



# Transmission of Less Stinging Trait in *Apis mellifera* Linnaeus Colonies through Selective Breeding

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**Abstract:** Studies on selective breeding for development of less stinging *Apis mellifera* colonies were carried out at Campus Apiary, Department of Entomology, Punjab Agricultural University, and at isolated mating yard at PAU Seed Farm, Ladhawal during 2021-2022. Ten least stinging colonies were selected as parent colonies from the stock of 100 colonies screened for defensive behaviour. Five daughter queens were reared from each mother queen colony. The drones were multiplied from only two of the 10 selected least stinging colonies. Selective mating between the newly reared gynes and drones was carried out at the isolated mating yard. The defensive behaviour of the colonies was assessed by alarm pheromone assay. The comparative assessment of the defensive behaviour between selected mother colony, F<sub>1</sub> colonies and control (unselected colonies) revealed that both mother and F<sub>1</sub> colonies had lesser number of stings/min, took more time to first sting and had lesser number of bees recruited for defense thus depicting defensive behaviour to be heritable. Among the 50 F<sub>1</sub> colonies, 62 per cent colonies showed improvement in this trait compared to just 10 per cent in the original stock (100 colonies). Correlation among the three defensive traits revealed that the number of stings/min was positively correlated with the number of bees recruited for defense and inversely with the time taken for first sting.

**Keywords:** Alarm pheromone assay, *Apis mellifera*, Defensive behaviour, Isolated mating, Selective breeding

Honey bees are important from economic, nutritional, pharmaceutical, and commercial point of view as they provide various valuable hive products like honey, wax, royal jelly, bee venom, pollen, propolis, etc. Thus, contribute in the pollination of fruits, vegetables, and field crop plants, thereby increasing the crop productivity and improving the quality of produce. Punjab is one of the leading states in beekeeping, it contributes 14.1 per cent to the total honey production in India (Anonymous 2024). In beekeeping, honey bee behaviour such as honey production and hygienic behaviour are commonly used selection criterion for commercial breeding programmes. Honey bees defend their nests mostly around the entrance, although aggressive bees may follow intruders for considerable distances away from the colony. The two tasks of defensive behaviour include guarding the nest entrance and flying out of the nest, as well as stinging. The defense of a colony is the consequence of both guarding and responding behaviour exhibited by the worker bees (Breed et al 2004, Stabentheiner et al 2007). Aggression is a sequence of behaviours started by the colony's first line of defense, the guard bees, in response to a probable risk posed to the hive (Arechavaleta-Velasco and Hunt 2003, Breed et al 2004, Hunt 2007). Defensive behaviour of honey bees which has been described as a highly heritable trait, with genetic dominance and paternal effect can also be used for breeding less defensive honey bees (Breed et al 2004). Selective bee breeding is a tool for improving traits of apicultural interest that have an important

impact on the beekeeping industry (Buchler et al 2013, Uzunov et al 2017). The benefits of selection are well recognized and can strongly affect the economic success of beekeeping. Honey bee colonies with less stinging behaviour would make the management of the colonies easier and would also promote beekeeping among the amateur beekeepers without the fear of being stung. Overall, in order to realize profits from beekeeping, a beekeeper needs the honey bee colonies with gentle temperament in terms of defensiveness. The benefits of selection for less defensive behaviour are well recognized and can positively impact the economic success of beekeeping. Thus, the present studies were conducted to develop less sting *Apis mellifera* colonies through selective breeding.

## MATERIAL AND METHODS

The studies were carried out at Campus Apiary, Department of Entomology, Punjab Agricultural University, and at isolated mating yard at PAU Seed Farm, Ladhawal during 2021-2022. Ten least stinging colonies were selected as parent colonies from the stock of 100 colonies screened for defensive behaviour (Madaan et al 2024). Daughter queens were reared from each of the ten selected least stinging colonies by using PAU modified doolittle grafting method. The drones were multiplied from two of the 10 selected least stinging colonies. Selective mating between the newly reared gynes and drones was carried out at the isolated mating yard at PAU Seed Farm, Ladhawal. The

defensive behaviour of the 10 parent *A. mellifera* colonies, 50 F<sub>1</sub> daughter colonies and the control colonies was assessed by alarm pheromone (Madaan et al 2024). The observations recorded were time taken for the first sting in seconds (score of 1 for < 5 sec, 2 for 5.00-9.99 sec, 3 for 10.00-14.99 sec, 4 for 15.00-19.99 sec, 5 for 20 sec), number of stings received per minute (score of 5 for < 5 stings, 4 for 5.00-9.99 stings, 3 for 10.00-14.99 stings, 2 for 15.00-19.99 stings, 1 for 20 stings per min) and number of bees recruited for defense per minute (5 for < 10 bees, 4 for 10.00-19.99 bees, 3 for 20.00-29.99 bees, 2 for 30.00-39.99 bees, 1 for > 40 bees), the scores were given on a five-point scale of 1 to 5 wherein score of 1 represented the most aggressive and 5 represented the gentlest colony. Data were statistically analysed in completely randomized design. Pearson correlation analysis was used to test the significance of relationship among various parameters and p value was used to test the significance of correlation coefficients

## RESULTS AND DISCUSSION

**Relative number of stings:** The least number of stings was 1.33 in M7D1 (Daughter colony No. 1 from M7) followed by 1.66 stings in M3D1 (Daughter colony No. 1 from M3) and M5D1 (Daughter colony No. 1 from M5) and revealed that the mother colonies maintained their position in score 5 as obtained in the screening experiment (Table 1). The performance of the F<sub>1</sub> daughter colonies was statistically on par with the mother colonies which had the score varied from 4-5. It was also observed that mother colonies and their daughter colonies had less number of stings as compared to the control colonies (10.80 stings), which showed that the trait is transmitted in daughter colonies.

