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DEFENSIVE RESPONSE VARIATION AMONG *Apis mellifera* LINNAEUS COLONIES

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ABSTRACT

Honey bees (*Apis mellifera*) defend their colonies and food reserves against intruders primarily through stinging behaviour. However, this defensive behaviour is often considered a limiting factor for the broader adoption of beekeeping practices. To initiate a breeding program aimed at developing less defensive honey bee strains, 100 colonies were screened for their response to external stimuli. The defensive behaviour of the colonies was assessed through a colony-level assay involving the application of 100 µl of alarm pheromone (isopentyl acetate, 98%) to suede leather wrapped around a rubber ball. This ball was then oscillated in front of the colony entrance to provoke a defensive response. Three key parameters were measured: (1) the mean number of stings per minute, (2) the time taken for the first sting to occur, and (3) the number of bees recruited for defense per minute. These parameters showed considerable variation across the colonies, with the mean number of stings per minute ranging from 1.33 to 22.67, the time to the first sting ranging from 3.67 to 23.00 seconds, and the number of bees recruited for defense varying from 3.67 to 41.67 per minute. To quantify the overall defensive response of each colony, a five-point scale was applied to each of the three parameters (with 1 indicating the most aggressive behaviour and 5 indicating the gentlest). The cumulative scores for the three parameters were summed to assess the colonies' overall defensive tendencies. Results revealed that 50% of the screened colonies received cumulative scores ranging from 10 to 12, indicating moderate defensiveness. In contrast, 34% of the colonies scored between 7 and 9, while 10% of the colonies were classified as least defensive, with scores ranging from 13 to 15. Only 6% of the colonies exhibited the highest level of defensiveness, scoring between 4 and 6. A correlation analysis indicated that the number of stings per minute was positively correlated with the number of bees recruited for defense, while it was inversely correlated with the time taken for the first sting. These findings highlight the variation in defensive behaviour across colonies and highlights the potential for selective breeding of less defensive strains. Nonetheless, further efforts are necessary to refine breeding strategies that incorporate the gentler colonies, ultimately contributing to the development of a less defensive honey bee stock for beekeeping programs.

Keywords: *Apis mellifera*, Alarm pheromone assay, Bee breeding, Defensive behaviour, Isopentyl acetate

Honey bees (*Apis mellifera*) are social insects that live in perennial colonies characterized by overlapping generations, cooperative brood care, and a high degree of polyethism. These colonies form complex societies with a wide range of behaviours designed to protect the colony from predators and diseases while ensuring its reproduction and survival (Winston, 1987). Among these behaviours, defensive responses are particularly critical, and they represent a complex trait that involves both individual worker bee actions and coordinated colony-level responses, resulting in a group effect. Colony defense primarily occurs around the entrance, though aggressive bees may pursue intruders over considerable distances. The defense of the colony is a highly social behaviour that requires a series of coordinated actions from multiple individuals, which can range from aggressive (e.g., guarding, recruiting, alerting, attracting, biting, stinging, and pursuing) to docile or gentle expressions of behaviour (Collins *et al.*, 1980; Kastberger *et al.*, 2009). The defense sequence typically unfolds in several stages. In the initial stage, bees adopt a characteristic alert posture, increase their movement, emit an alarm pheromone, and buzz their wings to alert and recruit nestmates. If a threat is perceived, the second stage is triggered, during which the bees fly or run towards the intruder. In the third stage, the bees intensify their aggression by flying around the target and making contact with it. The final stage involves the most extreme form of defense, with bees actively biting or stinging the perceived threat.

Alerted honey bees recruit nestmates for colony defense by releasing an alarm pheromone, which is produced by glands associated with the sting apparatus (Winston, 1991). The primary active component of this alarm pheromone is isoamyl acetate (also known as isopentyl acetate) (Free, 1987; Schmidt, 1998; Breed *et al.*, 2004; Hunt, 2007). Isoamyl acetate has been widely used in aggression studies as a surrogate for the entire

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alarm pheromone due to its ability to elicit a response similar to that induced by the complete pheromone blend, thereby triggering the same defensive reactions in the colony (Hunt, 2007).

