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Diversity of Stingless Bees (Apidae: Meliponini) in Thong Pha Phum District, Kanchanaburi Province, Thailand

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ABSTRACT

Stingless bees were observed in four types of undisturbed forest: dry dipterocarp forest (DDF), upper mixed deciduous forest (UMDF), lower mixed deciduous forest (LMDF) and dry evergreen forest (DEF) during April 2004 to March 2005 to determine the diversity and abundance of stingless bees. Monthly sampling was performed using honey-bait traps. Diversity was analysed using the Shannon-Weiner diversity index. Species abundance was computed for each forest type. Three genera and eleven species (*Trigona ventralis, T. collina, T. sirindhornae, T. terminata, T. apicalis, T. thoracica, T. canifrons, T. fuscobalteata, T. melina, Pariotrigona pendleburyi* and *Lisotrigona cacciae*) were recorded; *Trigona ventralis, T. sirindhornae, T. collina* and *T. terminata* were the most abundant. Species diversity indices ranged from 0.63 (DDF) to 0.86 (LMDF). Species richness and abundance data indicated the following clustering of forest types based on the stingless bee fauna: (((LMDF+UMDF) + DDF) + DEF). Stingless bees were most abundant during cooler, drier months and in seasons and forest types where the percentage of light transmittance was the greatest.

Key words: stingless bees, honey-bait trap, species abundance, diversity indices, forest types

INTRODUCTION

Pollination is one of the most critical reproductive processes in plants. One third of the human diet in tropical countries is derived from insect-pollinated plants. Stingless bees are a group of eusocial insects that play an important role in the pollination process of plant life, particularly plants in natural habitats in most tropical countries (Heard, 1999)

Stingless bees (Apidae, Meliponini) live socially in perennial colonies of a few hundred up to several thousand individuals (Wille, 1983). They are generalists that visit many different flower types. On the population level, some species are known to use floral resources from more than a hundred plant taxa over the course of several seasons in a given habitat (Wilms *et al*., 1996).

In Thailand, large areas of natural forest still remain, although changes in land use, such as the establishment of oil palm plantation, are increasing. In addition, selective logging has disturbed most of the remaining natural forest. These human disturbances not only directly affect the community structure of forest trees, but may also indirectly influence the pollinator community. This indirect effect is probably quite strong with respect to stingless bees, given that they depend on forest trees for both food (nectar and pollen) and habitat (nest site). Although decreases in the

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species richness and population density of stingless bees are proportional to the intensity of human disturbance, the mechanisms responsible for the decline in stingless bee communities are still unclear.

Species diversity is a parameter of community structure involving species richness and abundance (Wang *et al.,* 2000). In general, species diversity and the complex associations among species are essential for the stability of the community. Little is known on how extensive habitat loss will affect species diversity and ecological processes. Even less information is available regarding how stingless bees respond to forest disturbance imposed by human activities, although there is some indication that stingless bee abundance is greater in undisturbed, primary forests (Lee *et al.*, 2001). To date, only one study (Eltz *et al.,* 2003) has directly addressed ecological factors that are potentially limiting to stingless bee populations.

Stingless bees are dispersed throughout most parts of Thailand and form an important group of pollinators in agricultural and natural ecosystems. The objective of the study was to determine the species diversity and seasonal abundance of stingless bees in four types of forest in Thong Pha Phum District, Kanchanaburi Province, Thailand.

MATERIALS AND METHODS

The study area was located at Thong Pha Phum District, Kanchanaburi Province, western Thailand. The four sites studied intensively represented four major vegetation types, namely: dry dipterocarp forest (DDF) (449 m above sea level and 14.41°N, 098.29°E); upper mixed deciduous forest (UMDF) (549 m above sea level and 14.42°N, 098.28 °E); lower mixed deciduous forest (LMDF) (167 m above sea level and 14.39°N, 098.31 °E) and dry evergreen forest (DEF) (362 m above sea level, and 14.33°N, 098.33°E).

Bee surveys were performed using honey-baiting on vegetation. Honey-baits were formed by mixing honey and water in the ratio of 1:2 (honey:water, v:v) with salt (NaCl) added at the rate of 2 cm^3 per 500 ml of solution. Batches of bait solution were standardised using a hand refractometer. One to three transect lines of 0.5 – 1 km were setup at each site. Thirty jets (each of 100 ml) of bait solution were sprayed on the vegetation between 30 and 100 cm above the ground and marked with colored flagging tape every 20 m along the transects. The baited spots were about 1 m in diameter. Bees (principally Apidae) attracted to each of these baited spots were caught with a standard insect net during a maximum of five minutes. Spraying commenced between 8.00 a.m. and 9.00 a.m. and was completed after approximately 90 minutes. Bees were recorded at the spray stations during a second circuit started 150 minutes after spraying was initiated and was completed within 240 minutes. This time schedule was proven to yield the maximum number of species and individuals of stingless bees during the previous tests. Honey-spraying was generally restricted to clear or only slightly cloudy days. If there was afternoon rainfall before the completion of the survey, the accumulated data were discarded and the survey was repeated on a different day. Mostly, bees could be identified directly at the stations. In uncertain cases, samples were collected and later identified using published keys and descriptions (Schwarz, 1939; Sakagami, 1978) Repeated samples were taken every month from April 2004 to March 2005.

