Workers Reproduction and Ovary Difference in Normal Workersand Egg Laying Worker Honey Bees in *ApisCeranaindica*(F) (Indian Bee) Honey Bee Colony

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1. Abstract:

There were physiological and structural differences among the ovaries tissue of the*Apisceranaindica*(Indian honey bee)normal worker honey bees and egg laying worker honey bees. To determine the influence of Queenonthe ovary development of the worker bees, The queen was manually removed from a colony and the development of the ovaries of the worker bees in normal and queen removed colonies were examined by Histological studies. The worker honey bee ovary was incompletely developed in thequeenright colony. In queen less colony the worker bees have developed functional ovaries, and laid unfertilized eggs.

Key word: Honey bee -Physiology-Anatomy- -Histology- Tissue -Honeybee ovary.

2. Introduction:

Honey beeslive in all parts of the world except the Polar regions. They live in colonies in high degree of division of labors[1]. There are three castes system in a colony of bees, VizThe Queen, the workers and the drones. The *Apisceranaindica* is found almost in all parts of the India. It is noteasy to handle *Apisceranaindica*due to its mild temper nature. These bees are prone to heavy swarming, absconding, robbing and develop a large number of laying workers. Usually every colony has only one queen[2,3,4]. The queen is the largest bee in the colony and her thorax is larger than that of the workers and her duty in the hive is to lay eggs and controls the colony. The worker bees are the most important bees in the hive. They do all the work and they

rule the colony. The workers are undeveloped or sterile females. Generally they do not produce eggs[5].

In queenright honey bee colonies, *Apiscerana*(Indian bee), workers normally prevent each other from reproducing by worker policing workers eat worker-laid eggs[6,7,8,9]. Worker reproduction is minimal in queenright colonies. In the entire colony only 0.1% of the males are workers' sons [10] and only 0.01% of the workers have produced full-sized eggs in their ovaries [11]. In queenless colonies the situation is very different the colony which has lost its mother queen Worker policing is switched off [12] and 5–24% of workers have fully-active ovaries with full-sized eggs and lay eggs [13,14]so that the final cohort of workers' sons in the colony was reared [15] before dying due to its dwindling workforce.

3. Methods:

3.1. The comparative study of laying worker and normal worker:

3.1.1. Inducing development of laying worker:

In a healthy colony only two brood combs were selected and restricted the brood combs to pupa stage honey bees.The queen and the larval stage of bees were manually removed.The brood combs were carefully observed regularly.As the two brood combs contained only sealed pupa and adult workers bees were present (Fig 1). The workers were not able to develop emergency queen cells. As only 1-7thdays larva can be developed in to a queen.

3.2. Identifying the laying worker:

The egg laying worker bees can be easily identified based on the Morphology. The abdomen of the laying workers will be large compare to the normal workers. In a comb, the eggs were placed over the side of the cells and several eggs in a cell (Fig 2).The collected normal worker bees and the laying works were analyzed for their abdominal changes by the dissection procedure.

Matured experimental beeswere collected from the Apiary**.** The dissected portion the abdomen regionof the experimental animal was placed in 50% formalin for 24 hours.The tissue was transferred sequentially to 70%, 80%, 90% and 100% alcohols for dehydration. The dehydrated tissue was placed in xylene for cleaning.And transferred to molten paraffin wax in a hot plate for infiltration and impregnation.The tissue was transferred sequentially to wax three times, at the melting point of (58°C-60°C). Blocks were prepared by embedding the samples in

the Wax. Sections were prepared using a microtome (size 8µm).The hydrated section were subjected to the stain haematoxylin for about 5 mins, washed and then it was stained with Eosin for 30 seconds. And again it was washed in running tap water for 10 minutes.The sectioned tissue was visualized under the microscope.

3.3 Conversion Ratio of laying worker development:

To assess the number of worker bees getting transformed in to laying worker, randomly ten bees were collected thrice from the hive per day. From the each set of ten bees collected, the number of worker and number of laying workers were counted and recorded

4. Result:

4.1. Identification of laying workers:

In queenless colony, the workers tend to develop large number of 3-5 days young larvae into queens. If any young larvae found to be present in a queenless colony, the workers will feed them with large amount royal jelly to develop them into queen. But in the present study the young larvae were removed intentionally leaving only the pupa stage bees in the combs. This forces the workers to become a laying worker

The laying workers and the worker bees were differentiated anatomically. When compared to the normal workers, the abdominal regions of laying workers were significantly enlarged.The collected worker bees and the laying workers were analyzed for their abdominal changes by the dissection procedure.

4.2. Histology studies of laying worker and normal worker:

In the histology studies of the normal worker, the two ovaries were clearly seen as ring shaped structure(10X) in which one of the ovaries was significantly large and well developed than another one. There was also some remarkable amount of free interspace seen between the two ovaries.

The histology studies in laying worker showed that ovaries were enlarged in size when compared to the ovary of normal worker.The ovary was enlarged higher in the right side than the left side.

The interspace between the two ovaries in the egg laying worker was reduced to a great extent.

