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Heterotrigona itama workers bees homing ability as the basis for colony placement

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Abstract. *Rusdimansyah R, Khasrad K, Jaswandi J, Rusfidra R, Aulia D. 2024.* Heterotrigona itama *workers bees homing ability as the basis for colony placement. Biodiversitas 25: 2478-2483.* This study aimed to evaluate the ability of *Heterotrigona itama* worker bees to return to the nest from various test distances. This research is an observational study using the translocation method. There were 550 individuals of *H. itama* worker bees from 5 colonies reared for over two years. Each worker bee sample was identified with a mark in the form of water-based paint before being released at a testing distance every 100 meters until a distance where no more worker bees returned. The camera with a macro lens is installed at the entrance to the beehive to record worker bees returning to the hive. The video recording process was carried out over 90 minutes. The parameters observed included environmental temperature and humidity, the number of worker bees returning, and the time required for worker bees to return from each test distance. The data obtained were analyzed using Chi-square, Kruskal-Wallis, and Mann-Whitney U-test. The research showed that the maximum distance that *H. itama* worker bees can travel to return to the nest was 1000 meters. However, the most effective return distance was more than 500 meters because although some worker bees can return from greater distances, the number of returns significantly decreases after passing 500 meters. Therefore, the ideal distance for placing a colony of *H. itama* is less than 500 meters from the source of bee food plants. The minimum distance to move a colony from the initial location to a new location is around 1100 meters.

Keywords: Flight distance, kelulut, meliponicultur, meliponini, stingless bee

INTRODUCTION

Globally, wild bees face significant threats due to the continuous depletion of their natural habitats, jeopardizing the sustainability of plant diversity and agricultural productivity. From both ecological and economic perspectives, bees play a crucial role in the pollination process of plants (Jaffé et al. 2016). Social bees of the Apidae family are vital for pollinating wildflowers and crops, relying heavily on their visual capabilities to perform various essential behavioral activities (Kelber and Somanathan 2019). Stingless beekeeping represents a biocultural heritage that should be appreciated and preserved for future generations (Aldasoro-Maya et al. 2023).

Stingless bees, or *Meliponini*, are a type of bee native to tropical regions and are smaller than the more familiar honeybees (genus *Apis*) (Quezada-Euán 2018). More than 500 species of stingless bees worldwide (Engel et al. 2019). In Sumatra, 23 species of stingless bees have been identified, with *Heterotrigona itama* (Cockerell, 1918) being the most commonly kept species, followed by *Geniotrigona thoracica* (Smith, 1857) (Herwina et al. 2021).

Bees rely on plants for nectar, resin, and pollen to produce various bee products while flowering plants depend on bees for pollination. Although stingless bees produce less honey than honeybees, they generate more propolis, known for its health benefits (Quezada-Euán 2018). Notably, *Lactobacillus plantarum* SN13T, a candidate probiotic bacterium isolated from the honey of the stingless bee *Homotrigona binghami*, has potential applications as a starter for fermented milk processing in the food industry (Melia et al. 2022).

Many factors, including morphology, physiology, ecology, and phylogeny, influence the flight ability of bees (Baird et al. 2020). The flight distance from the nest determines the foraging range, with smaller stingless bee species covering 100-500 meters and larger species capable of foraging over 1000 meters (Ciar et al. 2013). Bees navigate back to the hive using polarized sunlight, natural landscape cues, or pheromones. Worker bees are generally attracted to food sources located lower and closer to the nest entrance. The average number of returning *Melipona fasciculata* bees decreases with increasing release distance while return time increases (Nunes-Silva et al. 2020). The effective return distance for *Melipona seminigra* is 1000 meters (Costa et al. 2021). Return distance variations among bees can be influenced by body size, environment, and forager experience (Rodrigues and de Fátima Ribeiro 2014).

