



Foraging and preferencing pollen of *Tetragonula biroi* (Friese) through melissopalynology

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Abstract

One species of stingless bee is *Tetragonula biroi*, a eusocial insect that produces honey and other beneficial products for health. This research aims to identify the foraging range and plant species as pollen sources in honey and bee bread through melissopalynology. Colonies samples were taken in West Pepuro Village, Wotu District, East Luwu Regency, South Sulawesi. The pollen samples in honey and bee bread were matched with fresh flower pollen found around the meliponary within a radius of 100 m. The pollen samples were extracted using the acetolysis method and observed under a light microscope at a magnification of 400 times, and their percentages were calculated. The research results indicate that *T. biroi* searches for food within the closest distance of a radius of 2-18 m from the hive. The highest percentage of pollen in bee bread (62.3%) and honey (54.8%) was from coconut, while in bee bread (40.2%) and honey (33.3%), it was from cocoa. Coconut pollen was the main food source with predominance, while cocoa pollen was the secondary pollen type. The majority of pollen in honey and bee bread were medium-sized grains. The appropriate information on the food sources can serve as a reference for plantation managers, such as coconut and cocoa plantations, to integrate with stingless bee cultivation. In addition to producing honey products, they also serve as important pollinators for plantation crops.

Keywords: Pollen, honey, Bee Bread, *Tetragonula biroi*

Introduction

Honeybees are eusocial insects with numerous benefits. Honeybees can be divided into two groups, the stinging bees (Subfamily Apinae) and the stingless bees (Subfamily Meloponinae) from the order Hymenoptera (Fadhilah and Rizkika, 2015; Avila *et al.*, 2018) ^[10, 2]. Meloponinae has several genera that are widely distributed in tropical regions. It has been reported that there are 183 species in South America and 42 species in Asia (Ruttner, 1988) ^[27]. As many as 37 species have been identified in Indonesia, which is spread in Java, Sumatra, Kalimantan, and Sulawesi (Fadhilah and Riskikah, 2015) ^[10]. In Sulawesi, there are 8 species, 4 of which are in South Sulawesi, including the species *Wallacetrigona incisa*, *Tetragonula laeviceps*, *Tetragonula biroi*, and *Heterotrigona itama* (Sayusti, 2019) ^[28]. In addition to *W. incisa*, the species commonly cultivated in the Luwu region is *T. biroi* (Octaviani *et al.*, 2020) ^[22], and its distribution reaches Central Sulawesi (Suriawanto *et al.*, 2017) ^[30], South Kalimantan (Purwanto *et al.*, 2022) ^[25], the Philippines (Mostoles *et al.*, 2015; Locsin *et al.*, 2021) ^[20, 14], and Malaysia (Jalil, 2022) ^[15].

The main factor for the success of beekeeping is the availability of food sources for any type of bee that will be cultivated. The main food source plants are those that produce nectar, pollen, resin, and honeydew. Abundant food source plants will have a significant impact on honey productivity. Knowledge of the types and maps of bee food sources will greatly help the farmer in planning the management of the bee colonies (Taha, 2015) ^[31]. The main food source is the plants that flower around the nesting or hive location. The source of nectar and pollen can be dominant or only one type of plant available, which will produce monofloral or uni floral honey. Bifloral honey if the food source is from only two types of plants and hetero-floral honey if the food source is from several types of

plants (Barth, 2004) ^[3]. Monofloral honey is identified as one type of pollen with a frequency of pollen >45 %, bifloral honey is identified as two types of pollen with a frequency of pollen >22.25 %, and multi-floral honey is identified as three or more types of pollen with a frequency of pollen <16 % (Wingenroth, 2001) ^[35].

In addition to planning and managing meliponiculture, knowledge of nectar and pollen sources will serve as a reference for important information about the quality of honey produced. The feeding sources can be directly observed by the types of plants in the meliponiculture location. Honey collected by the merchants or sold in the market without information on the honey source location will be difficult to determine its feeding source. Therefore, the geographical and plant origin analysis of nectar and pollen sources of honey can be determined by melissopalynology (Louveaux *et al.*, 1978) ^[17], which was initially a branch of palynology or the study of pollen and spores (Von Der Ohe *et al.*, 2004) ^[34]. Melissopalynology is a method for determining the bee's feeding source through the identification of pollen in bee bread and pollen contamination in honey.