Defending the honey bee colony and its resources is essential for maintaining colony integrity. While the defensive behaviour of honey bees contributes to their evolutionary and ecological success, it can pose significant challenges for human management and commercial beekeeping activities (Guzman-Novoa *et al.*, 2003). Highly defensive colonies are particularly difficult to manage, causing substantial inconvenience for beekeepers (Andere *et al.*, 2002). In contrast, selecting for less defensive honey bees would not only ease colony management but also encourage the practice of beekeeping, particularly among amateur beekeepers, by reducing the risk of stings. For beekeepers to realize economic success, it is essential to maintain colonies with a gentle temperament and low defensiveness. The benefits of selecting for traits such as reduced aggression are well recognized, and such efforts could positively impact the economic viability of beekeeping. *Apis mellifera* Linnaeus, an exotic honey bee species introduced to India in 1962, has become a key species in commercial beekeeping, yet there has been no prior research on selecting for less defensive behaviours in Indian colonies. Most studies on defensive behaviour have focused on *A. mellifera* hybrids, particularly the "scutellata-hybrid" or the formerly termed "Africanized" honey bees (Calfee *et al.*, 2020; Zarate *et al.*, 2023), with very limited information available on the globally most widely kept honey bee subspecies, *A. mellifera ligustica*. Therefore, the present study aims to fill this gap by quantitatively assessing the variation in defensive behaviour among *A. mellifera* colonies in India. This research, the first of its kind in the country, serves as a foundation for establishing a robust breeding program aimed at developing a less defensive honey bee strain. By identifying and selecting colonies with gentler temperaments, this study seeks to enhance colony management practices and promote the growth of sustainable, low-risk beekeeping in India.

MATERIALS AND METHODS

The study to assess and screen *Apis mellifera* colonies (n = 100; bee strength of 6-8 Langstroth frames) for less defensive characteristics was conducted at the Campus Apiary of the Entomological Research Farm, Department of Entomology, Punjab Agricultural University (PAU), Ludhiana, India, during August-September 2021.

Assessment of defensive characteristics

The defensive characteristics of *Apis mellifera* colonies were assessed based on the response of guard bees to an alarm pheromone, following the methodology of Boch and Shearer (1966). Three types of observations were recorded (Villa, 1988). The assay involved the use of a rubber ball covered with black suede leather, isopentyl acetate (IPA, 98%), a one-meter metallic wire, and a digital stopwatch. The rubber balls, measuring 16 cm in diameter and wrapped with black suede leather (22 cm in length and 20 cm in breadth), were marked with 100 µL of IPA and attached to one end of a metallic wire. The ball was then swung in front of the selected colony entrance (Figure 1). The oscillation was performed manually, maintaining a distance of approximately 10 cm from the hive entrance, and the ball was swung rhythmically for exactly 60 seconds, beginning once the first sting was observed (Breed, 1991). The test was conducted in the afternoon, between 1500 and 1700 hours, to coincide with the period of heightened defensive behaviour in worker bees and to minimize any confounding effects related to foraging activity. The assay was repeated three times for each colony at weekly intervals. To avoid contamination from residual alarm pheromones, a new ball covered with fresh suede leather was used for each trial on each colony. After each test, the ball was placed in a plastic bag to collect and count the stings in the laboratory. The stings were carefully removed from the ball using forceps, and the ball was then left in the sun for two hours to eliminate any remaining traces of the alarm pheromone from the leather.

Fig. 1. Ball wrapped in black leather marked with IPA to assess defensive behaviour

Observations recorded

Number of stings: The total number of stingers left on the ball by the experimenter during the 60-second test period, following the first sting, was counted.

Time taken for the first bee to sting: The time taken from the moment the stimulation was provided (i.e., when the oscillations of the treated ball began) until the first sting was recorded.

Number of bees recruited for defense: The number of bees attacking the ball, including both stingers and non-stingers, during the 60-second period following the first sting, was recorded.