The species and size of bees significantly affect their speed in locating food sources. For example, *Apis mellifera* (Linnaeus, 1758) has a flight speed of 4.6 m/s, while *Apis cerana* (Fabricius, 1793) flies at 9.1 m/s (Baird et al. 2020). To quantify the ability of bees to return without stinging, researchers use translocation experiments, where colored markers are affixed to the dorsal region of the bee, or Radiofrequency Identification (RFID) for larger bees

(Nunes-Silva et al. 2020; Costa et al. 2021; Wayo et al. 2022). Many studies have examined the return distance of stingless bees but lack data on *H. itama*. This research aims to determine the ability of *H. itama* worker bees to return to the nest from various test distances. The findings will serve as a reference for the placement of new colonies during colony propagation and the strategic placement of vegetation to optimize food sources and enhance the production of *H. itama*.

MATERIALS AND METHODS

Research material

The research bee colonies were placed in Edufarm, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia (0°54'43.26"S, 100°27'57.89"E). The red circle indicates the test radius for releasing worker bees (100-1100 meters), while the red point shows the direction of the worker bee release point (Figure 1).

Five colonies of *Heterotrigona itama* stingless bees in a cultivation box with a brood sell box size of 13 × 13 × 18 cm^3 with a honey box size of 27 × 27 × 6 cm^3 that had been maintained for more than 1 year at the research location. From each colony, a sample of 110 bee workers was taken (50 for each test distance). A set of insect nets, tweezers with soft foam-coated tips and a hollow jar wrapped in black cloth to catch and carry worker bees. Water-based color paint was used to color the sample bee *mesoscutum*. Thermohygrometer to measure temperature and humidity around the nest. A set of digital cameras with macro lenses and tripods were placed at the bee entrance.

Procedures

This research used observational using the method of translocation (moving) of *Heterotrigona itama* worker bees that had been given paint marks on the thorax (*mesoscutum*) from the nest to the test distance point (Campbell et al. 2019). Each worker bee sample was identified with a mark in the form of water-based paint before being released at a testing distance every 100 meters until a distance where no more worker bees returned (Nunes-Silva et al. 2020).

Worker bees were captured on the study day at 08.30 a.m. (Nunes-Silva et al. 2020). Worker bees caught were then given a paint mark (water base) on the thorax. The colors used were different at each test distance. The worker bees that had been put in the jar were then transported in the jar covered with a black cloth to create dark conditions so that the bees were calmer. Release worker bees at each test point at 11.00 in sunny weather conditions, with no strong winds. The test distance points were measured using GPS. The observations were made of worker bees that had been released through video recordings taken during the observation period (90 minutes) in front of the nest entrance; the observation time was 30 minutes longer than the research conducted by (Campbell et al. 2019). The parameters observed included environmental temperature and humidity, the number of worker bees returning, and the time required for worker bees to return from each test distance. Temperature and humidity measurements around the colony were carried out during the observation period.

