Pin Prick Method: An easy, viable and cost effective assay for screening hygienic stock of *Apis mellifera* Linnaeus

Bharathi Mohindru, Amit Chaudhary, Pardeep K. Chhuneja and Jaspal Singh

Beekeeping in India is an age-old practice; however, with the introduction of *Apis mellifera* Linnaeus, it has attained a status of commercial and industrial activity. Besides providing multitude of useful hive products, also contribute immensely vital ecological service i.e. pollination. Successful beekeeping and attaining high productivity depends upon the various bee husbandry practices. Among these, maintaining the health of a honey bee colony is a major aspect. Honey bees are highly social organism, headed by a queen bee whose prime function is to lay eggs. Hence, the qualities, especially, the behavioural traits like temperament, colony build-up, industriousness, resistance to pests and pathogens, tendency to swarm and abscond, etc. are passed onto progeny from the queen bee. Thus, the qualities inherited by a queen determine the performance of its colony.

Like other organisms, honey bees are also exposed to various pests and pathogens maladies. These cause the decline in colony growth performance and ultimately may lead to colony losses. One such example was the outbreak of the ectoparasitic mite, *Varroa destructor* Anderson and Trueman in North India in 2004-2006, which took a heavy toll on honey bee colonies.

To cope up pests and diseases, the beekeepers resorted to use of various chemical treatments, which though effective, are also problematic in the long term. Firstly, these chemicals offer only a short-term solution, and development of resistance in pests and diseases against chemicals over a period of use. Furthermore, pesticides can contaminate hive products (honey, bees wax), and some of them may potentially have side effects on the bees. Hence, one should look for an alternative safe and sustainable method to manage these biotic stresses. Breeding of a stock for traits such as natural disease resistance would be an ideal option. However, at present, beekeepers generally select the queens predominantly by their egg laying ability and body size and give only a meager weightage to other important behavioural traits. One such trait i.e. hygienic behaviour should also be utilized, which can benefit the beekeepers in terms of saving the money in managing the biological stresses and thereby help in maintaining stronger honey bee colonies, and ensuring higher quantity and quality of hive products. Unfortunately, this is not a common practice among bee breeders in their selection criteria.

Hygienic behaviour

It can be defined as the ability of bees to detect and remove diseased or parasitized or dead brood from the colony. It consists of two processes: uncapping and removal. Uncapping is the process in which the wax caps covering the brood cells are removed by some worker honey bees upon detecting dead or diseased larvae or pupae inside the brood cells. This is followed by the removal of the dead brood. Hygienic behaviour differs from the grooming behaviour which is the removal of foreign objects and parasites from oneself (auto-grooming) or removal of foreign objects and parasites from another worker honey bee in the nest (allo-grooming).

Significance of hygienic behaviour

Hygienic honey bees have the ability to detect, uncap, and remove diseased brood from their nest before the causative organism reaches the infectious stage. Because honey bees reuse brood cells, diseased brood must eventually be removed from the nest. As the bees eliminate the focal infection from the colony, hygienic behaviour has emerged as a general system of resistance to brood diseases. The colonies that express hygienic behaviour are economically important to beekeepers as this behaviour limits the multiplication and spread of infection within the colony.

Usefulness against *Varroa* mite infestation

Two important mite-resistant traits *viz*. hygienic and grooming behaviour are observed in honey bees. Hygienic behaviour can be used as one of the defense mechanism against *Varroa* mite as it has the potential to limit the mite multiplication. The bees remove the infested pupa from the cell and thus immature mites are left without food and die due to either shortage of food or ambient development conditions which were otherwise present in sealed cells. At times, the mite or its offspring are also removed along with the brood. Thus, the reduction in the average number of offspring per reproducing mite can be attained. Colonies with high levels of hygienic behaviour have a lower build-up of *Varroa* population. The grooming behaviour by the worker bees too leads to the mortality of the adult mites.

Assessment of hygienic behavior

In beekeeping, the traits of economic importance are the result of the behaviour of the whole colony. Therefore, the hygienic behaviour is assessed at the colony level. It is performed by middle aged in-house worker bees of 15 to 20 days old i.e. older than the typical nurse bees but younger than the typical foragers. Among various available assays for measuring this behavioural trait, the pin-killed brood method (Newton and Ostasiewski 1986) is simple, easy, not labour intensive and cost effective.

How to perform pin prick method?