Air temperature and humidity data were recorded for each forest type for each month using a thermohygrograph and wet-and-dry bulb psychrometer. Light levels were obtained using a thermohygrograph and fish-eye photography (Inson and Malaipan, 2006).

Data analysis

Bee diversity and abundance were analysed based on the following measures: the number of bee species per transect-pair (survey), the mean number of bee species per spray station and the mean number of bee individuals per spray station. All three measures were analyzed. ANOVA with a repeated measures design was used to test for the effects of forest type and year on bee diversity and abundance.

Stingless bee data from the four types of forest were subjected to a cluster analysis using PC-ORD version 2.0. This analysis used Jaccard distance and Ward's method. Jaccard's Index was calculated as follows: the number of species found in both pairs of samples was expressed as a percentage of the total number of species found in one or the other of the samples. According to Ward's method, algorithms join clusters in such a way that within-group variances are minimised. This method uses Euclidean distances, which, in this case, was the distance between forest types. (McCune and Mefford, 1995).

Species diversity of stingless bees was analyzed using Shannon-Weiner diversity indices, $H' = -\Sigma^{S}_{i=1} p_i \log p_i'$ method (Shannon and Weiner, 1949; Price, 1984), where s = the number of species, p_i = the proportion of the total number of individuals consisting of the *i*th species and H' = an estimate of the diversity of the total population of individuals. Diversity indices were calculated based upon the number of stingless bees of each species found in each type of forest.

Factors affecting catches of stingless bee species were investigated through canonical correspondence analysis (CCA); (Braak, 1986); in PC-ORD version 2.0 (McCune & Mefford, 1995) using data matrices of the log-transformed stingless bee species abundance (numbers of individuals) with the two environmental variables retained. Axis scores were standardized using Hill's (1979) method and scaled to optimize the representation of species.

The sampling periods were from April 2004 to March 2005. The dry season was between April to September 2004 and up to March 2005, while the wet season was from May 2004 to September 2004 (Figure 1b).

RESULTS

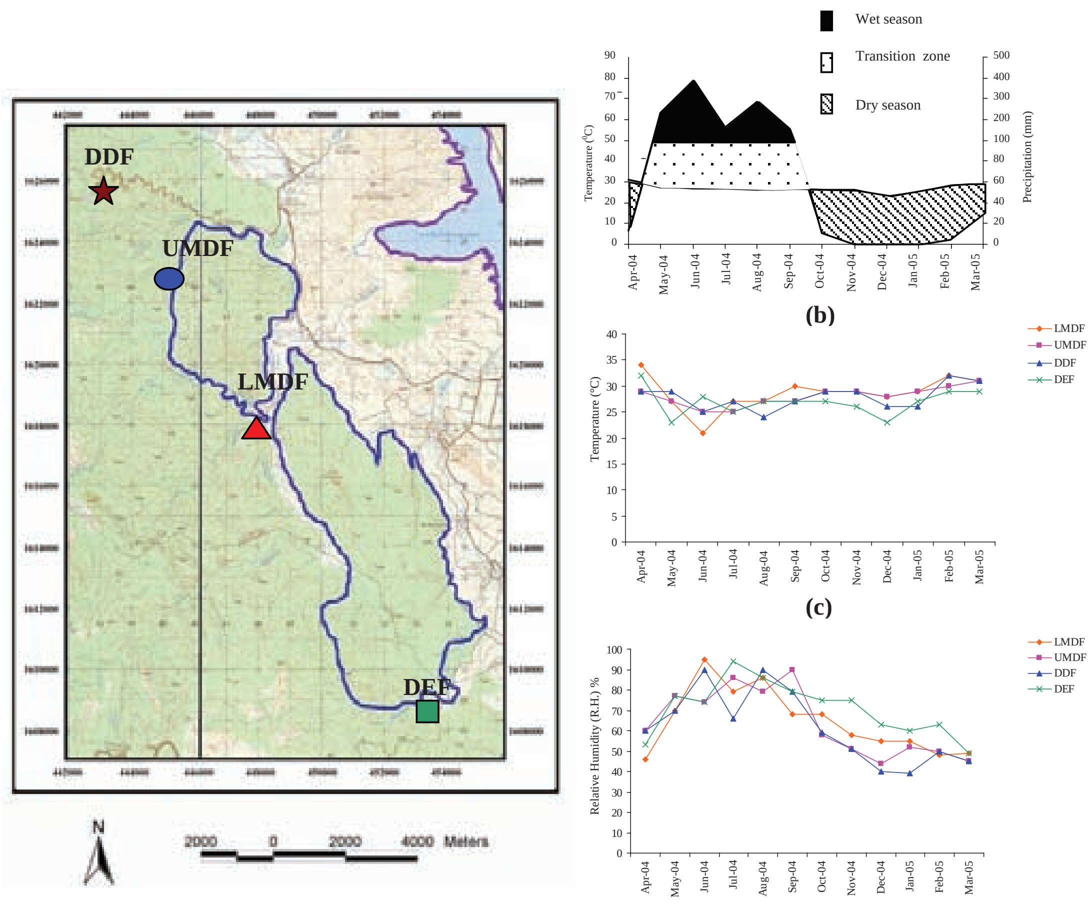
Eleven stingless bee species were found in each season from honey-bait trap sampling. The results showed the variation in species richness and abundance between the four types of forest and between the sampling seasons (Table 1).