Scoring of colonies

The mean values of the three defensive behaviour parameters, recorded across the three repeated experiments, were used to score all 100 colonies on a five-point scale (Table 1). Colonies were assigned scores ranging from 1 to 5 based on the recorded data for each of the defensive behaviour parameters. A score of 1 represented the most aggressive colony, while a score of 5 indicated the gentlest colony. The scores for each parameter of a particular colony were then summed to obtain a cumulative score for each of the 100 colonies. This cumulative score was used to categorize all the colonies screened in the program.

The arithmetic average values from the three repetitions for each of the three parameters were subjected to Pearson correlation analysis to determine the relationships among them. The significance of the correlation coefficients was assessed at both the 5% and 1% levels of significance, based on the corresponding *p*-values.

RESULTS AND DISCUSSION

Number of stings

The mean number of stings per minute recorded across the 100 tested *A. mellifera* colonies ranged from 1.33 to 22.67. Based on these values, the colonies were categorized as follows: 23 colonies had a sting rate between 0 and 4.99 stings/min (score 5), 49 colonies fell within the range of 5.0 to 9.99 stings/min (score 4), 18 colonies had a sting rate between 10 and 14.99 stings/min (score 3), 6 colonies ranged from 15 to 19.99 stings/min (score 2), and 4 colonies had a sting rate greater than 20 stings/min (score 1) (Table 2).

Time taken to first sting

The data presented in Table 3 revealed that the mean time taken for the first sting ranged from 3.00 to 23.00 seconds. Based on this, the 100 screened colonies were grouped as follows: the majority (54%) of colonies received a score of 2, indicating a moderate response time, followed by 26% of colonies with a score of 3, and 10% with a score of 4. Eight percent of colonies were categorized with the lowest score of 1, representing the quickest to sting, while 2% of colonies were the least defensive, receiving a score of 5.

Total number of bees recruited for defense

An analysis of the data presented in Table 4 revealed that the mean number of bees recruited for defense among the test colonies ranged from 3.67 to 41.67 per minute. The majority of colonies (50%) received a score of 4, followed by 37% of colonies that scored 3. Five colonies were assigned a score of 2, while 6% of colonies scored 5, indicating a lower level of defensive

Table 1. Categories for scoring *Apis mellifera* colonies on the basis of various defensive behaviour assessing parameters

Score	Mean number of stings/ min	Mean time taken to first sting(s)	Mean number of bees recruited for defense/ min
5	< 5.0	≥ 20.0	< 10.0
4	5.0-9.9	15.0-19.9	10.0-19.9
3	10.0-14.9	10.0-14.9	20.0-29.9
2	15.0-19.9	5.0-9.9	30.0-39.9
1	> 20	< 5.0	>40.0

Table 2. Grouping of the *Apis mellifera* colonies (n=100) based on the number of stings per min

Category	Score	Mean no. of stings/min	No. of colonies
< 5.0	5	1.23-4.67	23
5.0-9.9	4	5.67-9.67	49
10.0-14.9	3	10.00-13.67	18
15.0-19.9	2	15.55-19.67	6
≥ 20	1	20.67-22.67	4

Table 3. Grouping of the *Apis mellifera* colonies (n=100) based on the stinging response initiation

Category	Score	Mean time taken to first sting(s)	No. of colonies
≥ 20.0	5	21.67-23.00	2
15.0-19.9	4	15.33-19.00	10
10.0-14.9	3	10.67-14.00	26
5.0-9.9	2	5-9.67	54
< 5.0	1	3.00-4.00	8

Table 4. Grouping of the *Apis mellifera* colonies (n=100) based on the number of bees recruited for defense

Category	Score	Mean number of bees recruited for defense per min	No. or percent of colonies
< 10.0	5	3.67-8.67	6
10.0-19.9	4	10.33-19.67	50
20.0-29.9	3	20.00-29.00	37
30.0-39.9	2	35.33-37.33	5
≥ 40.0	1	41.67	2

recruitment. Only two colonies were classified in the most defensive category, with a score of 1.

