Chapter 31

Production of Quality Queens of *Apis mellifera* L. Under Mid-hill Conditions of Himachal Pradesh for use in Instrumental Insemination

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The first step in honeybee stock improvement entails the rearing of queens from desirable, selected stock. Superior stocks cannot be produced until desirable traits are produced in offspring brought about by systematic mating of selected individuals. Many characteristics of the honeybee are open to improvement. This approach towards stock improvement can be carried out if appropriate timings or seasons of queen production are known in a particular area. It requires standardisation of queen-rearing methods when multiplying colonies or replacing old and ineffective queens. Secondly, it is important to consider quality attributes like queen-weight when proposing queen production. Hoopinginner and Farrar (1959) and Woyke (1967, 1971) worked out a significant positive correlation between queen-weight and number of ovarioles, and suggested that queen-weight at emergence might be a useful index for selecting quality queens. Similarly Huang and Zhi (1985) reported a significant and positive correlation between queen-weight at emergence and number of eggs laid. Further Avetisyan *et al.* (1967) worked out a positive correlation between queen-cell volume and queen-weight as a parameter for culturing cells and selecting virgin queens. Jhajj *et al.* (1992) studied queen-weight and queen-cell volume of *Apis mellifera* raised during different seasons and they designated March and November as best periods of queen-rearing both for replacement of old queens and multiplication of stock. Other parameters such as acceptance and emergence of queens are also vital for queen-rearing success. These quality attributes have benefits in successful insemination. The present studies were aimed at working out the best period for quality-queen production under local conditions at Nauni and then using such queens for artificial insemination.

Materials and Methods

The studies were conducted in the apiary of the Department of Entomology and Apiculture, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan located at 1125 m. Standard method of queen-rearing involving grafting of appropriate-aged larvae was

followed. Queen cups were made by obtaining beeswax from old honeybee combs. The queen-cell cups were prepared with a cell-forming rod.

Collection of royal jelly

A strong colony with sufficient young brood, nurse bees and pollen stores was dequeened and allowed to raise queen cells. After 72 hours, the larvae from these cells were removed and a few drops of distilled water was added to each cell containing royal jelly. The diluted royal jelly was collected in a small vial and stored in a freezer. At the time of grafting, a small drop of diluted royal jelly was placed with a dropper at the bottom of the cell cup to facilitate priming of cell base. Immediately after priming, a 12-hour-old larva was grafted over the drop of royal jelly.

Collection of larvae of desired age

A brood frame from the centre of the breeder colony was removed and replaced with a frame containing empty worker cells. This comb was examined daily for the presence of eggs. Cells with eggs were marked for each particular date. Marked egg combs were introduced into a colony with sufficient nurse bees, pollen and sealed brood for development of the larvae. The incubator colony was regularly provided with 40% sugar syrup. After removing the first batch of eggs from the breeder colony, another empty comb was inserted in its place and this sequence was repeated to obtain a regular supply of eggs.

Grafting of larvae

A colony with sufficient nurse bees, sealed and emerging bees, pollen and honey stores was selected as a cell-breeder colony for rearing queens from grafted larvae. The colony was dequeened and the young brood, if any, was removed. Such a colony was liberally provided with enough provisions throughout the course of experimentation, starting from one day prior to the introduction of larvae. The percentage acceptance, sealing of accepted larvae and emergence of queens was recorded. Ripe cells were removed from the frames and placed in an incubator maintained at 33 ±1°C for emergence. Immediately after emergence 20 queens were weighed. The queens produced during April were artificially inseminated and their performance was tested. Colony parameters such as strength, honey and pollen stores, brood area and prolificness were measured throughout the year. The strength of colonies was estimated by counting the number of bee frames covered with bees. The amount of honey and pollen stored in the colony and brood area were recorded at intervals of 21 days by following the method of Al-Tikrity *et al.* (1971). The egg-laying rate was recorded every 21 days. The total brood area measured was multiplied by a factor of 3.9, which was the number of cells in 1 cm^2 of brood comb. The total number of brood cells was divided by 21 to obtain the daily rate of egg-laying by a queen (Sharma, 1958).

