Queen Cell Acceptance in Laying Worker Colonies of Russian and Italian Honey Bees

by ROBIN A. CARGEL[1] and THOMAS E. RINDERER[1]

Revised manuscript received for publication March 8, 2006

Abstract:
Honey bee colonies that have become queenless and develop laying workers are considered lost by beekeepers since they can rarely be requeened by introducing an adult queen. We tested the hypothesis that such colonies could be successfully requeened with queen cells. The results showed that both Russian and Italian colonies could be requeened with queen cells. Overall, about 60% of colonies were successfully requeened with equal success for Russian and Italian colonies.

Keywords: requeen, *Apis mellifera*, laying worker, queenless

Introduction

Honey bee (*Apis mellifera*) queens suppress egg laying by worker bees with pheromones. Queen substance ((E)-9-oxy-decenoic acid (9-ODA)) (Callow and Johnson, 1960, Barbier et al., 1960) is a major component in the mandibular gland secretion of queens. However, the queen mandibular gland produces other compounds (Butler and Callow, 1968). These compounds, as well as compounds produced by the queen's abdomen (Velthuis, 1970), have a variety of functions including inhibiting both the ovary development of workers and queen rearing by workers (Butlers et al., 1961). When a colony loses its queen, these inhibiting pheromones are also lost. The colony then makes queen cells, which normally lead to the production of a new queen. Immature queens in sealed queen cells produce a substance, which is known to inhibit further queen rearing (Boch, 1979). If the production of queen cells fails to lead to successful queen replacement, laying workers develop (Miller and Ratnieks, 2001).

Laying workers are sometimes called "false queens". They have developed ovaries (Koptev, 1957) and produce 9-ODA and other related compounds that the queen produces (Crewe and Velthuis, 1980, Crewe, 1988). In some cases their queen-like characteristics are pronounced and the behavior of other workers towards them resembles worker behavior toward queens (Sakagami, 1958). Usually, colonies having laying workers are very difficult to re-queen (Ribbands, 1953, Tucker 1958). Beekeepers typically do not try to requeen laying worker colonies since the rate of success is thought to be very low.

We received reports from beekeepers which suggested that queen-less colonies of Russian honey bees often could be re-queened by introducing queen cells to them (Tubbs et al. 2003). Hence, we conducted this study to determine if laying worker colonies could be re-queened with cells and if success with the procedure was specific to Russian honey bees. A literature search did not find any reports concerning re-queening laying worker colonies with cells.

[1] **USDA Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA. 70820 (e-mail: RCargel@ars.usda.gov, TRinderer@ars.usda.gov)**

Materials and Methods

Twenty Russian colonies and twenty Italian colonies were established and arranged randomly within the test yard. These colonies were composed of 0.5-0.7 Kg of bees with 3-4 frames of brood (Langstroth frames 16.8 cm deep) and were observed for a month. We made queenless splits to start, let these colonies remain queenless for about three days, and then introduced another queen under a push-in cage to re-establish the colony. These introduced queens were of the same stock as the worker bees and brood of the colony, either Russian or Italian, and were left in the colony to produce a brood nest having all stages of brood. These queens were then removed to encourage ovary development in workers.

Colonies were inspected twice a week for queen cells and any found were destroyed to make the colony "hopelessly queenless" (Gary, 1992). When 50 or more worker cells in a colony were observed to have worker laid eggs, the colony was considered to be a laying worker colony. Laying worker eggs are those laid on the side of the cell, at an angle or in clusters in the cell (Gary, 1992). These laying worker colonies were broodless except for the eggs which indicated their status.

As colonies became laying worker colonies, they were given Russian or Italian queen cells. These queen cells were introduced 1-2 days before they were expected to emerge. They were protected by plastic "cell protectors" which prevent bees from destroying the cells from the side, but allow the queen to emerge into the colony from the end of the cell. Because of queen cell availability, cells were distributed unevenly. Eleven Russian colonies were given Russian queen cells, the other nine Russian colonies were given Italian queen cells, 13 Italian colonies were given Russian cells and the other seven were given Italian queen cells.

Colonies were inspected for the presence of the emerged virgin queens two days after the introduced queen cells were expected to emerge. The virgin queens were paint marked for later identification. Nine days after virgin queens emerged, the colonies were again inspected to determine if the queens had mated and begun to lay eggs. Re-queening these laying worker colonies was considered successful when the introduced cell produced a queen which mated and then produced a brood nest with all stages of brood.