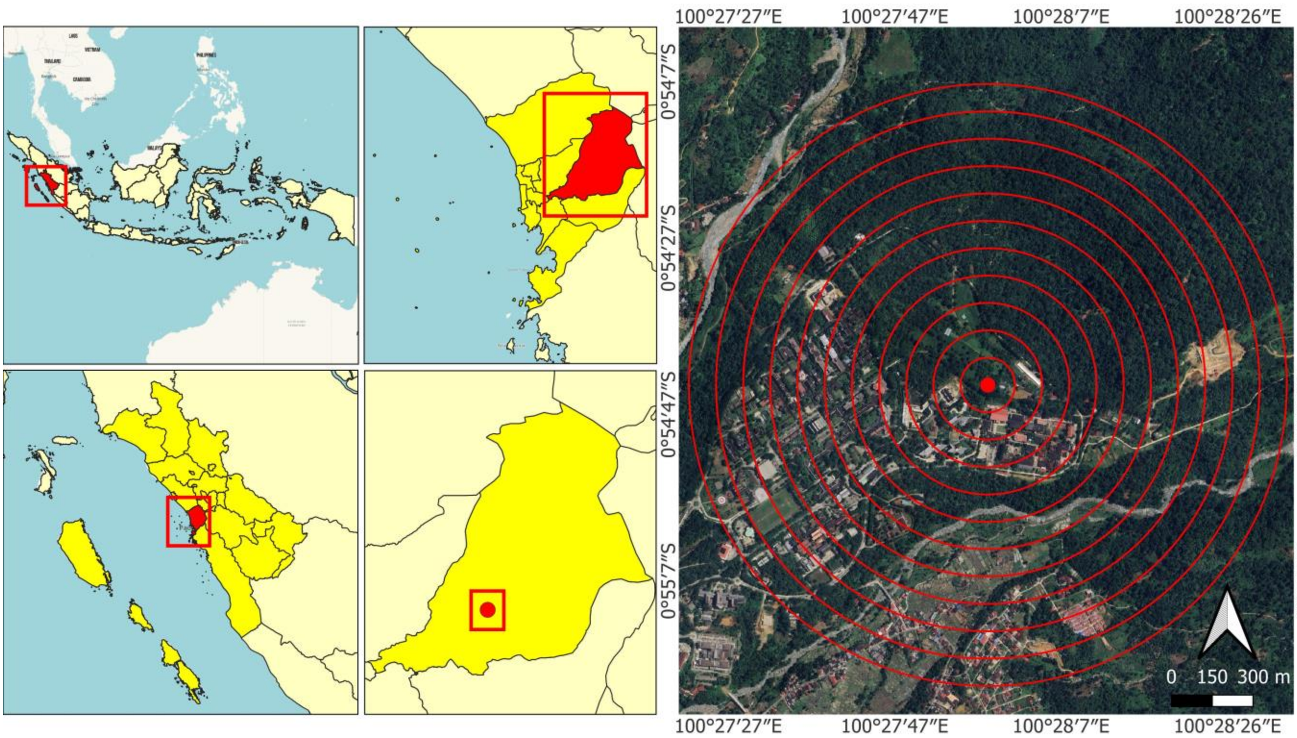

Figure 1. Landscape map of the radius of release of *Heterotrigona itama* workers

Data analysis

Statistical analyses were conducted to compare the number and percentage of worker bees that successfully returned to the nest using the Chi-square test. To determine the effective distance, the maximum distance at which 50% of worker bees were able to return to the nest was used as the criterion and analyzed using the Chi-square test. The Kruskal-Wallis test was employed to analyze the variable of return time for worker bees. In cases where significant differences were detected, multiple pairwise comparisons were performed using the Mann-Whitney U-test. These analyses were carried out with the assistance of IBM SPSS Statistics for Windows, Version 25.

RESULTS AND DISCUSSION

Ambient temperature and humidity

Based on observations, the average ambient temperature and humidity during observation outside the nest (Figure 2). The recorded temperature and relative humidity constitute the dataset acquired during the release of the bees. These measurements were conducted under rain-free meteorological conditions between 11 a.m. and 12 p.m. The Average ambient temperature during observation was 29,92±0,71°C, and humidity was 60,80±0,92%. The acquired data exhibits a relatively uniform distribution, as indicated by low coefficients of variation, precisely 2.37% for ambient temperature and 1.52% for humidity.

Average number and percentage of returns of worker bees from the test point

Figure 3 illustrates the outcomes derived from the analysis of video recordings conducted during the observation period.

There was a decrease in the percentage of bees returning to the nest as the distance between bees released increased. The furthest distance was 1000 m, where 20% of the worker bees from colony 2 were still returning to the nest. Based on the Kruskal Wallis test, there was no significant difference ($p>0.05$) in the percentage of bees returning to the nest in different colonies. At a distance of 100 meters, all 50 bees released exhibited a complete return to the nest, with a return rate of 100%. However, as the testing distance was extended, the proportion of bees returning to the nest progressively diminished. The maximum range observed for bee return was 1000 meters. Beyond this distance, specifically at 1100 meters, no bees returned to the hives.

A Chi-square test revealed no significant difference in the number of worker bees returning at test distances of 100 meters versus 200 meters ($p>0.05$). However, there was a noticeable decline in the number of returning bees at distances of 300, 400, and 500 meters, though the differences among these distances were not statistically significant ($p>0.05$). A further reduction in the percentage of returning bees was observed at distances between 600 and 800 meters. Notably, there was a significant difference in the number of bees returning from 500 to 600 meters ($p<0.05$). The Chi-square test also indicated that the decline in return rates between 600 and 800 meters was statistically significant ($p<0.05$).