Artificial insemination

The queens were inseminated with 2 µl of semen. To assess the success of insemination, after two days the last abdominal segment of each queen was detached by grasping its ventral part with fine forceps and the spermatheca removed. The trachea from the surface of the spermatheca was placed in a dish containing 0.1 ml of 0.9% NaCl. The spermatheca was pierced with a fine needle to liberate sperm and the empty spermatheca was removed after thorough washing. The sperm were dispersed by drawing and expelling the fluid with a dropper. The number of sperm was counted with a haemocytometer.

Results and Discussion

In order to find out the most appropriate period of larval grafting for quality-queen production, 20 larvae aged 12–24-hours were grafted every month from February until October under environmental conditions prevailing at Nauni. Data revealed that during the first year, acceptance of the larvae varied in the range 28.83–95.0%. Different steps in queen-rearing such as percentage acceptance of larval grafts, percentage sealing of grafted larvae, percentage

Table 1: Effect of one day storage of semen under different storage temperatures on the number of spermatozoa entering the spermathecae of *Apis mellifera* L. queens

Semen stored at different Temperature (°C)	Quantity injected (microlitre)	No. of spermatozoa in spermathecae (in millions)
10	2	0.29
15	2	1.13
0	2	0.83
Room temperature (26–32)	2	0.51
CD 0.05		0.08

emergence of queens and quality of queens depends upon seasonal variations. The success of queen-rearing with reference to the above parameters was found to be significantly greater during March, April and May (both years of experimentation). However, these criteria were not significantly different in June and October. Weight of newly emerged queens varied from 156.7 to 221.7 mg/queen during first year and from 167.7 to 205.0 mg/queen during the second year. During the first year, heavier queens were reared in March and April when the weight of queens was 218.0 and 221.7 mg respectively. Similarly during the second year, significantly heavier queens were reared during March to May when the weight ranged from 205 to 192 gm/queen. Lightest queens were reared in August during both year (average weight 156.7 mg and 167.7 mg). Queen-weight is considered to be largely determined by genetic makeup (Abdellatif, 1967). However, in the present study queens reared from the same stock differed in weight indicating that factors other than genetic makeup also play a significant role in determining queen-weight.

The variability in the weight of queens during different months can be attributed to climatic conditions, incoming nectar and pollen stores, and bee population in the colonies. This is evident from the fact that heavier queens were produced during the period of main nectar flow in both the years; whereas lightest queens were produced during dearth periods, i.e., June and September. The lower weight of queens can also be attributed to prevailing low temperature in October and high temperature in June. Cale (1983) reported that lightweight queens were produced under unfavourable conditions such as low temperatures, lack of incoming food and insufficient population of bees. The prevalent climatic conditions, availability of bee flora, number of nurse bees and state of colony are major factors contributing towards the development of quality queens (Taranov, 1959). With the approach of spring, bees are able to maintain high brood temperature (Zhdanova, 1967). This factor coupled with sufficient inflow of pollen and nectar might be contributing to the production of heavier queens (Abdellatif, 1967; Taranov, 1959). Better colony conditions with regards to nectar, pollen stores, brood area, foraging efficiency and prolificness during main nectar flow were observed between end of February to mid-May. These factors might have contributed to the production and better performance of queens reared during these months.

The experiment conducted at Nauni revealed that queens can be reared efficiently in the months of March to May. These months are the nectar-flow period and can be designated as breeding period under local conditions. The loss of a queen during autumn can be made good by rearing new queens in September and October and colonies can be given a new prolific queen in order to face effectively the coming winter season. However, March to May is the best period for breeding for replacement of old queens and multiplication of stock. The insemination of quality-queens produced during April was successful since spermatheca contained 0.29 to 1.13 million sperm/queen. However, lower-weight queens were more difficult to inseminate and spermatheceae of such queens did not show the presence of sperm.

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