Figures 2 and 3 represent data for the 11 species of stingless bees collected by the honey-bait trap method in four types of forest.

In order to consider the temporal effects, which might affect stingless bee richness and abundance, these data were grouped according to sampling season. The grouping of these data showed little difference between the sampling seasons in all forest types, as shown in Figures 3. The abundances of stingless bees in the dry and wet seasons were significantly different among the types of forest ($\chi^2 = 3382.71$, df = 3, P>0.001 for day season and $\chi^2 = 1768.38$, df = 3, P>0.001 for wet season).

Thus, while species richness varied only slightly between dry and wet seasons, the stingless bees were less abundant during the wet season across all forest types. All species were observed in both wet and dry seasons, except for one species (*T. fuscobalteata*) which was observed only in the dry season in DDF (Figure 4).

Figure 5 shows seasonal changes in abundance (catches of all species combined) during the study period in the four types of forest. While bee populations of each type of forest were discovered to fluctuate inconsistently during the study period, some common trends could be seen. In general, the number of stingless bees was considerably lower during the wet season and the population trends upwards as the dry season set



(a) (d)

Figure 1 (a) Study sites; dry dipterocarp forest (DDF), upper mixed deciduous forest (UMDF), lower mixed deciduous forest (LMDF) and dry evergreen forest (DEF). (b) Seasonal change in air temperature and precipitation. (c) Air temperature obtained from four types of forest. (d) Seasonal change in Relative Humidity during the study period.

Table 1 Species richness and abundance of stingless bees in four types of forest, April 2004 to March 2005.

Type of forest	Species richness		Abundance[1]		Total
	Dry season	Wet season	Dry season	Wet season	
Dry dipterocarp (DDF)	9	8	5,238	2,190	7,428
Upper mixed deciduous (UMDF)	7	7	2,985	1,094	4,079
Lower mixed deciduous (LMDF)	9	9	1,724	840	2,935
Dry evergreen (DEF)	5	5	676	271	947
Total			**10,623**	**4,395**	**15,389**

[1] Number of individual

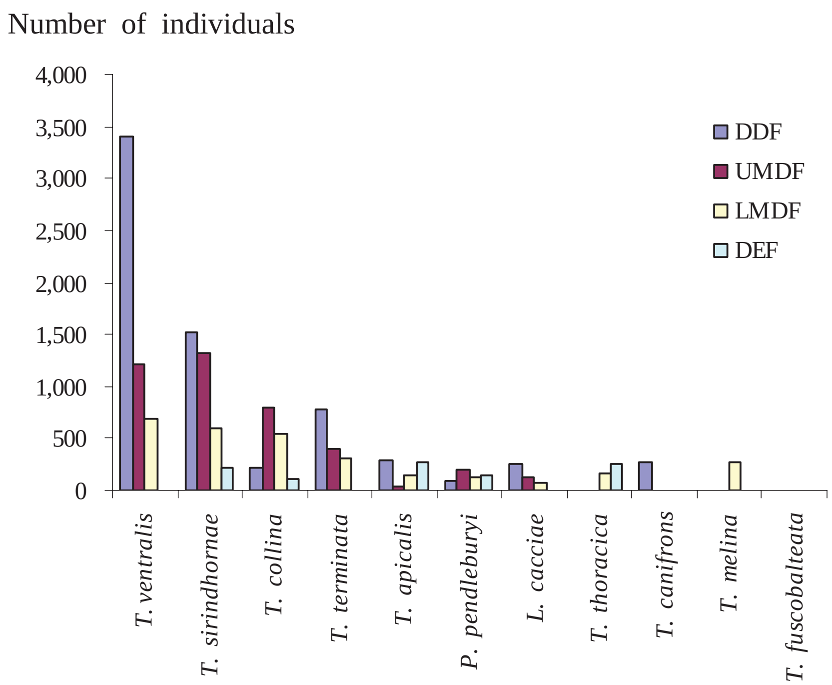


Figure 2 Species richness and abundance of stingless bees (number of individuals) visiting honey-bait traps in four types of forest, April 2004 to March 2005.