At the test distance of 900 meters, only worker bees from colonies 2, 3, and 5 managed to return, with return percentages of 30%, 20%, and 10%, respectively. Notably, colonies 1 and 4 did not have any returning bees (Figure 3). At 1000 meters, only worker bees from colony two returned, with a return rate of 10%. At a test distance of 1100 meters, no worker bees from any colony returned to the nest (Figure 3).

Data regarding the return time of worker bees based on the test distance is shown in Figure 5. There was a significant difference in the return time of worker bees at different test distances ($p<0.05$) based on the Kruskal-Wallis test. Further, the Mann-Whitney U test was carried out and showed that the bees returning at a distance of 100 and 200m had no significantly different times ($p>0.05$). However, the return time for worker bees at a test distance of 100 and a distance of 300-500m was significantly different. There was also no significant difference at the test distance of 300-900m ($p>0.05$). The 1000m distance had the longest return time compared to the return time at the test distance closer to the nest.

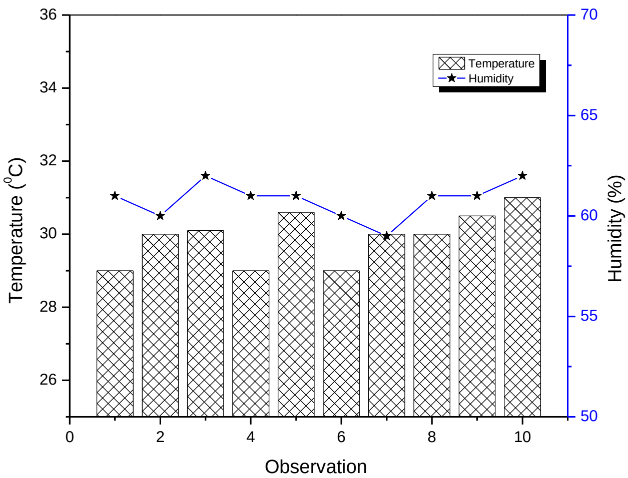

Figure 2. Ambient temperature and humidity during observation

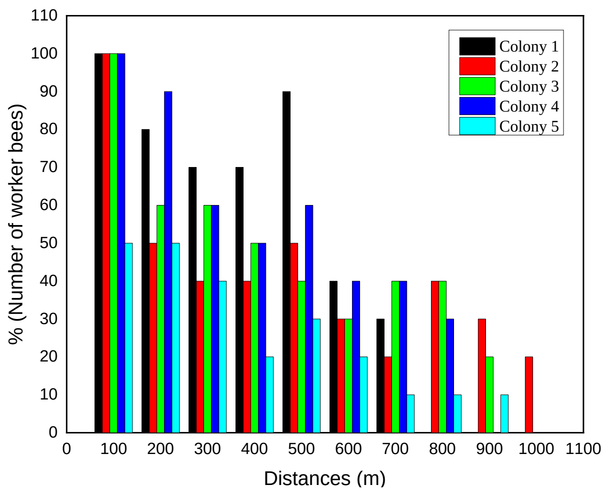

Figure 3. Percentage of worker bees returning to the nest based on test distance and colony

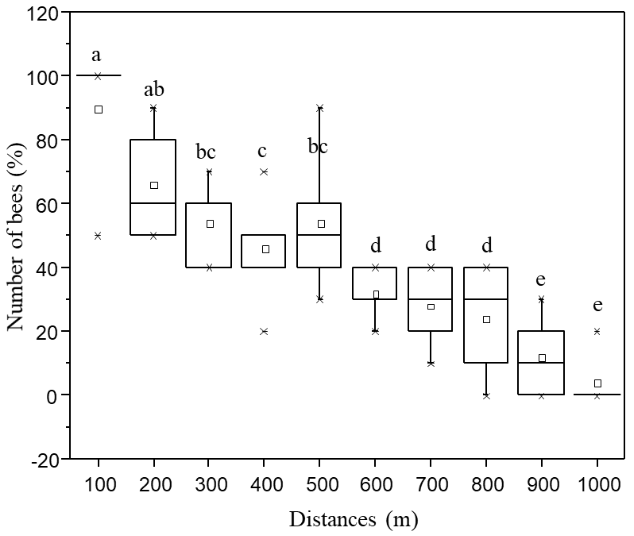

Figure 4. The average percentage of worker bees returning to the nest based on test distance ([a,b,c,d] Different letters for each test showed a significant difference ($p<0.05$) based on Chi-square analysis