First of all, select the sealed worker brood at pink eye pupal phase. Take out the sealed brood comb and pierce the sealed brood by using a number 1 size entomological pin (Gramacho and Spivak 2003) and mark the pricked area by coloured pins (Fig. 1). Care must be taken that the brood must be pierced by inserting the pin upto the midrib of a comb, thus deliberately killing the brood.

Fig. 1. Mark comb area having 100 sealed brood cells and pin prick the brood cells

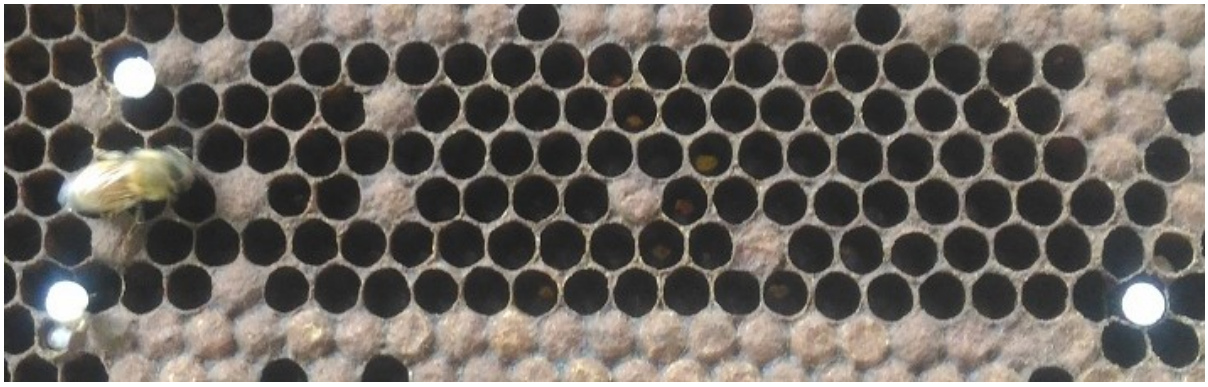

Fig. 2. After 24 h, record the number of cells uncapped and cleared

After pricking, put the comb back into original colony.

Recording the hygienic behavior

Record the percentage of brood removal (opening the cell and removal of pin pricked pupa from it) from each colony after 24 h (Fig. 2). The test in each colony will be performed thrice at 24 h interval to account for the variability in the existing intra-colony sub-families w.r.t. their hygienic behaviour.

Research work conducted at Dr A. S. Atwal Apicultural Laboratories, Punjab Agricultural University, Ludhiana revealed that 45 per cent of the colonies were hygienic (removal of dead brood > 80 per cent) 42 per cent were intermediary (removal of dead brood between 70 and 80 per cent) while 13 were non-hygienic (removal of dead brood < 70 per cent). The hygienic colonies also showed high percentage of removal of brood inoculated with *Varroa* mite (94.33%) as compared to the non-hygienic colonies (69.57%). Hence, the colonies showing mean scores i.e. removal of 80 per cent or more must be selected and marked as hygienic colonies.

Conclusion

Hygienic behavior in honey bees is a heritable trait. Thus, beekeepers can potentially concentrate this in apiary by screening colonies for hygienic behaviour. These colonies can be taken as parent stock for breeding queen bees that express high levels of hygienic behaviour. This can be obtained by performing stock selection ever for high hygienic behaviour in the successive generations. Queens from colonies exhibiting hygienic behaviour can be used as breeder colonies for the next

generation of bees. New colonies are made from these daughter queens and again these colonies are screened for hygienic behaviour. Since the hygienic behaviour is a heritable trait, these daughter queens can further be used as breeder queens for the production of next generation queens. Consequently, by performing stock selection every year for this trait, hygienic behaviour can be fixed in the colonies over the time period. Hence, selection for hygienic behaviour should be a routine component of bee breeding.

Overall, hygienic behaviour of honey bees would benefit the beekeepers with no apparent negative characteristics that accompany the trait. Therefore, selection for hygienic behaviour of honey bees to control the brood diseases and devastating mite pests can be an effective alternative to use of antibiotics and pesticides in bee colonies to maintain the colony health and to check contamination of hive products.

References

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AUTHORS

Bharathi Mohindru (Corresponding author)**, Amit Chaudhary, Pardeep K Chhuneja and Jaspal Singh,** Department of Entomology, Punjab Agricultural University, Ludhiana 141004, India email: deekshamudagadde@gmail.com