4.3. Conversion Ratio of laying worker development:

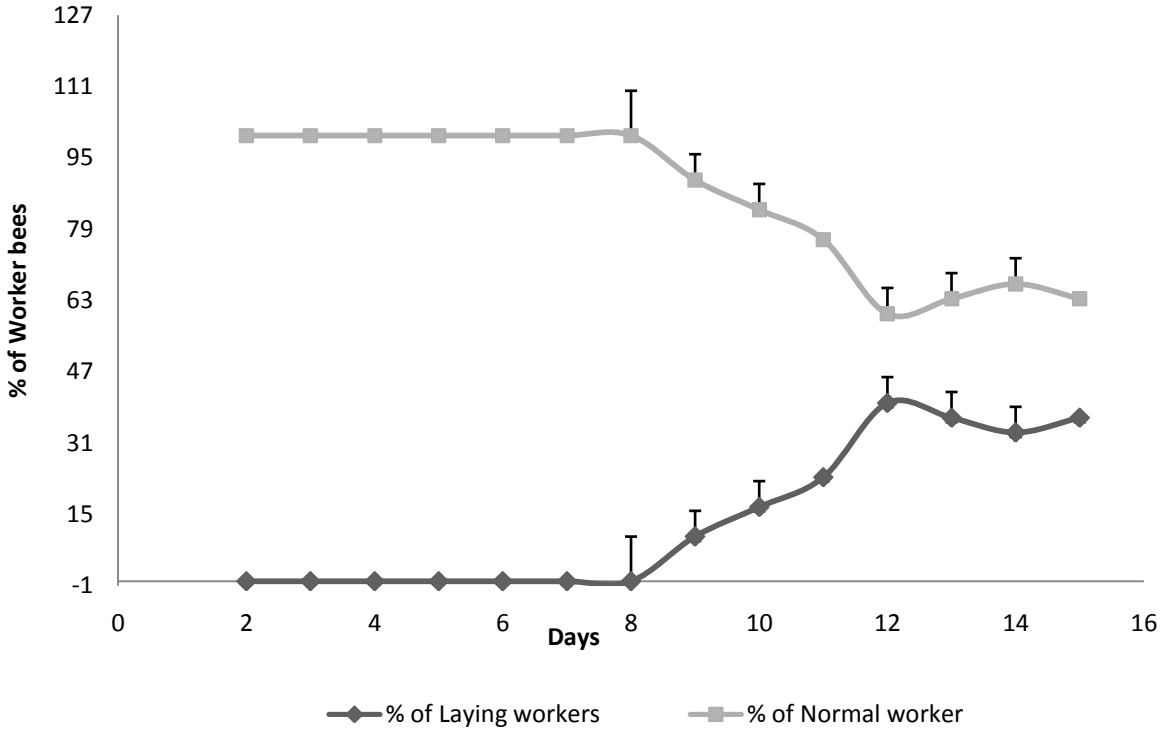
The conversion ratio of the worker bees in queenless colony was checked, the physiological changes in the abdominal cavity of the worker bees were taken into consideration. The appearance of enlargement in the abdominal cavity of a bee makes it categorize it as laying workers. In three sets of experiments conducted in two sets the lying workers started to appear on the 8^{th} day, in another set it was at 9^{th} day. The rate was conversion is at exponential phase during the period between 8^{th} and 12^{th} day after removing the queen from the colony. the percentage of laying worker converated from normal worker stands at 63.3±5.7 on average at the 12^{th} day. from the 12^{th} day the exponential rate of conversion becomes stationary, checks the converted rate around 65%, by keeping a 35% of the workers as normal worker bees (Table – 1 & Fig – 1).

Table – 1: Development of laying worker

Days	SET I		SET II		SET III		% of LW	% of NW
	N W	LW	NW	LW	NW	LW		
1.	10	0	10	0	10	0	0	100
2.	10	0	10	0	10	0	0	100
3.	10	0	10	0	10	0	0	100
4.	10	0	10	0	10	0	0	100
5.	10	-	10	-	10	-	0	100
6.	10	-	10	-	10	-	0	100
7.	10	-	10	-	10	-	0	100
8.	8	2	9	1	10	-	10±10	90±10
9.	8	2	8	2	9	1	16.6±5.7	83.3±5.7
10.	7	3	8	2	8	2	23.2±5.7	76.6±5.7
11.	6	4	6	4	6	4	40±0	60
12.	7	3	6	4	6	4	36.6±5.7	63.3±5.7

13.	7	3	7	3	6	4	33.3±5.7	66.6±5.7
14.	6	4	7	3	6	4	36.6±5.7	63.3±5.7

Fig – 1: Development rate of laying worker in queenless colony



5. Discussion:

Inqueenless colony of *Apiscerana* (Indian bee), workers starts to lay eggs in theabsence of queen. Approximately 60 – 65 % of the workers had developed active ovaries and starts to lay eggs from 12th day of queen absent in a colony.

Châline*et al.*, [12] suggested that in queen right colonies of *Apismellifera*, worker policing normally eliminates worker-laid eggs thereby preventing worker reproduction. However, in queenless colonies that have failed to rear a replacement queen, worker reproduction is normal. Worker policing is switched off, many workers have active ovaries and lay eggs and the colony rears a last batch of male brood before dying out.