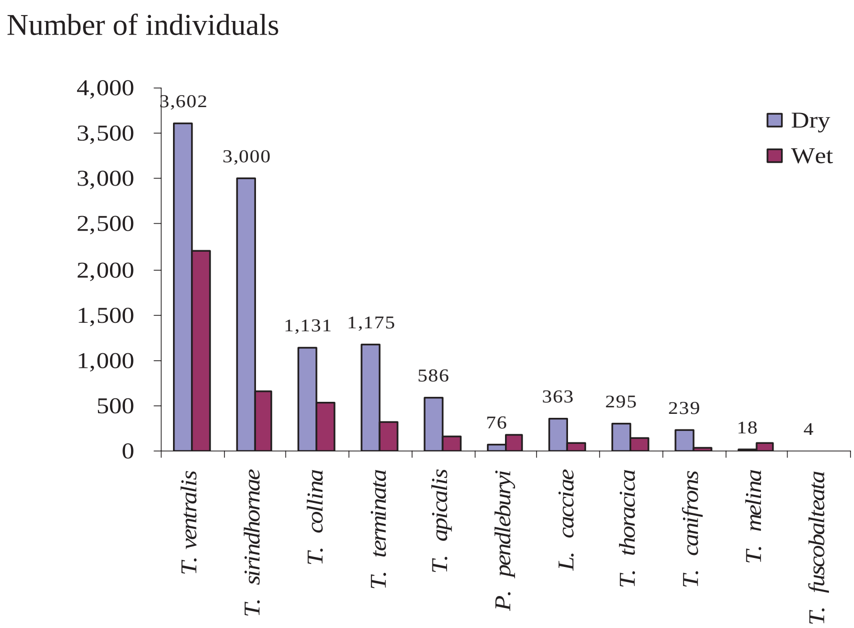


Figure 3 Species richness and abundance of stingless bees visiting honey-bait traps from four types of forest during the dry and wet season.

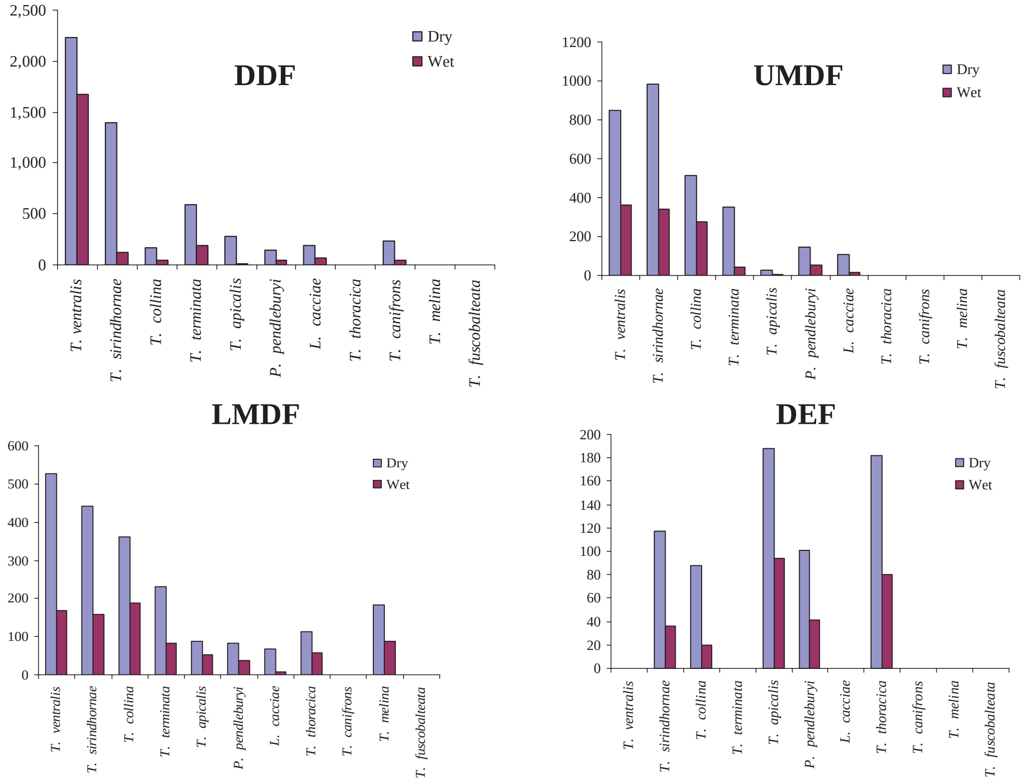


Figure 4 Species of stingless bee and their abundance at visited honey–bait traps from four types of forest during the dry and wet season.

