been suggested that their reduced ability to effectively thermoregulate their nests and their reproductive diapause may be related to body size (Blochtein et al., 2008). In stingless bee inhabiting southern Brazil, there is a progressive increase in worker size from *Plebeia* spp. (with obligatory diapause), through *M. obscurior* (with facultative diapause) to *Melipona bicolor schencki* Gribodo (with no dia-

Fig. 1. Colony reproductive phase: Plebeia emerina (Hymenoptera: Apidae: Meliponini) nest showing many workers over brood combs, some brood cells being built in the periphery. Involucrum layers can already be seen around combs.

Fig. 2. Diapause phase: Plebeia emerina (Hymenoptera: Apidae: Meliponini) nest showing brood combs entirely covered by involucrum layers. Below right: some honey pots.

pause) (Blochtein at al., 2008) (Table 1). It is thought that in cold regions, such as southern Brazil, there could be a relationship between bee body size and their ability to withstand low temperatures (Blochtein et al., 2008).
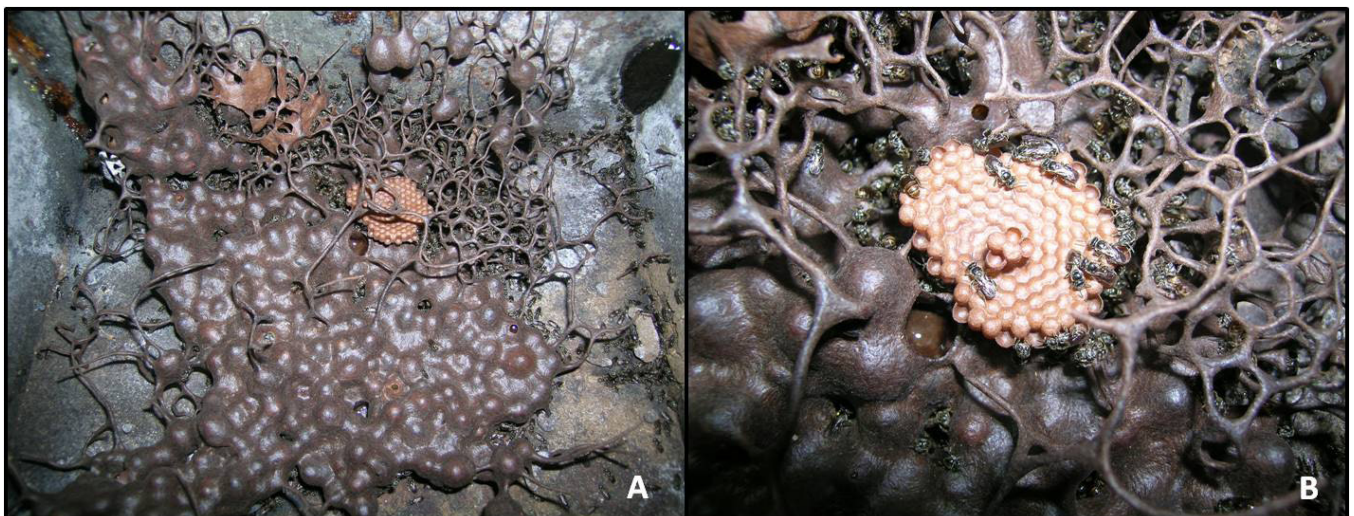
The diapause phase

Many populations of *Plebeia* spp. (*P. droryana*, *P. emerina*, *P. julianii*, *P. nigriceps*, *P. remota*, *P. saiqui* and *P. wittmanni*) in southern South America (Table 1) may be exposed to quite rigorous winters that may reach temperatures at or below zero in June to July (Instituto Nacional de Metereologia [INMET], 2014). In preparation for these cold winters, the construction rate of brood cells falls gradually until it stops entirely. Consequently, the queen's egg-laying is interrupted in many *Plebeia* colonies during the milder autumn months of March to May (Juliani, 1967; Terada et al., 1975; Imperatriz-Fonseca & Oliveira, 1976; Kleinert-Giovannini, 1982; Wittmann, 1989; van Benthem et al., 1995; Pick & Blochtein,

2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010). This kind of diapause in stingless bees has been called reproductive diapause (Juliani, 1967; Terada et al., 1975; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010).