Data concerning both the presence of virgins from the queen cells and the presence of the marked queens producing brood were analyzed by Fisher's exact tests. Russian and Italian colonies were compared for their acceptance of cells and the later presence of mated and laying queens. Also, Russian and Italian cells across all colonies were compared both for their acceptance and the subsequent presence of a mated queen with a brood nest.

Data concerning the number of days it took for Russian and Italian colonies to become laying worker colonies were analyzed by a t-test.

Results

The average number of days it took to become a laying worker

colony after the queen was removed was 34 ± 1.9 (x ± SEM) for the Russian colonies and 36 d ± 2.5 for the Italian colonies (t-test, df=38 t=0.71, P =0.48).

Cell acceptance based on the presence of recently emerged virgins was remarkably high and the acceptance of Russian and Italian cells was similar, regardless of colony type. The overall initial acceptance of Russian and Italian cells in all colonies was 92%. In the Russian colonies with Russian cells, ten out of 11 (91%) were accepted; in the Russian colonies with Italian cells, eight out of nine (88%) were accepted; in the Italian colonies with Italian cells, seven of seven (100%) were accepted, and in the Italian colonies with Russian cells, 11 out of 12 (91%) were accepted.

In Italian colonies, no difference was found between the acceptance of Russian and Italian cells (Fisher's exact test, P=0.63 sample size=19). In Russian colonies, there was also no difference comparing Russian and Italian cell acceptance (Fisher's exact test, P=0.52 sample size=20).

We further observed these colonies to see if these virgins would be fully accepted and successful by producing a brood nest with all stages of brood.

Russian and Italian colonies did equally well, accepting cells and virgin queens. They also did equally well in continuing to accept those queens through their mating and establishment of a brood nest (Table). The Fisher's exact test was used to compare the final acceptance rate of queens in Russian and Italian colonies. Three colonies were lost prior to the final evaluation and were not included in the analyses. The causes of these losses are unknown. However, they started as small colonies and after several weeks without emerging brood may have become too small to control nest parasites.

Of the cells introduced, 60% resulted in successful requeening. In the Russian colonies with Russian cells, six out of ten (60%) were successfully requeened; in the Russian colonies with Italian cells, six out of eight (75%) were requeened; in the Italian colonies with Italian cells, five out of seven (71%) were requeened, and in the Italian colonies with Russian cells, six out of 11 (54%) remained established.

Discussion

Surprisingly, 60% of the laying worker colonies were successfully requeened. Since laying workers have queen-like characteristics (Crew, 1982), we expected most colonies would not accept cells or virgins which emerged from them. However, this result compares favorably with the success rates encountered in commercial queen production (Laidlaw, 1979). This high success rate is contrary to other reports (Ribbands, 1953, Tucker 1958) which are based on attempts to requeen colonies with mated queens. Both our Russian and Italian colonies were requeened with similar success using the cell technique. Also, both Russian and Italian cells produced similar success rates. Therefore, the requeening success is not related to either the stock of cells or the stock of the worker bees in the colonies. Some queens were lost between emergence and the time they could have produced a brood nest (Table 1). However, these losses were low and similar to those observed in commercial queen rearing (Laidlaw, 1979).

We established that a minimum of 50 cells with laying worker eggs would indicate a laying worker colony. The number of days it took for laying workers to develop in queenless colonies averaged 34d for Russian bees and 36d for Italian bees. This is similar to studies that report successful worker oviposition occurs in *A. mellifera* 20-40 days after dequeening (Miller and Ratnieks, 2001, Page and Erickson, 1988, Ruttner and Hesse, 1979).

Cell protectors and a quick release allowed virgins to emerge into the colonies. We used cell protectors since this is a common practice for ourselves and others for introducing cells. The cause of the success of the virgin queen is unknown, but changes in pheromone production seem to be most likely. The virgins slowly produce 9-ODA (queen substance) which peaks near mating time. This gradual change may allow the virgin queen to, in effect, "supersede" the laying workers. However, the success of the virgins may have resulted from something other than pheromone production such as behavior.

Generally, commercial beekeepers do not make an attempt to salvage colonies with laying workers. This study indicates that such colonies can often be recovered by the introduction of queen cells that are near emergence. In practice, only some beekeepers can take advantage of this technique since it is based on the availability of queen cells, which are close to emerging when a laying worker colony is discovered.