Pollen is collected and stored in pot-pollen by stingless bees, which previously mix it with various enzymes and honey, causing it to undergo lactic acid fermentation, referred to as bee bread (Markiewicz-Żukowska *et al.*, 2013) ^[19]. Pollen can also be found dissolved in honey that has been contaminated during the bee's collection of nectar into the storage pots. The cell wall of the pollen has a unique characteristic consisting of a layer made up of sporopollenin. Sporopollenin consists of an outer layer called exine made of cellulose and pectin materials (Hesse *et al.*, 2009) ^[12]. This layer is resistant to harsh chemical processes and even digestive processes that involve enzymatic reactions. This makes the shape of the pollen

unaltered (Mackenzie *et al.*, 2015) [18]. This makes pollen a useful indicator for determining the plants that serve as a food source for the bees. This study aims to determine the types of plants that can serve as the main food source for *T. biroi* bees.

Material and Method

Study Site

The study was conducted in West Pepuro Village, Wotu District, East Luwu Regency, South Sulawesi meliponary

(Figure 1), in January 2020. The collected samples were honey and bee bread from three different colonies. The shape of the pollen found in the honey and bee bread samples was matched with the fresh flower pollen in the surrounding meliponary. The sample collection of fresh flower pollen was taken within a radius of 100 m in the morning or according to the blooming time of the flowers. The source of food plants around the meliponary was measured by the nearest distance from the bee hives.



Fig 1: The study locations in West Pepuro, Wotu District East Luwu Regency

Preparation of pollen slides

Preparation of Pollen Slides 10 ml of honey sample and 4 g of bee bread were taken as pollen samples. The honey and bee bread was diluted by adding 1-2 milliliters of glycerin. The pollen staining was carried out using 1 ml of safranin with a 2 % concentration. The samples were placed on a glass slide and covered with a cover slip. The observation of the samples using a light microscope was performed at a magnification of 400x on three separate slides for each colony sample. The number of pollen observed under the microscope was counted to analyze the percentage of the main flower pollen feed.

Analysis

The extraction and observation of flower pollen were based on the acetolysis method (Erdtman, 1960; Vit *et al.*, 2022) [8, 33]. The percentage of the total pollen was calculated as follows:

$$X = \frac{\sum A}{\sum B} \times 100\%, w$$

Here X represents the percentage of one plant species pollen in honey or bee bread (%), A represents the amount of one plant species pollen (grains), and B represents the total amount of pollen observed from all plant species in one preparation (grains).

The identified pollen is calculated and classified into Predominant Pollen Type (>45 %), Secondary Pollen Type (16-45 %), Important Minor Pollen Type (3-15 %), and Minor Pollen Type (<3 %) (Priambudi *et al.*, 2021) [24]. The pollen grain is determined by measuring the longest diameter of the equatorial section. There are 5 types of grain diameter based on this measurement, which are very small granules <10 μm , 10-24 μm small grains, 24-49 μm medium grains, 50-99 μm large granules, and > 100 μm giant grains (Erdtman, 1972) [9].

Results

Pollen in bee bread and honey

The percentage of plant pollen identified in honey and bee bread can be seen in Figure 2. The pollen identified in honey and colony bee bread was found in *C. nucifera*, *T. cacao*, and *C. pulcherrima*. Pollen was only found in bee bread from *Jasminum* sp flower and *H. rosa-sinensis*. Meanwhile, pollen that was only found in honey was identified from *L. esculentum*, *P. rubra*, and *Ixora* sp plants. The highest percentage of pollen in bee bread came from coconut 62.3 % and cocoa 40.2 %. Similarly, the highest percentage of pollen in honey also came from coconut 54.8 % and cocoa 33.3 %. Coconut flower pollen became the main predominant pollen type. Meanwhile, cocoa in bee bread and honey is considered a secondary pollen type.