One of the more conspicuous signs that a given colony of *Plebeia* spp. is preparing for reproductive diapause is the progressive construction of a multi-layered involucrum covering the brood comb area, as observed in *P. droryana* and *P. emerina* (Drumond et al., 1996) and in *P. remota* (Ribeiro et al., 2003) (Figs 1 and 2). In other cases, a substantial increase in the number of cerumen pillars may occur, also over the brood combs, as in *P. remota* (Ribeiro et al., 2003) (Fig 3a, b; Table 1). At the peak of reproductive diapause, when all of the brood has already emerged ("winter workers"), brood cells or combs are no longer constructed and no combs are found in the nest (Ribeiro et al., 2003). Many workers and the queen can be seen clustered in specific regions of the nest, under or over food pots or among the pillars (or lamellae), where they exhibit lethargic movements or remain completely immobile (Ribeiro et al., 2003). These "winter workers" are longer lived than the workers emerging at other times (Table 1) (Blochtein et al., 2008; Nunes-Silva et al., 2010).

During reproductive diapause, queens of *P. remota* may lose weight, as evidenced by their reduced physogastry (Ribeiro et al., 2003). This lower queen physogastry observed during diapause has been ascribed to a regression in ovarian development due to the cessation of egg-laying (Ribeiro et al., 2003). However, little is known about this phenomenon in stingless bees, although it is possible that glycogen and body fat reserves also significantly decrease during this period. Reproductive diapause in stingless bees has been recorded as lasting from 2 up to 6 months (Juliani, 1967; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010) (Table 1). However, colonies inhabiting the same locality may not always commence their reproductive diapause in synchrony (Juliani, 1967; van Ben-

Fig. 3. Post-diapause phase: Plebeia remota (Hymenoptera: Apidae: Meliponini) nest shortly after terminating diapause. A - Note that there is a large number of food pots, several wax pillars and an incipient brood comb being built by some workers; B – Detail from A with some removed pillars.

Table 2. Overall behavioral characteristics concerning to the reproductive diapause process in stingless bees (Hymenoptera: Apidae: Meliponini). Note: diapause phases following Koštál (2006).

Pre-diapause		Diapause		Post-diapause
Induction	**Preparation**	**Initiation/maintenance**	**Termination**	
Environmental stimuli (e.g. photoperiod and temperature) achieve critical levels according to physiological sensitivity from bees.	Progressive reduction in brood cell building with a decrease in provisioning and oviposition process; Increased presence of cerumen involucrum layers or pillars over brood cells; Shift in foraging pattern (1): larger input nectar and less pollen; Shift in foraging pattern (2): flight activity concentrated around noon.	No brood cell actively built; Emergence of the last brood, winter workers.	Some brood cells start to be built; eventually this can occur over food pots or cerumen layers.	Full removal or significant reduction in involucrum layers or pillars; Usual provisioning and oviposition process (POP) is implemented.
Autumn / Winter		**Spring / Summer**		

them et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010).

While some colonies may start diapause early, during the milder months (March, April), others may not start until the winter months (May, June) (Juliani, 1967; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010). In contrast to this variability in preparation for diapause, which occurs over a prolonged period and independently among colonies at the same site, termination of diapause among *Plebeia* colonies seems to be faster and synchronous during the first weeks of spring (in August, September or eventually in October) (Juliani, 1967; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Alves et al., 2009; Nunes-Silva et al., 2010).

Despite the arrest of POP throughout reproductive diapause in stingless bees, the nest population is not entirely lethargic or immobile. Other tasks, e.g., manipulating waste, storing food (nectar, pollen), or foraging still occur (Juliani, 1967; van Benthem et al., 1995; Pick & Blochtein, 2002b; Ribeiro et al., 2003; Nunes-Silva et al., 2010). Regarding foraging, the activity of workers outside of the nest is more intense when ambient temperatures are milder and the wind is light or absent (Pick & Blochtein, 2002b; Ribeiro et al., 2003; Nunes-Silva et al., 2010).