In queenless (absence of queen in a colony) honey bee colonies, *Apismellifera*, workers normally prevent each other from reproducing by worker policing[16,17,18,19,20,21]. Worker reproduction is minimal in queenless colonies. Only 0.1% of the adult males are workers [(13)] and

only 0.01% of the workers have full-sized eggs in their ovaries [15]. In a "hopelessly queenless" colony, that is one which has lost its mother queen and has failed to rear a replacement, many workers (5–24%) have fully-active ovaries with full-sized eggs and lay eggs [17].

Visscher (1989) reported that the worker reproduction is minimal in queen right colonies. Only 0.1% of the adult males are worker's sons and only 0.01% of the workers have full-sized eggs in their ovaries. But our observations were contradictory to the above detailed research articles. In our study, the queenless colonies have produced an hoping 60 to 65% of Worker bees converted to laying worker bees which is a huge amount when compared to the other authors who reported an average of 12.6% of worker bees converted to laying worker bees.

Ovary size of laying worker varies from the normal worker structurally which was observed on histopathological analysis.

6. References:

1. Aeppler. M. (1922), S Gleanings in Bee Culture, 50,151.
2. Barron, A.B., B.P. Oldroyd and F.L.W. Ratnieks, 2001. Worker reproduction in honey bees (*Apis*) and the anarchic syndrome: a review. Behav. Ecol. Sociobiol. *50*: 199–208.
3. Benjamin P. Oldroyd · Luke A. Halling., (2001), Worker policing and worker reproduction in *Apiscerana.*BehavEcolSociobiol**50**:371–377.
4. Christian W. W. Pirk ., (2003), Egg viability and worker policing in honey bees.
5. Cruz Landim., (2003), Ultrastructural and cytochemical aspects of metamorphosis in the midgut of *Apismelliferа*L. (Hymenoptera: Apidae: Apinae). Zoological- Science,**20**: 1099-1107.
6. Hahn DA, Wheeler DE., (2003), Presence of a single abundant storage hexamerin in both larvae and adults of the grasshopper, *Schistocercaamericana*.J Insect Physiol, **49**:1189-1197.
7. Halling L, Oldroyd BP, Patimus B, Wattanachaiyingcharoen W, Barron, AB, Nanork P, Wongsiri S., (2001), Worker policing in the bee *Apisflorea.* BehavEcolSociobiol**49**:509–513.
8. Isabel c. Boleli, zila´ luzpaulinosimoes, and klaushartfelder., (1998). The Stomatogastric Nervous System of the Honey Bee (*Apismellifera*) in a Critical Phase of Caste Development. Journal Of Morphology.**236**:139–149.

9. Mahmoud E. Zakaria., (2010), The Physiological Structure Differences of the Honey Stomach Tissue at Different Developmental Stages of Worker Honey Bees (*Apismellifera L.*)Journal of Applied Sciences Research, **6(1)**: 45-49.
10. Miller, D.G. and F.L.W. Ratnieks, 2001. The timing of worker reproduction and breakdown of policing behaviour in queenless honeybee (*Apismellifera*L.) societies. Insect. Soc. *48*: 178–184.
11. Miller, D.G. and F.L.W. Ratnieks., (2001). The timing of worker reproduction and breakdown of policing behaviour in queenless honey bee (*Apismellifera*L.) societies. Insect. Soc. ***48***: 178 – 184.
12. N. Châline, S.J. Martin and F.L.W. Ratnieks., (2004), Worker policing persists in a hopelessly queenless honey bee colony (*Apismellifera*). Insectes Soc. **51:** 1 – 4.
13. Norbert Hrassnigg, Karl Crailsheim ., (2005), Differences in drone and worker physiology in honeybees(*Apismellifera*)Apidologie**36**: 255 – 277.
14. Page, R.E. and E.H. Erickson, 1988. Reproduction by worker honey bees (*Apismellifera*L). Behav. Ecol. Sociobiol. *23*: 117–126.
15. Page, R.E. and R.A. Metcalf, 1984. A population investment sex-ratio for the honey bee (*Apismellifera*L). Am. Nat. *124*: 680–702.
16. Ratnieks, F.L.W. and P.K. Visscher, 1989. Worker policing in the honeybee. Nature 342: 796-797.
17. Ratnieks, F.L.W., 1993. Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav. Ecol.Sociobiol. *32*: 191–198.
18. Seeley, T.D., 1985. *Honeybee Ecology*. Princeton University Press. Princeton, NJ. 201.
19. Visscher, P.K., (1989), A quantitative study of worker reproduction in honey bee colonies. Behav. Ecol. Sociobiol. **25**: 247–254.
20. Wheeler DE, Tuchinskaya II, Buck NA, Tabashnik BE., (2000), Hexameric storage proteins during metamorphosis and egg production in the diamondback moth, *Plutellaxylostella*(Lepidoptera). J Insect Physiol, **46**:951-958.
21. Winston, M.L., 1987. *The Biology of the Honeybee.* Harvard University Press. Cambridge, MA. 281 pp.