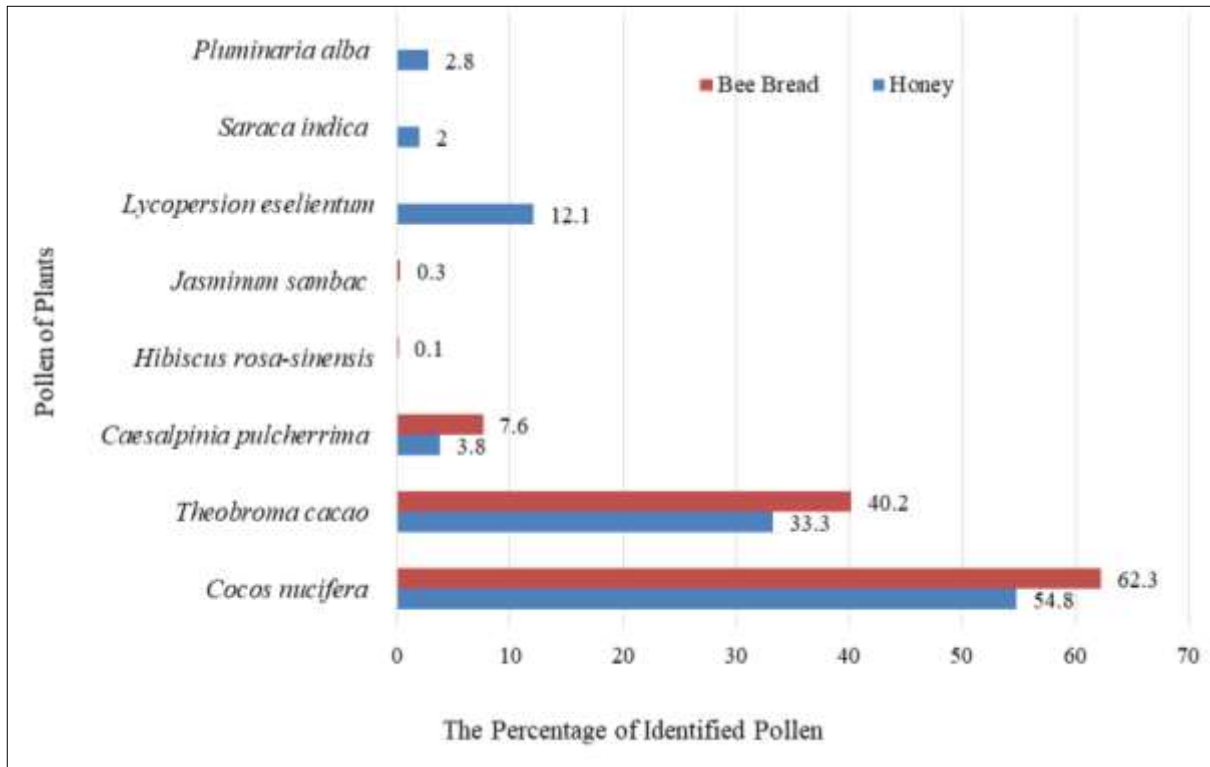


Fig 2: Pollen found in honey and bee bread.

Available Pollen around Meliponary

The available pollen in the vicinity of the meliponary area can be seen in Table 1. There are 24 species of plants that produce pollen around the hives with the closest distance of

similar plants to the hive ranging from 1.5 - 18 m. The classification of pollen grain type based on the equatorial diameter size of the pollen ranges from very small to giant.

Table 1: Pollen feed was identified in bee bread and honey around the meliponary, P=Pollen, (-) = not found pollen, PPT=Predominant Pollen Type, SPT=Secondary Pollen Type, IMPT=Important Minor Pollen Type dan MPT=Minor Pollen Type