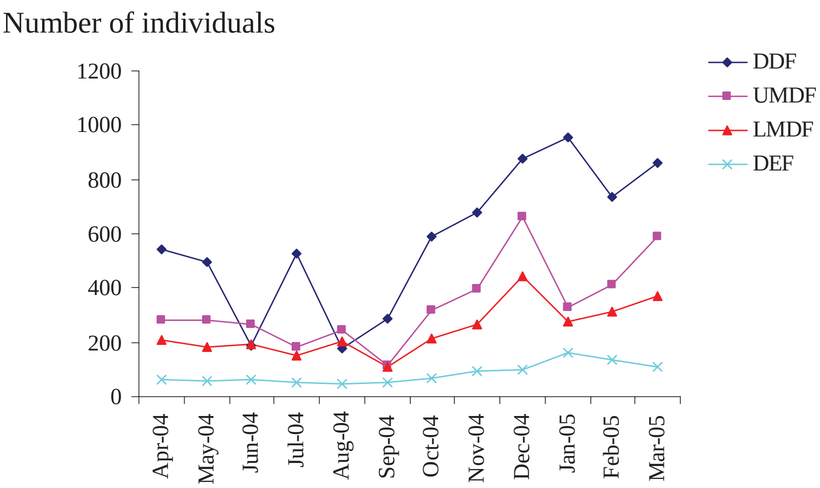


Figure 5 Seasonal changes in the population numbers of stingless bees in the four types of forest from April 2004 to March 2005.

in. The lowest population levels in any type of forest were recorded in the early wet season (May–August) and increased steadily with the onset of the dry season (September–December), with some fluctuations continuing until March. The highest number (954 bees collected) was recorded in DDF in January 2005. The population of stingless bees in all four types of forest in the dry season was higher than in the wet season. The number of individuals showed clear seasonal variation, although catches from day-to-day might have depended on weather conditions. The population of stingless bees declined in number through the wet season and then increased during the dry season.

To examine the effect of the type of forest on the relative abundance of dominant species, these samples were grouped according to the type of forest (Figure 6).

Dry dipterocarp forest

The dominant species in DDF during the sampling period was *Trigona ventralis* followed by *T. sirindhornae* and *T. terminata*, accounting for 49.10, 21.89 and 11.23% of total stingless bee numbers, respectively. The population of each species fluctuated inconsistently. The population of each species in the dry season was higher than in the wet season, although *T. ventralis* was an interesting exception with a population peak (439 bees captured) in May 2004 during the wet season.

Upper mixed deciduous forest

The dominant species in UMDF during the sampling period was *T. sirindhornae* followed by *T. ventralis* and *T. collina*, accounting for 32.46, 29.71 and 19.44% of total stingless bee numbers, respectively. The population of each species fluctuated inconsistently. The population of each species was higher in the dry season than in the wet season. *T. sirindhornae* was exceptional in that wet season (June) catches indicated higher populations than in some dry season months (November 2004 and January 2005). Nevertheless, the highest number of *T. sirindhornae* (201individuals) was recorded in the dry season

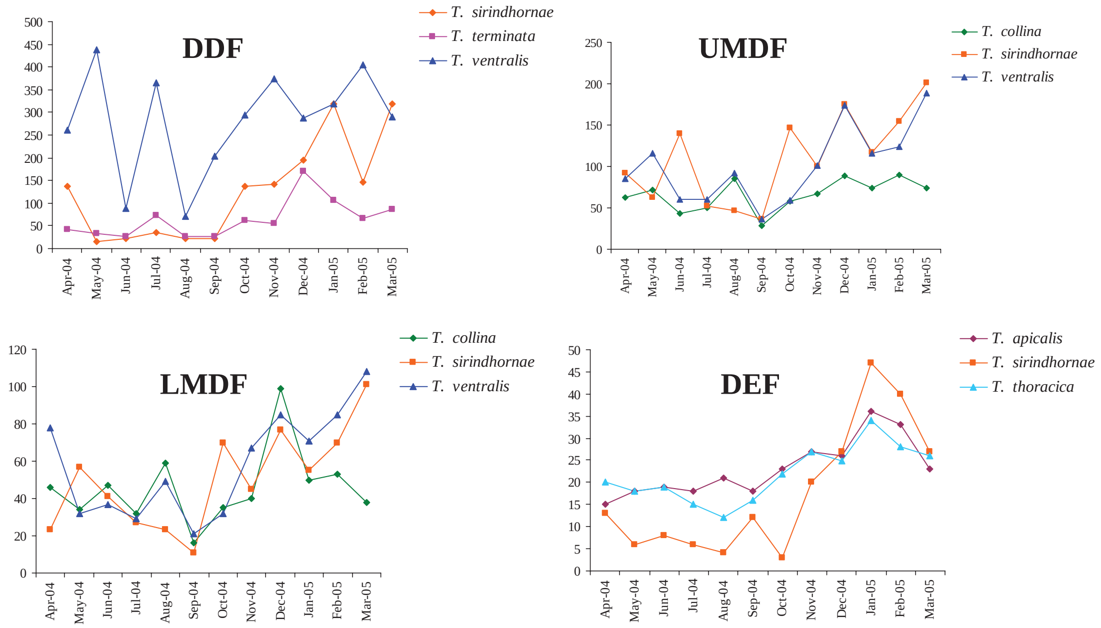


Figure 6 Seasonal trends of the abundance of dominant species in four types of forest during the study period.

(March 2004).

