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Evaluation of hygienic bees behavior and quality queens production through Doolittle method in honey bee [*Apis mellifera* (L.)]

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Abstract
This experiment was carried out in Apiary, Modern Bees Garden Research and Training Centre, Department of Entomology, G.B.P.U A&T, Pantnagar, Uttarakhand during August 2013 to May 2014, to determine hygienic bees behavior to evaluate the good cleaning ability or hygienic cleanness behavior of the honey bees colonies and production of quality queens in queen less colonies through Doolittle method to establish healthy queen for stock improvement as well as study of biological steps in queen rearing. This study was performed on ten colonies which were selected randomly in the apiary. The results had shown a variation in the removal of dead brood from the colonies. Higher mean percentage of cleaning of comb cells were observed 9***8***% in C_9 followed by 9***1***% in C_2 and C_3 while minimum in C_{10} (8***7.8***8%) colonies. Average numbers of larval grafts were 20 out of which 10 accepted, whereas sealed cells were 8, 6 queens emerged and 4 were established. The average success rate in cell acceptance was 52%, sealed cells 75%, queen emergence 72% and mated queen 74%. The percentage success rate of established queen was recorded highest 83% in C_2 followed by 80% in C_9 and the lowest 66% in C_{10} colonies.

Keywords: *Apis mellifera*, hygienic behavior, queen production, Doolittle method

Introduction
A newly honey bee queens can be produce without human intervention as long as fertilized eggs are present in the colony. Beekeepers have different techniques to produce large numbers of quality queen honey bees to re-queen colonies regularly (every year or two), for the purpose of reduce swarming, to increase brood and honey production, to start new colonies, and to change certain genetic characteristics (Laidlaw and Page, 1997; Ruttner, 1983) [1, 2]. A queen honey bee possesses colony's heritable genetic traits. These genetic traits may influence in many aspects of colony behaviors, *viz.* their defensiveness, parasite tolerance and disease resistance, rate of population growth, and the efficiency of winter food consumption. The importance of a quality queen bee cannot be ignored by beekeepers. Artificially rearing of queen compels the researcher and breeders to select suitable stock and to explore honey bees behavior and genetics. It will enable to select the specific queens with desired characters such as high honey production, high brood viability, early spring build up, good temperament, clearing behavior, incidence of diseases, swarming and colour. There are many artificial methods for the production of queens in the colonies. Among all these methods, grafting is the simple process of transferring larva from the worker cell of breeder hive to an artificial queen cell (Jonestone, 2008) [7]. Queen rearing is one of the major objects of apiaries especially for the commercial beekeepers, and it is the main factor for successful in beekeeping (Morse, 1994). Rearing of honey bee queen occurs when the colony is in the process of swarming, supercedure or when the queen has been accidentally lost or killed (Seeley, 1985) [20]. Although the rearing of queen bees can be performed in the presence of the queen in a nurse colony however a higher effectiveness can be achieved in queenless colonies (Morse, 1994; Crailsheim *et al.*, 2013) [8, 22] and in the absence of emergency queen cells (Free,1987) [23]. Gilbert Doolittle (1889) [21] in the USA developed a comprehensive system for rearing queen bees which serves as the basis of current queen production.
Hygienic behavior play a very important role for population dynamics of bees because it can avoid/ hinder the development of brood disease, dead larvae, being considered the primary defense of honey bee against different disease like American foul brood, European foul brood,

sac brood etc. According to Rothenbuhler (1964) [19] hygienic behavior is genetically controlled by two pair of recessive genes (u= uncapping & r=remover). It is known that the hygienic behavior is governed by genes highly influences by the climatic conditions such as humidity, temperature, as well as colony conditions (Message, 1979; Gramacho *et al.*, 1999) [18, 16]. Hygienic behavior is considered as a natural antiseptic defence behavior against the brood diseases, *viz.* American foulbrood and chalk brood, and against varroa (Boecking and Spivak, 1999; Evans and Spivak, 2010; Spivak and Reuter, 2001; Wilson-Rich *et al.*, 2009) [11-14] and thus may be relevant in breeding programmes for resistance to these pathogens and parasite.