No.	Pollen Source Plants	Pollen Samples in		Pollen Category	Types of Grain Pollen	Distance of plants from hives (m)
		Bee bread	Honey			
1	<i>Cocos nucifera</i> L. (Arecaceae)	P	P	PPT	medium	8
2	<i>Theobroma cacao</i> L. (Malvaceae)	P	P	SPT	medium	2
3	<i>Hibiscus rosa-sinensis</i> L. (Malvaceae)	P	-	MPT	giant	12
4	<i>Caesalpinia pulcherrima</i> L. (Fabaceae)	P	P	IMPT	large	15
5	<i>Jasminum</i> sp L. (Oleaceae)	P	-	MPT	medium	10
6	<i>Lycopersion esculentum</i> Mill. (Solanaceae)	-	P	IMPT	small	5
7	<i>Ixora</i> sp. (Rubiaceae)	-	P	MPT	giant	10
8	<i>Plumeria rubra</i> L. (Apocynaceae)	-	P	MPT	medium	18
9	<i>Clerodendrum thomsoniae</i> Balf.F. (Lamiaceae)	-	-	-	medium	13.5
10	<i>Mimosa pudica</i> L. (Fabaceae)	-	-	-	very small	1.5
11	<i>Orthosiphon</i> sp (Lamiaceae)	-	-	-	giant	1.8
12	<i>Thevetia peruviana</i> (Apocynaceae)	-	-	-	medium	17
13	<i>Amarathus spinosus</i> L. (Amaranthaceae)	-	-	-	small	5.6
14	<i>Gardenia jasminoides</i> J. Ellis (Rubiaceae)	-	-	-	large	13.8
15	<i>Sauropus androgynus</i> L. (Phyllanthaceae)	-	-	-	medium	14
16	<i>Bougainvillea peruviana</i> Ch. (Nyctaginaceae)	-	-	-	medium	9
17	<i>Vitex trifolia</i> L. (Lamiaceae)	-	-	-	medium	15.5
18	<i>Ageratum conyzoides</i> L. (Asteraceae)	-	-	-	medium	4.8
19	<i>Phyllanthus urinaria</i> L. (Phyllanthaceae)	-	-	-	medium	1.8
20	<i>Capsicum annum</i> L. (Solanaceae)	-	-	-	medium	6
21	<i>Manilkara zapota</i> L. (Sapotaceae)	-	-	-	small	12
22	<i>Vernonia cinerea</i> L. (Asteraceae)	-	-	-	medium	2.3
23	<i>Carica papaya</i> L. (Caricaceae)	-	-	-	medium	7
24	<i>Cananga odorata</i> Hook.f. and Thomson (Annonaceae)	-	-	-	medium	18

Discussion

The high percentage of coconut pollen is due to several factors, including the fact that coconut is the dominant plant growing near the hive location. Coconut is widely planted by villagers on the edge of the field, near their homes, or growing near the river. Coconut is used by the community

for various needs such as food (fruit), handicrafts (leaves and stems), and religious rituals (leaves). The coconut tree is a flowering plant that blooms all year round and is the predominant pollen type that can reach 73 % of *T. biroii* in the Philippines (Balderas, 2016) [2]. Coconut is a multi-flowering plant with a beautiful form. Coconut flowers have

a yellowish, shiny color with white inner parts. The aroma of coconut flowers is also very distinctive and has its attraction for *T. biroi* bees. This is in line with the statement by Pratama *et al.* (2018) ^[23] that *T. biroi* bees are more attracted to flowers with bright petals like white and yellow, with a distinctive aroma and open form. Unlike its sibling *T. pagdeni* in Thailand, it prefers to collect pollen from other types of plants even though coconut plants are available. Unlike its sibling, *T. pagdeni* in Thailand collects pollen from *Nephelium lappaceum* as the Predominant Pollen Type and pollen from *M. pudica* and *C. nucifera* as the Secondary Pollen Type (Thakodee *et al.*, 2018) ^[32].

The second plant frequently visited by *T. biroi* is cocoa which belongs to the category of secondary pollen type. Cocoa is the main plantation plant in Pepuro West Village, Luwu Timur Regency (Nurqadri *et al.*, 2020) ^[21]. Pollen from other plants such as *H. rosa-sinensis*, *C. pulcherrima*, *Jasminum sp.*, *L. esculentum*, *Ixora sp.*, and *P. rubra* are only categorized as important minor to minor pollen types. Meanwhile, 16 other types of plants that produce pollen were not found in the samples of bee bread and honey. These pollen-producing plants include vegetables (*C. annuum*, *A. spinosus*, *S. androgynus* and *C. papaya*), medicinal plants (*Orthosiphon sp* and *M. zapota*), as well as ornamental plants (*G. jassmoides*, *T. peruviana*, *C. thomsoniae*, *C. odorata*, and *B. peruviana*), and weeds (*M. pudica*, *A. conyzoides*, *V. trifolia*, and *V. cinerea*)