Lower mixed deciduous forest

The dominant species in LMDF during the sampling period was *T. ventralis* followed by *T. sirindhornae* and *T. collina*, accounting for 23.65, 20.44 and 18.71% of total stingless bee numbers, respectively. The population of each species fluctuated inconsistently. However, the population of each species was higher in the dry season than in the wet season. *T. collina* was the exception in that slightly higher numbers were recorded in the wet season (August) than in most dry season months. The most abundant bee species (with 108 individuals collected in March 2005) was *T. ventralis*.

Dry evergreen forest

The dominant species in DEF during the sampling period was *T. apicalis* followed by *T. thoracica* and *T. sirindhornae*, accounting for 23.65, 20.44 and 18.71% of total stingless bee numbers, respectively. The population of each species fluctuated inconsistently. In general, the population of each species was higher in the dry season than in the wet season. *T. sirindhornae* was notably scarce in the wet season. In contrast, the highest catches (47 individuals) were of *T. sirindhornae* in January 2005.

The hierarchical clustering routine was applied to the stingless bee data to compile similarity of abundance measures for the four types of forest. The resulting dendrogram (Figure 7) illustrates that DEF was clearly distinguished from other types of forest (100% dissimilarity) and that stingless bee diversity and abundance were most similar in UMDF and LMDF (100% similarity). The cluster UMDF+LMDF+DDF was more heterogeneous and the stingless bee fauna of DEF was the least similar to those of the other forest types.

Table 2 presents the species diversity indices of stingless bees collected from the four types of forest, calculated for combined data and separated for wet and dry seasons. The LMDF had the highest diversity index, followed by UMDF, DEF and DDF, respectively.

Presence-absence data were used to compile (similarity) indices of stingless bees in the four types of forest. The resulting dendrogram (Figure 8) illustrates DEF to be clearly distinguished from other types of forest, DDF to form a separate cluster the most similar to those observed in LMDF and UMDF, and UMDF and LMDF to be similar.

A CCA was performed on a primary data matrix consisting of the log-transformed numbers of 11 stingless bee species from all four types of forest and a secondary matrix with two environmental variables (relative humidity and temperature). The analysis made it possible to distinguish groups of stingless bees. In particular,

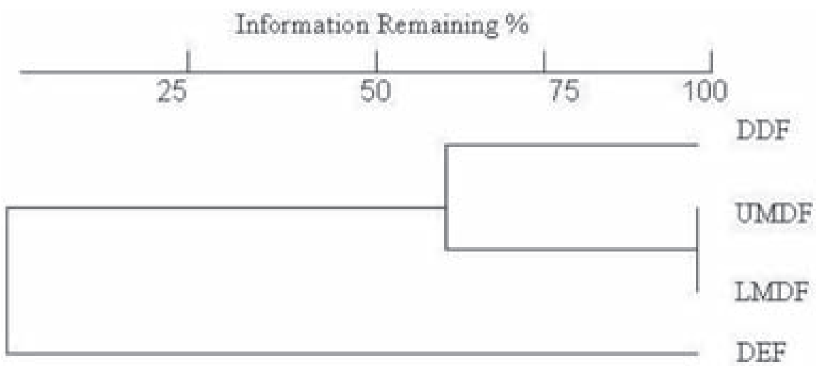


Figure 7 Dendrogram of the similarity of stingless bee in th four types of forest.

the group comprising *T. terminata, T. ventralis, L. cacciae* and *T. sirindhornae* increased in abundance with rising temperature and decreased in abundance with rising relative humidity (Figure 9).

DISCUSSION

The data analysed in this paper appeared to produce reasonable indice of stingless bee diversity and abundance in the four types of forest at Thong Pha Phum. It was possible that some rare species escaped detection or that canopy-level pollinators were undersampled. However, the data were generally supported by the nest census data. The honey-bait sampling methods employed in this study were particularly effective for surveying bees "recruited" to food sources, i.e. social species such as stingless bees and honey bees (Lee *et al.*, 2001). The diversity of stingless bees recorded at Thong Pha Phum was comparable to that reported from sites in Sabah (Eltz *et al.,* 2003) and was greater than that reported from sites in Malaysia and Singapore (Lee *et al*., 2001). However, the community structure of stingless bees at Thong Pha Phum was clearly different from that found at sites in peninsular Malaysia, Singapore, Sumatra and Sabah. Cluster analysis (Jaccard, Figure7; Bray Curtis, Figure 8) both indicated that the stingless bee fauna was the most similar to those observed in LMDF and UMDF, that LMDF and UMDF and DDF formed a cluster and that the bee fauna observed in DEF was the least similar to those of the other three forest types. The results showed variation in species richness and abundance (number of individuals) among the four types of forest and between the sampling seasons. The stingless bees had their highest species

Table 2 Diversity index of stingless bee in the four types of forest during the dry and wet seasons from March 2004 to April 2005.