In the light of above facts regarding the importance of queen in colony management, the specific criteria of quality queen production has been determined in present investigation to enlighten the bee keepers to improve their breeder stock as well as working force. The improved stock will ensure the minimal loss/disease in the colonies and maximum production of honey and other products.

Materials and Method

The present investigation was carried out in the Apiary, Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnagar (Udham Singh Nagar) Uttarakhand, India during August 2013 to May 2014. For this experiment the test insect was [*Apis mellifera* (L.)] and numbers of colonies were selected ten. Following materials were required: Royal jelly, Honey, Queen rearing frame, Grafting needle, Frame containing <24 hours old larvae, Distilled water, 00 point brush, Queen cage, Melted bee wax, Petri plate, Needle and Forceps.

Methodology

1. Hygienic behavior of *Apis mellifera*

To observe the hygienic cleanness behavior "pin killed/ pricking methods" was used with slight modification in the method developed by Newton and Ostasiewski (1986) [17] and further modified by Gramacho *et al.* (1999) [16]. Three frames containing capped brood cells were taken out from every colony. In these combs, the area contained six brood cells around the central killed by pin. The pin killed cells were marked for the purpose of identification and observations were recorded after 24 hours at 2 hours interval and continue up to 28 hours.

2. Production of quality queen:

The Doolittle method of larval grafting was adopted for queen rearing devised by G. M. Doolilltle (1915) [24].

2.1. Preparing cell builder colonies (starter hive)

The cell builder colony (starter hive) was prepared four days prior to larval grafting by dequeening a strong colony. The starter hive was crowded with mostly young, healthy and well fed-workers. Eight to twelve (8-12) days old nurse bees produced the royal jelly for the queen grafts. They were usually found on combs of open brood. In case of a colony with super box (double hive) the queen was found and confined to the brood chamber with a queen excluder. The two frames of honey, one frame of pollen and one unsealed larvae 3-5 days old age were placed in centre of the super above the excluder. A space was left between two frames to make room for the frame holding the bar of grafted queen cells.

2.1.1. Grafting method of larvae and checking of grafting

The 0-24 hour's old worker larvae were transferred into plastic cell cups by grafting tools, as carved duck or goose feather or 00 point brush or grafting needle. Before transferring the young larvae into the queen cell cups, the cell cups with few drops of honey was supplied to a colony for an overnight for the bees to work on the cells in order to make them more acceptable. All the selected larvae of about 24h old or less age was removed by placing the nib of the grafting tool under the middle of the larvae and scoop up some royal jelly with the larvae. Then the tip of the nib was in the centre of the bottom of the Queen Cell cup and nib pulled back away from larvae. To float them off on the royal jelly diluted with equal amount of distilled water (1:1). After doing this the frame with grafted larvae was placed to the cell builder colony. The next morning (around 24-30 hours after grafting), number of accepted ells were checked. Accepted cells had larvae in a pool of royal jelly and wax secretion on the rim of the cell cap was observed. Grafting the larvae from the worker comb to the queen cells was done rapidly and with suitable environmental conditions (24-26 ºC and RH > 50%).

2.2 Finisher colony

A finisher hive was stronger and queen right with the resources and population to care for many developing queens simultaneously. Queen excluder sheet was kept between super chamber and brood chamber. At least two frames of open brood were kept in upper hive body to draw nurse bees and in the centre of the between two frames of the open brood one empty space made where these grafts were placed. Frames containing pollen or bee bread on the other side of these brood frames were placed. Grafted queen cells frame are taken from cell builder colonies to finisher hive after 24-30 hours of grafting. The capping of the queen cells were done after 5 days. Cells on 10 or more days after grafting were referred as ripe. If it was not done at time when queen emerge earlier than expected she seek out and destroy all other sealed queen cells. New produced queen cells were transferred into queen less colonies by 10 days after grafting. The queen sealed cells were kept in the vertical position, as they hang in the hive. If they are kept inverted, the wing buds may be damaged.

2.3 Number of emergence and number of mated queen.

After transferring of queen cells in queen less colonies queens were emerged by 15 days and 5 to 7 days of after emergence the queens were mated. In this experiment number of emergence and number mated queens were recorded.