The stingless bee *T. biroi* chooses to visit flowers to obtain food, as well as to act as a pollinator and spreader of pollen as a reward for the plant. According to Rismayanti (2015) ^[26], the main attraction for bees to a particular plant is the amount of pollen and nectar produced by that plant. Coconut flowers produce enough pollen and nectar to be a food source for bees. Meanwhile, according to Hempel de Ibarra & Vorobyev (2009) ^[13], bees choose to visit flowers based on differences in color and size. Bees use brightness vision mediated by green (or L-) receptors to detect flowers. Bees prefer flowers with a darker color reflection in the center and surrounding brightness. However, there is a slight difference of opinion regarding flower color preference by Bisui *et al.* (2019) ^[5], who state that Meliponini bees are more attracted to brighter-colored flowers such as yellow, white, or cream. In this case,

coconut and cocoa flowers are brighter in color (cream and white) and contain sufficient amounts of nectar and pollen. Recent research by Forster *et al.* (2023) ^[11] has proven that color differences in flowers will be disregarded by bees if high-quality flowers with high nectar content are available.

The morphological observation of pollen from 24 species of plants found around the *T. biroi* bee hive can be seen in Figure 3. Pollen from eight plant species was identified in honey, bee bread, or both, 50 % included the medium pollen grain group. Meanwhile, according to Carballo *et al.* (2022) ^[6], that stingless bees generally prefer flowers with small pollen sizes (10 to 25 μm) from the Families Amaranthaceae, Myrtaceae, and Fabaceae. In addition, Meliponini bees pollinate flowers according to their small body size in flowers that have small to medium pollen sizes with short stigmas (Eltz *et al.*, 2001) ^[7].

The pollen from wild plants such as *M. pudica*, *Orthosiphon sp.*, *P. urinaria*, *A. conyzoides*, and *V. cinerea*, which are available within a radius of 5 meters from the beehive, represents a potential source of pollen. However, the distance of the food source from the beehive is not the main reason why bees choose their food while still within their flying range. Stingless bees (*Melipona subnitida*) in the dry tropical forests of Brazil tend to prefer food sources closer to their hives (less than 100 m) in order to avoid the consequences of increased body temperature when flying long distances (Souza-Junior *et al.*, 2020) ^[29]. According to Kasiera *et al.* (2023) ^[16], stingless bees cultivated in urban landscapes and natural landscapes have different foraging ranges. In urban landscapes, the foraging range is closer, ranging from 400-800 m, due to various obstacles such as buildings and traffic noise, compared to natural landscapes where the foraging range is further, ranging from 800-1,800 m. The simulation experiment on distance and food concentration for *Heterotrigona itama* conducted by Basari *et al.* (2018) ^[4] concluded that bees prefer flowers with nectar concentrations of 35 % and above, with feeders located at similar distances visited by bees within 1 to 7 meters from the hive. Thus, the characteristics of flower shape and color, pollen size, and distance to food sources are not reasons for bees to choose their food but rather depend on the availability of food types surrounding the hive.