The foraging pattern and food sources harvested by foragers also change from the reproductive phase to reproductive diapause, at least in *P. saiqui* (Pick & Blochtein, 2002a, 2002b) and *P. remota* (Nunes-Silva et al., 2010). During the reproductive phase, *P. remota* forage constantly throughout the day and both nectar and pollen are collected (Nunes-Silva et al., 2010). However, during diapause foraging does not commence until noon, when temperatures are higher, and the collection of pollen is greatly reduced compared to the reproductive phase (Nunes-Silva et al., 2010). A decrease in pollen

collection and a shift in the time of peak foraging activity between phases has also been observed for *P. saiqui* (reproductive period: peak 11:00-13:00; diapause: peak 13:00-14:00) (Pick & Blochtein, 2002a, 2002b).

It has therefore been suggested that, at least for *P. remota*, the higher nectar input during diapause affects the potential energy storage by bees, whereas reduced collection of pollen (a protein source) causes no harm because there is no egg-laying or production of new individuals during this period (Nunes-Silva et al., 2010). Nevertheless, comparative observations should also be made for other *Plebeia* and stingless bee species that show diapause. An overview of the process of reproductive diapause in stingless bees is presented in Table 2.

Factors determining diapause in stingless bees

Our knowledge regarding which environmental cues (e.g., photoperiod and temperature) are indeed significant in determining the onset, permanence and termination of diapause in stingless bees is almost absent. These social insects live inside cavities in trees or dead wood, and also nest in soil or other cavities, so how could the population in the nest, especially nurse workers and the queen, foresee the onset of adverse climatic conditions? Furthermore, how could they (or foragers) communicate and/or induce reproductive diapause in their nestmates? Unfortunately, there are few studies addressing these questions. For example, an experiment with *P. remota* showed that workers have a significant role in promoting diapause behavior (Ribeiro, 2002). By introducing *P. remota* queens from colonies in diapause into colonies that were in the reproductive phase (and vice versa), it was possible to observe that diapausal queens did not reinitiate egg-laying (Ribeiro, 2002). Moreover, it remains unknown just how individuals that have never left their nests, and thus

have not been exposed to external environmental cues, are induced to enter, continue and terminate diapause.

Studies with other social insects might provide some clues. For example, it has been suggested that foraging honeybees may somehow synchronize their biological rhythms and inform nestmates about external environmental conditions through their body movements (Bloch et al., 2013). Another possibility could be some sort of chemical signaling that would induce entire nest populations to enter or terminate diapause. Evidence for this has been obtained from *Myrmica rubra* (Linnaeus) ants in which larvae remain in diapause and do not pupate, while simultaneously queens do not lay eggs (Kipyatkov, 2001). In this study, it was observed that when diapausal nests were interconnected to non-diapausal nests, and odors from the latter could enter the former, diapause was terminated in the diapausal colonies, most probably due to worker primer pheromones. However, the reverse effect was not observed (Kipyatkov, 2001). Unfortunately we do not yet know if this system could also apply in stingless bees, even though chemical communication is known to also play a pivotal role within their colonies.

Conclusions and perspectives

Possibly because nest thermoregulation in stingless bees is somewhat passive and not highly efficient, species exposed to winters that are more rigorous in southern Brazil have apparently evolved reproductive diapause behavior as a strategy for overwintering. This strategy interrupts egg laying for up to 6 months, even though this is a critical activity promoting the growth and maintenance of their nests. Stingless bees are subject to several environmental stresses (Freitas et al., 2009).

Therefore, it is reasonable to assume that stingless bee colonies could be more susceptible to strong environmental pressures during diapause, due to the lack of population resilience. Furthermore, very little is known about how climate change may affect the distribution of stingless bees which show diapause behavior. The future of stingless bee populations which show reproductive diapause will depend on how the scientific community, policy makers and society act to effectively preserve the natural populations of these pollinators.

In conclusion, we have much to learn about reproductive diapause in stingless bee species. The use of a combination of methods involving molecular, biochemical, physiological and ecological techniques could allow new investigations on the mechanisms underlying the regulation of diapause in stingless bees. We hope that this review will stimulate further studies that address these questions and that it will lead to better conservaion practices to preserve these bee species.

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