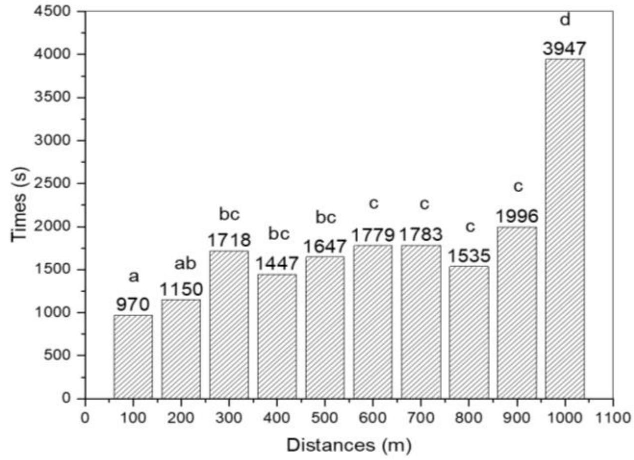

Figure 5. Worker bee return time (s) according to the distance of the release test (m) ([a,b,c,d] Different letters for each test showed a significant difference ($p<0.05$)

Discussion

Based on Figure 2, the average temperature and humidity of the outer environment of the nest were 29.92°C±0.71 and 60.80%±0.92%, respectively. Therefore, the temperature and humidity of the environment of the study site were the normal temperature and humidity needed by stingless bees to support their activities and productivity.

Temperature influences the flying activity of worker bees; when the temperature is below or above normal temperature, the flying activity of worker bees in foraging will be disturbed. Even worker bees cannot fly, so the bees only do the activities in the hive. When the temperature in the hive exceeds its normal range, several worker bees will gather at the hive's entrance and flap their wings until the temperature in the hive drops. If the environmental temperature increases to 40°C, bees will increase in temperature in the head and abdomen. Bees can anticipate this condition by collecting nectar at a temperature of 32°C, but if they fly in these conditions any further, the bees will not be able to cope (Souza-Junior et al. 2020). The stingless bees need more energy and may cause dehydration when flying at high ambient temperatures. Responding to an ambient temperature increase, numerous bees exploit the utmost sugar concentration available. *Heterotrigona itama* prefers nectar with a sugar concentration exceeding 35%. The foraging activity experiences decline as the environmental temperature progressively rises towards midday, between 12:00 a.m. and 01:00 p.m. (Basari et al. 2018).

The average return number of worker bees in *Heterotrigona itama* decreases with increasing test distance from the hive, except at a distance of 500 m (Figure 4). According to the research conducted on *Melipona mandacaia*, the number of returning worker bees decreases with the increase of distance apart from the hive, except at a distance of 2000 m, there is an increase in the number of returning bees until it finally decreases. The most effective and maximum return distances are 500 m and 1000 m (Rodrigues and de Fátima Ribeiro 2014). The maximum return distance is at which 90% of the released bees do not return to the hive (Campbell et al. 2019).

The number of worker bees back at the point of 500 m occurs because, at this point, there is in the Faculty of Agriculture, where on the land there are flowering plants (a source of bee feed) such as jewawut, chili, tomatoes, and other plants with a plant height of 0.5-1.5 m. At 400 m, there are not many flowering plants; the vegetation there is in the form of large and tall trees like pine plants. The primary determinant for the foraging preference of honey bees towards pollens would be the nutritional composition of protein and the accessibility of the less nutritious floral resources (Ghosh et al. 2020). There existed considerable divergence in the foraging distances undertaken by workers across colonies of identical species, a phenomenon markedly impacted by the quantity and spatial arrangement of the accessible foraging habitats (Redhead et al. 2015). In order to locate nourishment, return to their dwelling, and meticulously regulate their aerial movement amidst these two areas, many insects heavily depend on their visual capabilities (Chakravarthi et al. 2017). Bees prefer to visit areas with many flowering plants rather than very tall plants in other areas. It is to the statement that *Tetragonula biroi*, bees like flowering plants with a height of less than 3 m (Ciar et al. 2013). Therefore, the bees have more experience at the point of 500 m, so the bees can return more than at a distance of 400 m. Stingless bees have been documented to frequent a wide range of plant genera that are not native to their region, even in areas with native vegetation (Wilson et al. 2021).