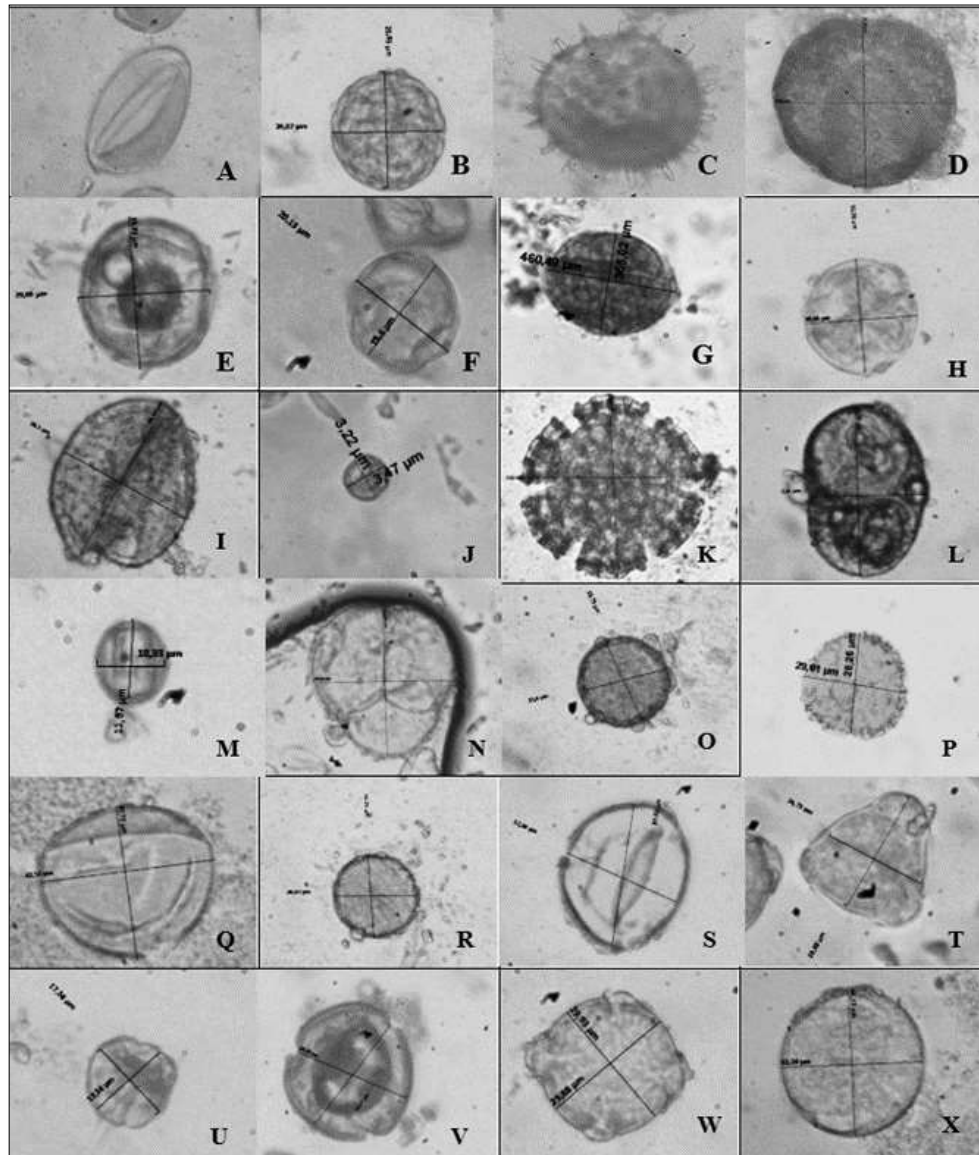


Fig 3: Types of pollen identified in meliponariy with equatorial axis diameter. A. *Cocos nucifera* (Bar = 42.3 μm), B. *Theobroma cacao* (Bar = 24.4 μm), C. *Hibiscus rosa-sinensis* (Bar = 220.6 μm), D. *Caesalpinia pulcherrima* (Bar = 77.2 μm), E. *Jasminum* sp (Bar = 32.9 μm), F. *Lycopersion esculentum* (Bar = 20.3 μm), G. *Ixora* sp. (Bar = 460.5 μm), H. *Plumeria rubra* (Bar = 36.1 μm), I. *Clerodendrum thomsoniae* (Bar = 43.3 μm), J. *Mimosa pudica* (Bar = 3.8 μm), K. *Orthosiphon* sp. (Bar = 120.8 μm), L. *Thevetia peruviana* (Bar = 31.2 μm), M. *Amarathus spinosus* (Bar = 11.7 μm), N. *Gardenia jasmoides* (Bar = 54.5 μm), O. *Sauropus androgynus* (Bar = 24.4 μm), P. *Bougainvillea peruviana* (Bar = 29.6 μm), Q. *Vitex trifolia* (Bar = 42.6 μm), R. *Ageratum conyzoides* (Bar = 24.3 μm), S. *Phyllanthus urinaria* (Bar = 37.6 μm), T. *Capsicum annuum* (Bar = 29.9 μm), U. *Manilkara zapota* (Bar = 17.6 μm), V. *Vernonia cinerea* (Bar = 40.0 μm), W. *Carica papaya* (Bar = 29.9 μm), X. *Cananga odorata* (Bar = 44.4 μm).

Conclusion

The research results indicate that *T. biroi* searches for food within the closest distance of a radius of 2-18 m from the hive. The pollen from coconut is more prevalent in honey (54.8%) and in bee bread (62.3%), while the pollen in honey (33.3%) and bee bread (42.2%) comes from cocoa. Coconut pollen serves as the main food source with a predominance of coconut pollen type and secondary pollen type from cocoa. Coconut pollen is the predominant pollen type, while cocoa is categorized as the secondary pollen type. The size of pollen found in bee bread and honey is mostly medium-sized.

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