Result and Discussion

It is pretty clear from the data embodied in table 1. Indicates that 28 hours after pin pricking of capped brood, hygienic bees cleaned all the perforated capped brood cells while, unhygienic bees did not remove all the damaged cells. In this experiment worker bees removed 98% of perforated cells within 28 hours. So, the mean percentage hygienic response (cell cleanness) have been found maximum in the colony C_9 (98.05%) followed by C_3 (91.67%) and C_2 (91.66), while the minimum was in C_{10} (87.49%). The colonies that removes <80% of dead brood cells selected for queen and drone production. Therefore, it could be concluded that colonies removed all dead brood <80% selected for breeding yard for queen production. It is clear from the above investigation that the average value of hygienic response after 28 hours of pin pricking, was recorded maximum 95.14 followed by 93.79 in

the April first week while, 93.00 in March fourth week. The minimum average value of cell cleanness was observed 78.42 in May fourth week. So, it is pretty clear that hygienic behavior of worker bees decline in month of May. Hence, the present finding are in confirmation with those of Placio *et al.* (2000) [15] evaluated the hygienic behavior in honey bee colonies and observation was recorded after 24 hrs of pin pricking. The similar finding was observed by Gramacho and Gonvalves (2009) [10] that the capped worker brood cells aged 12 to14 days old were perforated with the pin-killing method and the observation were recorded 24 hrs of pin-killing. Adjlane and Haddad (2014) [5] evaluated the hygienic behavior of *Apis mellifera intermissa* after 24 h of pin pierced and found that the bees removed 83.55% of the cells to the test in September while was 91.56% in March. According to the finding of Balhareth *et al.* (2012) [4] the cell cleanness percentage was 43.84% for *Apis mellifera carnica* and 85.28% for *Apis mellifera jemenitica,* whereas, Kamel *et al.* (2003) [3] found it to be 72.5% for the *A. mellifera jemenitica* and 35.6% for *A. mellifera carnica*.

According to the studies carried out for queen rearing data presented in the table: 2., it indicates that among the number of 20 grafted larvae, numbers of accepted cells were recorded maximu in the colonies C_9 (16) followed by C_2 and C_4 (15). While, minimum was found in colony C_3(6). The percent of grafted cells acceptance was found 80% in C_9 followed by C_2 (75%) and 30% in C_3 colonies.

Number of sealed cells were recorded maximum 13 in C_2 followed by 12 in C_9, whereas, minimum was 4 in C_3 colonies. Percentage of sealed cells were found 90% followed by 86% in C_1, C_2 colonies, whether 75% in C_9 colonies. Number of emerged queens and percentage emergence was observed in C_1 (12, 92%) followed by C_9 (10, 83%), while in C_3 (3, 75%). Number of mated queen and percentage mated queen were recorded in colonies C_2 (10, 83%) followed by C_9 (8, 80%), while in C_2 and C_3 were (2, 66%). Average numbers of larval grafts were 20 out of this 10 were accepted whereas 8 cells were sealed and 6 queens were emerged and 4 were established. The average success rate in cell acceptance was 52%, sealed cells 75%, queen emerged 72% and queen establishment was 74% during this study period. These results are in accordance with those obtained by Abrol *et al*., (2005) [6] in which maximum acceptance (72.22%) was found in *Apis cerana* (female queens). Thus, the present finding are in confirmation with those Ahmad *et al.* (2013) [9] found that the average numbers of larval grafts were 52 from which 35 accepted whereas 28 queens emerged and 26 were established. Whereas, the average success rate in cell acceptance was 67%, in queen emergence 78% and in queen establishment was 94%. Flores *et al.* (1998) used the Doolittle method based mainly on transferring (a few hours old) worker larvae to artificial queen cells and larvae were reared in queen less colonies. Mean percent of live queens during a season reached 80 per cent.