Based on Figure 3, it can be seen that there are variations in the number of worker bees in each colony. The maximum distance of bee returns in colony 1 was 700 m, while in colony 2, it was 1000 m; in colony 3, it was 900 m; in colony 4, it was 800; and in colony 5, it was 900m. There were variations in the return number of worker bees from the point of release, as a whole, because of the differences in the discharge site's topography, differences in each worker bee's experience, and the direction of release. The foraging behavior exhibited by stingless bees possesses distinct characteristics. It is in accordance with the experience of the explorer bees and differences in the direction of release; most likely, the bee did not forage circularly, but it is determined by the availability of feed in the maximum flight range (Costa et al. 2021). There is an inversely proportional relationship between the average number of returning bees and the return time of *Heterotrigona itama* workers' bees to distance. The farther the discharge distance from the hive, the fewer worker bees can return, whereas the farther the distance from the hive, the more time it takes for the worker bees to return to the hive. The number of artificially released *Melipona fasciculata* worker bees with radiofrequency signs continues to decrease with increasing test distance, and the return time increases with increasing test distance (Nunes-Silva et al. 2020). The average return time of worker bees varies; the lowest average was 8.13±8.52 minutes, and the highest average was 65.46±31.48 minutes. The average return time of the bees increases with increasing test distances. Bees need more time to return from a greater distance than at a closer distance to the hive. The return time of the bee *Melipona fasciculata* increased with increasing test distance from the hive (47 minutes from a distance of 100 m and 120 minutes from a distance of 1600 m) (Nunes-Silva et al. 2020).

Furthermore, the same was discovered in the neotropical bee *Scaptotrigona aff. postica* that the time it takes for worker bees to fly back to the hive is longer over distances compared to closer distances (Campbell et al. 2019). Variations in the return time of worker bees occur because the bees have never roamed at the release site, so the bees have no knowledge of the area and need time to find their way to the hive. It is consistent with the statements that time variations again occur due to differences in the experience of explorers (Rodrigues and de Fátima Ribeiro 2014).

Furthermore, worker bees are tasked with collecting feed to meet the entire colony's needs. The time bees visit a sprig of flowers per unit of time depends on the amount of feed ingredients available on each flower. The lateral position is regulated by maintaining the highest optic flow equilibrium in the frontal visual field. It will guarantee that even minuscule obstacles in the flight trajectory will be identified and utilized to regulate the position, thereby facilitating prompt and efficient evasion of collisions (Lecoeur et al. 2019).

Based on the data on the average return time (Figure 5), the return speed of the worker bees can be calculated, but this speed is not the flying speed of the worker bees. The speed of returning home also includes time to find a way back to the nest, collecting feed, and resting. If we calculate the average return speed of the worker bees according to the test distance, then at a distance of 100 m, the average rate of the bees is 0.103 m/s or 6,188 m/min. This figure is much lower than the speed of *Apis cerana* worker bees, namely 9.1 m/s (Baird et al. 2020). It happens because of differences in species, body size, and the ability of *H. itama*, which tends to fly low and pass between trees to avoid the wind.

Determining the release point for bees can only be done to the east because the west area is a building area; in the north area, large trees increase the risk of bees being trapped by predators. While the southern area is residential. Further studies need to be conducted where it is possible to release bees from all directions.

In conclusion, the maximum return distance for *Heterotrigona itama* worker bees is 1000 m, but the most effective return distance is up to 500 m. This shows that the most effective distance for bees in finding food sources is less than 500 m, so it is the basis for determining the distance between a colony and plants as a source of food for bees. Meanwhile, if we move the bee colony to a new place, the distance from the original location must be further than 1100 m so that the bees do not return to their original location.

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