**Relative time taken to first sting:** Variation among *A. mellifera* selected mother and their 50 F<sub>1</sub> daughter colonies in initiation of stinging response revealed the M10D4 (Daughter colony No. 4 from M10) took a maximum time of 23.00 s to initiate stinging response followed by 22.33 s in M2D1 (Daughter colony No. 1 from M2) and M2D2 (Daughter colony No. 2 from M2) (Table 1). The mother colonies registered a score of 4 with respect to time taken to first sting. Performance of the F<sub>1</sub> daughter queens' performance was better or on par as compared to the mother colonies, registering a score of either 4 or 5, respectively.

**Relative number of bees recruited for defense:** The least number of bees recruited for defense was 5.33 bees observed in M6D4 (Daughter colony No. 4 from M6) followed by 6.00 bees in M10D5 (Daughter colony No. 5 from M10). Mother colonies lie in the score of 4 while F<sub>1</sub> daughter colonies in the score of 4 or 5. Thus daughter colonies performed better than or on par to the mother colonies. The

mother and F<sub>1</sub> daughter queen colonies recorded less number of bees recruited for defense when compared with control colonies (20.73 bees). On the basis of cumulative score, six F<sub>1</sub> daughter colonies lie in score 15, eight colonies in score 14, 27 in score 13, eight colonies in score 12 and eleven colonies in score 11 (Fig. 1).

### Improvement in less stinging tendency in F<sub>1</sub> colonies:

The selective breeding for less stinging honey bee colonies resulted in overall reduction in aggressiveness of colonies reared from the selected mother colonies as 62 per cent of the F<sub>1</sub> daughter colonies lie in the highest score category (13-15) as compared to only 10 per cent in the initial stock of colonies (Fig. 2). The highest cumulative score (all the three parameters) of 15 was achieved by 12 per cent F<sub>1</sub> colonies compared to only one colony from original stock. In original stock 62 per cent of colonies were in high stinging range (score 4-10) whereas selective breeding resulted in not even a single colony falling in this score range. Correlation among the three defensive traits revealed that the number of stings/min was positively correlated with the number of bees recruited for defense and inversely with the time taken for first sting (Table 2) indicated that a colony that inflicts more number of stings on the leather ball will have more number of bees recruited for defense and these will take less time to initiate defense.

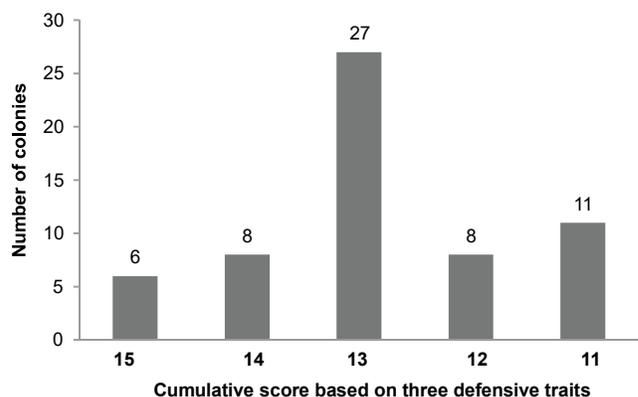


Fig. 1. Grouping of mother and F<sub>1</sub> colonies based on the cumulative score of three defensive traits

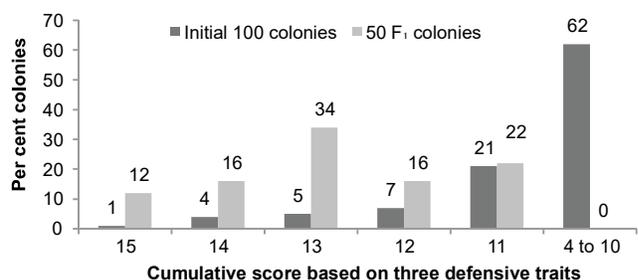


Fig. 2. Comparative cumulative score based on three defensive traits in F<sub>1</sub> and initial stock of *A. mellifera* colonies