Type of forests	Diversity index[1]	Dry season	Wet season
Deciduous dipterocarp (DDF)	0.63	0.69	0.41
Upper mixed deciduous (UMDF)	0.68	0.69	0.62
Lower mixed deciduous (LMDF)	0.86	0.85	0.84
Dry evergreen (DEF)	0.67	0.68	0.64

[1] Shannon - Weiner diversity index (*H'*) values

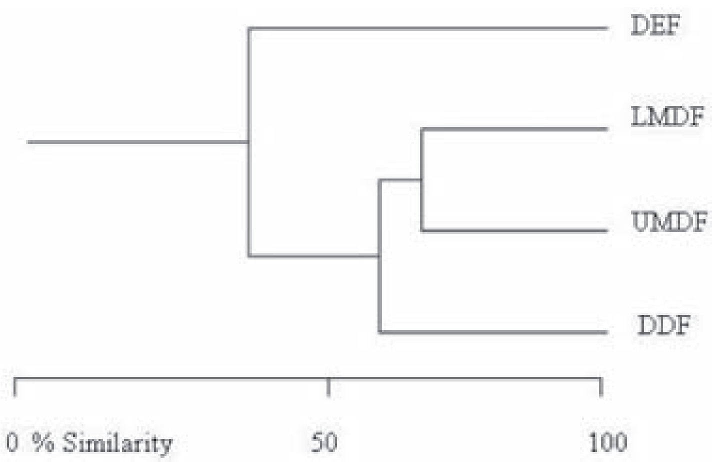


Figure 8 Dendrogram of the diversity indices of stingless bees in the four types of forest.

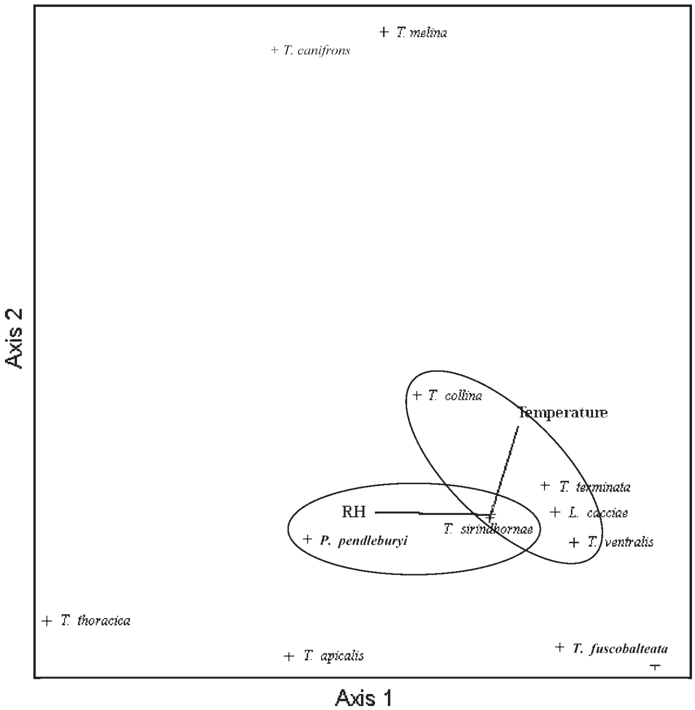


Figure 9 Ordination diagram from the CCA of a primary matrix of the log-transformed totals of stingless bees collected from four types of forest in Thong Pha Phum District, Kanchanaburi Province and a secondary matrix of two environmental variables.

richness (absolute number of species, Figure 1) in DDF and LMDF, which was relatively greater than species richness in UMDF followed by that in DEF. Stingless bees were most abundant in DDF followed by UMDF, LMDF and DEF. These studies suggested that LMDF provided a favourable habitat for stingless bees. This was considered to be due to the fact that the microclimate was more sutitable for nesting or successful foraging, because either food resources were richer, or there were more nesting trees. Lee *et al.* (2001) found that there were more stingless bees where forest trees were larger, where ambient conditions were more constant and cooler and where somewhat counter-intuitively, flowering intensity was generally less.

Abiotic factors

Several abiotic factors influenced the activity of the bees. Temperature has been mentioned as the most important factor and the activity of some species appears to be limited by relative humidity (Corbet *et al.*, 1993). A review of several authors (Fowler, 1978; Kleinert-Giovannini, 1982; Heard and Hendrikz, 1993) indicated that the flight activity of bees was influenced both by the internal conditions of the colonies and the environmental conditions. Temperature, relative humidity, light intensity, rainfall and wind speed influenced the flight activity of stingless bees, although not always with the same intensity. Temperature and relative humidity data revealed that the forest types at Thong Pha Phum had somewhat different microclimates.

Light: Light levels are commonly expressed in terms of "transmittance". Growing-season transmittance is widely used in forestry because of its strong relationship to tree growth and survival. Transmittance in the four forest types was recorded by hemispherical canopy (fish-eye) photography. This technique was used to measure subcanopy light conditions and the results obtained from the present study were confirmed by Insoon and Malaipan (2006). The data showed that the percentage of transmittance in the wet season in LMDF was 7.73 followed by DDF, UMDF, DEF (6.60, 3.93 and 0.89% respectively). In the dry season, the percentage of transmittance for LMDF was 15.88 followed by DDF, UMDF and DEF (12.35, 11.36 and 1.26% respectively). These figures indicated a positive correlation between percentage transmission of light and catches of stingless bees, for all species and forest types.