Conclusion

The present study reveals that the colonies show excellent hygienic behavior are considered to be able to resist the different insect and non-insect pests as well as various diseases. Colonies breed for hygienic behavior are able to detect, uncap and remove brood from the colonies. The importance of selecting hygienic colonies is that they have similar adult population and brood areas, produce as much honey, and have less brood disease than unselected colonies. So, it could be concluded that testing the hygienic behavior in colonies is the important factor to reduce the incidence of brood diseases and pests. Hence, this trait can be used as criteria in further selection in breeding yard. In the case of queen production number of accepted cells out of grafted cells depend on some of the important factors *viz.* quality, strength and developmental stage of the nurse bees in colonies, age of the workers, age of the grafted larvae, presence or absence of queen in the rearing colony and duration of the queen less stage, presence of open brood in the cell-starting colonies, number of grafted cells, rearing sequence and method of rearing. Other important factor is the environmental condition for success of final queen rearing. These essential factors are: regulation of humidity and temperature by the rearing colony and vitality of queen cells and the feed supply (nectar flow, supplemental feeding) of the nurse colony. There is also some indirect influence of the weather conditions and of the season. Under well managed conditions at least 80% of the larvae should be accepted even in bad weather conditions.

Table 1: Swiftness in hygienic response of various colonies at different dates

	Swiftness in hygienic response of various colonies at different dates										
Colony no.	**17/03/2014**	**24/03/2014**	**3/04/2014**	**10/042014**	**17/04/2014**	**24/04/2014**	**02/05/2014**	**10/05/2014**	**18/05/2014**	**26/05/2014**	Mean (%)
C_1	81.48	97.22	91.67	100.00	91.67	80.44	80.55	100.00	91.67	67.59	88.22
C_2	83.33	91.67	91.67	94.44	94.66	91.67	83.33	100.00	91.67	97.22	**91.66**
C_3	90.74	94.66	100.00	91.67	87.04	100.00	87.04	91.67	85.37	81.48	**91.67**
C_4	91.67	91.67	100.00	91.67	80.55	100.00	91.67	94.44	91.67	84.26	90.96
C_5	100.00	91.67	91.67	83.33	94.44	100.00	94.44	80.55	83.33	66.67	91.76
C_6	91.67	94.44	97.22	90.74	87.44	77.78	90.74	90.74	87.04	72.22	88.61
C_7	90.74	85.37	94.66	91.67	80.55	100.00	97.22	91.67	70.36	100.00	88.00
C_8	90.74	91.67	87.33	100.00	91.67	97.22	91.67	97.22	100.00	61.11	90.22
C_9	100.00	100.00	100.00	100.00	97.22	94.44	97.22	100.00	100.00	91.67	98.05
C_{10}	91.67	91.67	97.22	94.44	94.44	87.04	83.33	81.48	91.67	62.03	87.49
average	91.20	93.00	95.14	93.79	89.96	92.85	89.72	92.77	89.27	78.42	

Table 2: Performance of colonies in terms of queen production

Colony no.	No. of grafted cells	Gyne cells				No. Queen emerged	% emergence	No. of queen mated	% mated queen
		No. of cell accepted	% acceptance	No. of sealed cells	% sealed cells				
C_1	20	10	50	09	90	08	88	06	75
C_2	20	**15**	**75**	13	86	12	92	10	**83**
C_3	20	06	30	04	66	03	75	02	66
C_4	20	15	75	10	66	08	80	06	75
C_5	20	08	40	06	75	04	66	03	75
C_6	20	10	50	07	70	05	71	04	80
C_7	20	10	50	08	80	04	50	03	75
C_8	20	07	35	05	71	03	60	02	66
C_9	20	16	80	12	75	10	83	08	80
C_{10}	20	07	35	05	71	03	60	02	66
Average	20	10.4	52	7.9	75	6	72.5	4.6	74.1