Cumulative score for defensive behaviour

Based on the cumulative scores, the genetic pool of the 100 colonies was categorized into four groups: 4-6, 7-9, 10-12, and 13-15. Ten percent of the colonies fell into the cumulative score range of 13-15, 50% into the 10-12 range, 34% into the 7-9 range, and 6% into the 4-6 range (Table 5). Correlation analysis (Figure 2) revealed a statistically significant positive correlation between the number of stings and the number of bees recruited for defense. Additionally, there was a statistically significant negative correlation between the number of stings and the time taken for the first sting, as well as between the number of bees recruited for defense and the time to first sting.

Table 5. Grouping of the *Apis mellifera* colonies (n=100) based on cumulative score for the three defensive behaviour assessing parameters

Category	Percent of colonies
13-15	10
10-12	50
7-9	34
4-6	6

In the present study, an average of 8.45 stings per minute was recorded, which is comparatively lower than the findings of Stort (1974), who recorded a mean of 26.4 stings per minute on a leather ball during the first 60 seconds in Italian honey bees. In contrast, the most defensive Africanized bees exhibited an average of 61.2 stings. Aleme *et al.* (2017) assessed the defensive behaviour of *Apis mellifera* Weyi Gambella honey bee race in Ethiopia and found that the race was aggressive, with sting counts ranging from 19.54 to 80 on the black ball. Stort (1975b) also observed an average of 61.15 stings for African bees and 48.13 stings for Africanized bees. Similarly, Collins *et al.* (1982) reported that Africanized honey bees exhibited a faster and more intense defensive response compared to European honey bees, producing 8.2 and 5.9 times the number of stings in two separate studies. Faita *et al.* (2014) observed that the average number of stings on the black leather ball (2 cm) varied from 13.2 ± 0.83 to 28.4 ± 2.50 in Africanized honey bees. In terms of the time taken to deliver the first sting, African honey bee colonies averaged 3.15 seconds, while Africanized colonies took 12.86 seconds (Stort, 1975a). Faita *et al.* (2014) reported a time range of 1.4 ± 0.54 s to 6.0 ± 3.60 s for the first sting in Africanized honey bees. Gbate (2014) observed that *Apis mellifera* adansonii in Nigeria delivered the first sting in just 2.65 seconds. In the present study, based on cumulative scores from three defensive behaviour parameters, 50% of the colonies were classified with scores between 10 and 12, while 10% of the colonies were in the least defensive category with a score between 13 and 15. Our findings showed that more aggressive colonies took less time to sting, recruited more bees, and left more stings on the leather ball, whereas gentler colonies exhibited the opposite behaviour, taking more time to sting and recruiting fewer bees. This study represents the first research on honey bee defensive behaviour conducted in India. Of the 100 colonies screened, 10 were categorized as least defensive. These colonies will now be subjected to productivity assessments, and those demonstrating