**Table 1.** Relative defensive behaviour of *A. mellifera* mother and F<sub>1</sub> colonies

Colony No.	Mean no. of stings per minute	Score	Time taken for first sting (s)	Score	No. of bees recruited for defense	Score
M2D1	2.00	5	22.33	5	9.67	5
M2D2	2.00	5	22.33	5	7.67	5
M3D5	2.33	5	20.33	5	9.67	5
M4D2	2.67	5	21.33	5	9.67	5
M8D5	2.00	5	21.00	5	8.00	5
M10D5	2.00	5	20.33	5	6.00	5
M3D1	1.67	5	17.67	4	6.67	5
M4D4	4.33	5	20.67	5	11.33	4
M6D4	2.33	5	16.00	4	5.33	5
M7D1	1.33	5	15.67	4	6.67	5
M8D2	2.67	5	21.67	5	11.33	4
M9D2	3.00	5	21.33	5	11.67	4
M10D4	3.33	5	23.00	5	14.00	4
M3D3	3.33	5	15.00	4	8.67	5
M1D2	4.00	5	13.00	3	7.33	5
M1D4	5.33	4	22.00	5	10.00	4
M4D5	3.33	5	14.00	3	9.33	5
M5D1	1.67	5	14.67	3	7.67	5
M5D4	2.67	5	18.67	4	11.00	4
M7	4.67	5	16.67	4	14.33	4
M7D5	2.67	5	13.33	3	9.33	5
M8D4	3.33	5	16.00	4	13.00	4
M1	4.33	5	15.66	4	12.33	4
M2	2.67	5	16.66	4	12.67	4
M3	4.67	5	19.00	4	15.00	4
M5	4.33	5	15.00	4	15.33	4
M8	4.67	5	15.33	4	15.67	4
M9	4.33	5	15.33	4	14.33	4
M6	4.33	5	17.66	4	15.33	4
M10	3.33	5	17.00	4	12.33	4
M4	4.66	5	19.33	4	13.00	4
M1D1	5.33	4	16.00	4	9.33	5
M1D5	3.67	5	15.66	4	15.66	4
M2D3	3.67	5	15.66	4	14.67	4
M2D4	2.33	5	18.00	4	12.33	4
M2D5	4.33	5	15.00	4	13.00	4
M6D1	2.66	5	17.66	4	12.67	4
M6D3	3.33	5	16.33	4	15.33	4
M6D5	4.33	5	18.00	4	14.67	4
M7D2	4.67	5	16.67	4	16.66	4
M9D1	3.67	5	16.00	4	11.67	4
M7D3	3.00	5	13.66	3	11.00	4
M8D1	4.33	5	13.00	3	14.33	4

Cont...

**Table 1.** Relative defensive behaviour of *A. mellifera* mother and F<sub>1</sub> colonies

Colony No.	Mean no. of stings per minute	Score	Time taken for first sting (s)	Score	No. of bees recruited for defense	Score
M8D3	4.00	5	13.33	3	14.67	4
M9D4	4.67	5	14.33	3	15.67	4
M9D5	3.33	5	13.00	3	14.00	4
M10D1	3.00	5	13.33	3	10.33	4
M6D2	5.00	4	15.33	4	15.33	4
M10D3	3.66	4	15.33	4	16.00	4
M1D3	5.67	4	14.33	3	10.00	4
M3D2	5.00	4	14.33	3	14.33	4
M3D4	5.33	4	14.00	3	15.00	4
M4D1	4.66	4	14.33	3	16.67	4
M4D3	5.33	4	14.00	3	15.00	4
M5D2	5.00	4	14.66	3	16.33	4
M5D3	7.67	4	14.66	3	16.00	4
M5D5	5.33	4	13.66	3	14.33	4
M7D4	6.33	4	13.66	3	15.66	4
M9D3	5.33	4	13.66	3	13.00	4
M10D2	5.00	4	14.00	3	12.67	4
Control	10.80	3	10.26	3	20.73	3

M1 to 10 Mother colony; D1 to D5 Daughter colony No. 1-5 from respective mother colony Scoring

Mean no. of stings/min: 5 for < 5 stings, 4 for 5.00-9.99 stings, 3 for 10.00-14.99 stings, 2 for 15.00-19.99 stings, 1 for 20 stings/min

Time to first sting (s): 1 for < 5 sec, 2 for 5.00-9.99 sec, 3 for 10.00-14.99 sec, 4 for 15.00-19.99 sec, 5 for 20 sec to first sting

No. of bees recruited: 5 for < 10 bees, 4 for 10.00-19.99 bees, 3 for 20.00-29.99 bees, 2 for 30.00-39.99

**Table 2.** Correlation coefficients among the number of stings, time taken to first sting and number of bees recruited for defense in F<sub>1</sub> colonies

Trait	No. of stings/min	Time taken (s)	Recruitment of bees (No.)
No. of stings/min	-	-0.402**	0.643**
Time taken to first sting (s)	-0.402**	-	-0.364**
Recruitment of bees (No.)	0.643**	-0.364**	-

n = 150; p (0.01): 0.211; \*\* r significant at 1 % level of significance

## CONCLUSIONS

The successful transmission of less stinging trait in F<sub>1</sub> and thereby significant reduction in stinging tendency of bees has been achieved. The information generated from the study on the less stinging behaviour will be helpful in further bee breeding work towards developing less stinging *A. mellifera* strain.

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