References

1. Laidlaw HH, Page RE. Queen rearing and bee breeding. Wicwas Press; New York, USA, 1997, 224.
2. Ruttner F. (ED.). Queen rearing: biological basis and technical instruction. Apimondia Publishing House; Bucharest, Romania, 1983, 358.
3. Kamel SM, Strange JP, Sheppard WSA. scientific note on hygienic behavior in *Apis mellifera lamarckii* and *A. m. carnica* in Egypt. Apidologie. 2003; 34:89-190.
4. Balhareth HM, Alqarni AS, Owayss AA**.** Comparison of hygienic and grooming behaviors of indigenous and exotic honeybee, *Apis mellifera* races in central Saudi Arabia. Int. J Agric. Biol. 2012; 14:1005-1008.
5. Adjlane N, Haddad N. The first data on hygienic behavior of *Apis mellifera intermissa* in Algeria. J. Biol. Earth. Sc., 2014; 4(1):B1-B5.
6. Abrol DP, Bhagat RM, Sharma D. Mass rearing of *Apis cerana* F. queen. Journal of Asia-Pacific Entomology, 2005; 8(3):309-317.
7. Jonstone M. Rearing queen bees. Primefact 828. Rearing queen bees, Honey Bees, Richamond, USA. www. nsw dpi.au, 2008.
8. Morse RA. Rearing queen honeybees. Bd. Wicwas Press; Cheschire, Ct; USA, 1994, 64-72.
9. Ahmad JK, Shafiq M, Abbasi KHR, Assif Saleem M, Ullah MA. Production of quality queens by artificial rearing technique (larval grafting). An International Quarterly Journal of Life Science. 2013; 2(2):26-29.
10. Gramacho KP, Goncalves LS. Sequential hygienic behaviour IN Carniolan honey bees (*Apis mellifera* Carnica). *Genetics and Molecular Research*. 2009; 8(2):655-663.
11. Boecking, Spivak. Boecking O, Spivak M. Behavioural defences of honey bees against *Varroa jacobsoni* Oud. Apidologie. 1999; 30:141-158.
12. Evans JD, Spivak M. Socialized medicine: individual and communal disease barriers in honey bees. Journal of Invertebrate Pathology. 2010; 103:62-72.
13. Spivak M, Reuter GS. Resistance to American foulbrood disease by honey bee colonies, *Apis mellifera*, bred for hygienic behaviour. Apidologie. 2001; 32:555-565.
14. Wilson-Rich N, Spivak M, Fefferman NH, Starks PT. Genetic, individual, and group facilitation of disease resistance in insect societies. Annual Review of Entomology. 2009; 54:405-423.
15. Palacio MA, Figni EF, Ruffinengo SR, Rodriguez EM, Del Hoyo ML, Bedascarrasbure EL. Changes in a population of *Apis mellifera* L. selected for hygienic behaviour and its relation to brood disease tolerance. Apidologie. 2000; 31:471-478.
16. Gramacho KP, Gonçalves LS, Rosenkranz P**.** Study of the temperature of brood killed by the pin-killing method in worker bees of *Apis mellifera* carnica. Apiacta. 1999; 33:33-41.
17. Newton DC, Ostasiewski NJA. A simplified bioassay for behavioural resistance to American foulbrood in honey bees (*Apis mellifera* L). Am. Bee J. 1986; 126:278-281.
18. Message D. Effect of environmental conditions on hygienic behavior in honeybees africanizaddas *Apis mellifera*. Master's thesis, College of Mediciana Preto Rivulet, University of Sao Paulo, Rivulet Preto, 1979.
19. Rothenbuhler WC**.** Behaviour genetics of nest cleaning in honey bees responses of four inbred lines to disease-killed brood. *Anim. Behav*. 1964; 12:578-584.
20. Seeley TD, Visscher PK. Survivall of honey bees in cold climates: the critical timing of colony growth and reproduction. Ecological Entomology. 1985; 10:81-88.
21. Doolittle GM. *Scientific queen rearing*. Thomas G Newman & Son; Chicago, USA. 1889, 169.
22. Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, Jutta Vollmann J *et al*. Standard methods for artificial rearing of Apis mellifera larvae. Journal of Apicultural Research. 2013; 52(1):1-16
23. Free JB. Pheromones of social bees. London, UK: Chapman and Hall, 1987.
24. Doolittle GM. Scientific queen-rearing as practically applied; being a method by which the best of queen-bees are reared in perfect accord with nature's ways. American Bee Journal; Hamilton, USA, 1915, 126.