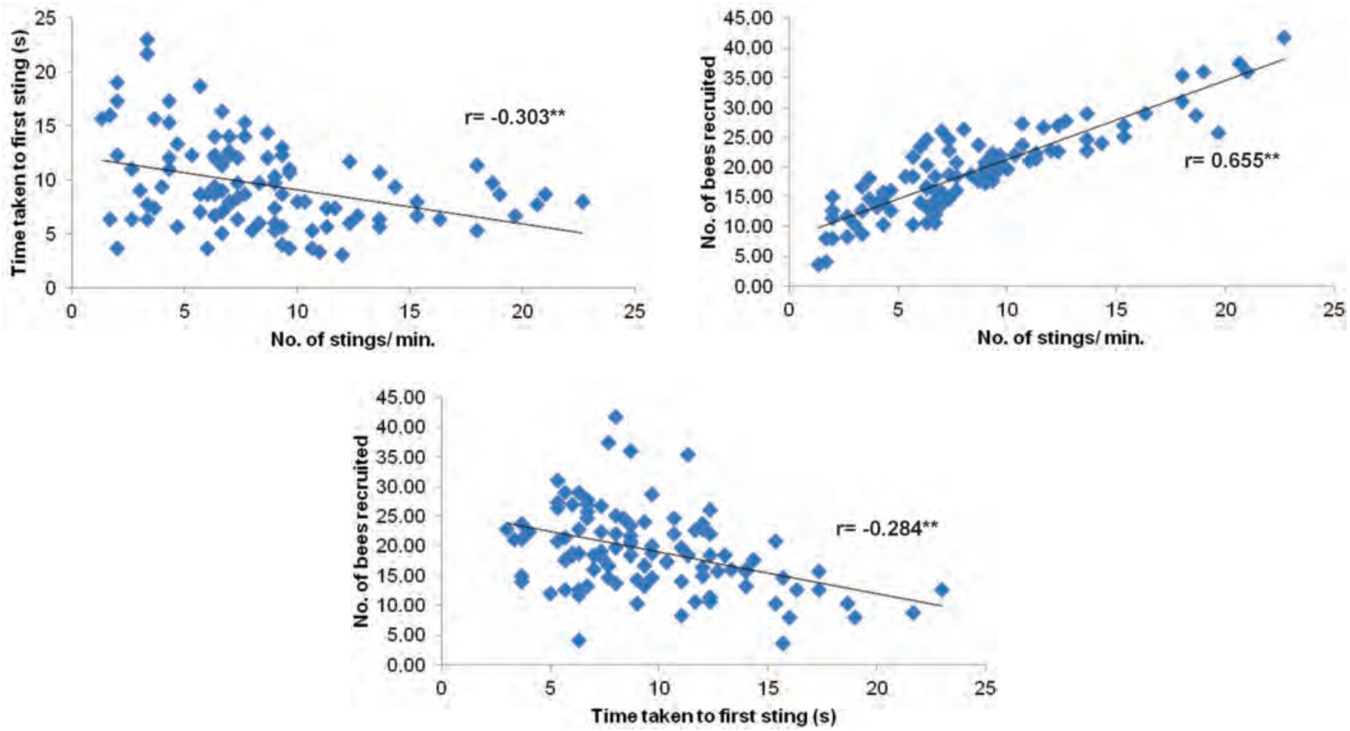

Fig. 2. Correlation between various defensive behaviour parameters
**significant at 1% level of significance

higher productivity will be selected as breeder colonies for inclusion in a breeding program aimed at developing less defensive honey bee strains.

CONCLUSION

This study represents the first comprehensive assessment of the defensive behaviour of *Apis mellifera* colonies in India, focusing on traits such as the number of stings, the time to first sting, and the recruitment of bees for defense. The findings revealed considerable variation in defensive behaviours among the 100 colonies screened, with significant differences observed in terms of sting frequency, response time, and the number of bees engaged in defense. The data indicated that more aggressive colonies tended to sting more quickly, recruit more bees, and inflict a higher number of stings, while gentler colonies exhibited the opposite pattern, taking longer to sting and recruiting fewer bees. By categorizing the colonies into four distinct groups based on cumulative scores from the defensive parameters, this study provides valuable insights into the temperament of *A. mellifera* colonies in India. The 10% of colonies identified as the least defensive will form the basis for future selection in a breeding program aimed at developing honey bee strains with reduced defensive behaviour. Such colonies will be evaluated further for their productivity traits, with the goal of identifying high-yield, less defensive colonies that are better suited for sustainable beekeeping practices.

The results of this study offer a crucial first step towards improving honey bee management in India, where the gentle temperament of bees can significantly enhance both commercial and amateur beekeeping. Moreover, these findings contribute to the broader understanding of honey bee behaviour, providing a foundation for future research and breeding programs aimed at enhancing the economic and practical viability of beekeeping globally.

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Authors' contribution

Conceptualization and designing of the research work (BM, JS and PKC); Execution of field experiments and data collection (AM, BM); Analysis of data and interpretation, Preparation of manuscript (AM, BM, AC, JS, PKC).

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