Temperature and relative humidity: Temperature and radiation are commonly cited as important variables that affect insect flight activity. Temperature influenced the intensity of activity above certain thresholds. Cloud cover and wind speed had no significant effect on flight activity in this study. Canonical correspondence analysis (Figure 9) was performed on a primary data matrix consisting of log- transformed numbers of stingless bee individuals from all four types of forest for 11 stingless bee species, and a secondary matrix with two environmental variables (relative humidity and temperature). The analysis made it possible to distinguish groups of stingless bees. In particular, the group comprising *T. collina*, *T. terminata*, *T. ventralis*, *L. cacciae* and *T. sirindhornae* had a positive relationship with temperature, as the species of the group increased in abundance with rising temperature and decreased in abundance with increasing relative humidity. Another group, comprising *T. sirindhornae* and *P. pendleburyi*, had a positive relationship with relative humidity, increasing in abundance with rising relative humidity and decreasing in abundance with rising temperature. T. melina, *T. canifrons*, *T. thoracica*, *T. apicalis* and *T. fuscobalteata* had no consistent relationship with the two environmental variables. Data for these variables showed that DEF, the forest type least favored by foraging stingless bees, had the lowest relative humidity and also had the highest air temperatures, in general, of all the forest types. These results concurred with those of Heard and Hendrikz (1993), who reported factors influencing flight activity of colonies of the stingless bee *T. carbonaria* in Australia. They considered temperature and light intensity as important factors that determined the flight activity of these bees. Colonies of *T. carbonaria* were active throughout the year at their experimental sites, but the daily activity period was longer in the warmer months, while the relative humidity did not show any significant effect.

Biological factors

Body size and flight range: Catches of stingless bees at honey baits may be influenced by the flight range of the different bee species. Roubik (1989) reported that stingless bees had more compact flight ranges than honey bees. The workers of honey bees can forage over two to three km, while those of stingless bees can forage over one km at most. There are some correlations between flight range and the body size of the workers. The results of the study showed that *T. ventralis* was the most abundant, followed by *T. sirindhornae*. Both of these are medium-sized bees. These species would likely have a greater flight range than small–sized bees (*L. cacciae*, *P. pendleburyi* and *T. fuscobalteata*). Kerr and Kerr (1999) found that small bees like those of the subgenus *Plebeia* (body length 3 – 4 mm), had a flight range of about 300 m; medium-sized bees, such as species of the subgenus *Torigona* (5 mm), about 600 m; large bees (10 mm) about 800 m; and very large bees, 13 – 15 mm, e.g. *Melipona fuliginosa,* about 2,000 m. Foraging bees of *T. carbonaria* fly over a maximum distance of 500 m and usually prefer to fly over a distance of about 100 m from the nest. Not only do larger insects tend to fly further, but they also can be active in cooler conditions. Kapyla (1974) remarked that external activity depended on the body size of the insect, because larger species flew in lower temperatures than smaller ones. At Thong Pha Phum, large and medium-sized bees might be more likely to be active and thus drawn to honey baits than the smaller species.

Biological factors that might influence stingless bee diversity and abundance across forest types at Thong Pha Phum included the availability of suitable nesting sites and food resources, especially floral resources. However, it was worth noting here that preliminary analysis of these data suggested that nest density was higher in LMDF (15.66 nest/ha) than in the other forest types. Nest densities in UMDF, DEF and DDF were 11, 6.66 and 0.66 nests/ha, respectively. Since the population in one stingless bee nest could vary from about 6,000 to 20,000 individuals, the differences in nest density could have a major impact on the total number of bees present in a particular type of forest. Nest density data may provide further insights into the influence of flight range on catches of foraging stingless bees at Thong Pha Phum. The information presented here offered new insights into the ecology of stingless bees and provided new perspectives that can be applied widely to conservation methods and to strategies for developing native bees as agricultural pollinators. In addition, this information provided baseline data on Thailand’s bees essential for monitoring populations in the future. Information acquired in this study formed a basic and supporting knowledge resource for the development of an integrated pollination management program in Thailand. This paper has presented the initial results from an intensive study that was understood to be the first investigation of the biodiversity of stingless bees in western Thailand. It was also the first major application of honey-baiting techniques in Thailand. More specifically, this paper has examined whether vegetation type and microclimate were correlated with the distribution of stingless bee species.

CONCLUSION

Species richness and abundance of stingless bees varied over the seasons and stingless bees were most abundant during the cooler, drier months of the year. Overall, species diversity was directly affected by the fluctuation in populations of individual species and several factors influenced the activity of the bees such as temperature, relative humidity, transmittance, body size and flight range.

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