Table. Comparisons of the acceptance of Russian and Italian queen cells in Russian and Italian colonies. Acceptance includes the presence of virgin queens two days after emergence and the presence of the same queens at a later date after they have mated and produced a brood nest having all stages of brood. Probabilities are derived from Fisher Exact Tests.

	Acceptance of Queen cells				Presence of Laying Queen with brood nest		
Comparison	Cell Type	Accepted	Rejected	P	Yes	No	P
Acceptance of Russian and Italian cells in Russian colonies	R	10	1	0.52	6	4	0.38
	I	8	1		6	2	
Acceptance of Russian and Italian cells in Italian colonies	R	11	2	0.63	6	5	0.22
	I	7	0		5	2	
Acceptance of Russian and Italian cells in all colonies	R	21	2	0.44	12	9	0.16
	I	15	1		11	4	

*Three colonies (one each in the colony/cell distribution of R/R, R/D, and D/R) died between the acceptance of the queen cell and the final evaluation of queen acceptance due to small populations and wax moth. These are not included in the analysis of final queen acceptance.

References

Barbier, M., E. Lederer, T. Reichstein, O. Schindler. 1960. Auftreunnung der sauren Anteile von Extrakten aus Bienenkoniginnen (*Apis mellifera* L.): Isolierung des als Koniginnen-Substanz bezeichneten Pheromones. *Helv. Chim. Acta* 43: 1682-1689.

Boch, R. 1979. Queen substance pheromone produced by immature queen honey bees. *J. Apic. Res.* 18(1): 12-15.

Butler, C.G., R.K. Callow, N.C., Johnson. 1961. The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honeybee pheromone. *Proc R Soc Lond* B155:417-432.

Butler, C.G., R.K. Callow. 1968. Pheromones of the honey bee (*Apis mellifera* L.) the 'inhibitory scent' of the queen. *Proc. R. ent. Soc. Land.* (B) 43 (4-6): 62-65.

Callow, R.K., N.C. Johnson. 1960. The chemical constitution and synthesis of queen substance of honey bees (*Apis mellifera*). *Bee Wld* 41(6): 152-153.

Crew, R.M., H.H.W. Velthuis. 1980. False queens: a consequenee of mandibular gland signals in worker honeybees. *Naturwissenschaften* 67: 467-469.

Crew, R.M. 1988. Natural history of honey bee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: "Africanized Honey Bees and Bee Mites". *ed.* Needham, G.R., Page, R.E., Delfinado-Baker, M., Bowman, C.E. pp.149-158. Chichester, England: Ellis Horwood Limited.

Gary, N.E. 1992. Activities and Behavior of Honey Bees. In: "The Hive and the Honey Bee". *ed.* Graham, J.M. pp. 269-361. Dadant & Sons. Hamilton, Illinois.

Keeling, C.L., Slessor, K.N., Higo, H.A. and M.L. Winston. 2003. New components of the honey bee (*Apis mellifers* L.) queen retinue pheromone. *Proceedings of the National Academy of Services.* 100 (8): 4486-4491.

Koptev, V.S. 1957. Laying workers and swarming. *Pchelovodstvo* 34: 31-32 (in Russian).

Laidlaw, H.H. 1979. Contemporary Queen Rearing. Dadant & Sons. Hamilton, Illinois.

Miller, D.G.III and F.L.W. Ratnieks. 2001. The timing of worker reproduction and breakdown of policing behaviour in queenless honey bee (*Apis mellifera*) societies. *Insectes soc.* 48: 178-184.

Page, R.E.Jr. and E.H. Erickson. 1988. Reproduction by worker honey bees (*Apis mellifers L.*). *Behav. Ecol. Sociobiol.* 23: 117-236.

Ribbands, C.R. 1953. The behavior and social life of honey bees. Bee Research Association, London.

Ruttner, F. and Hesse. 1979. Rassenspezifiche Unterschiede in Ovarentwichung Und Eiblage von weisellosen arbeiterinnun die Honigbiene. *Apis mellifera* L. *Apidologie* 12: 159-183.

Sakagami, S.F. 1958. The false queen: fourth adjustive response in dequeened honeybee colonies. *Behaviour* 13: 280-296.

Tubbs, H., Harper, C., Bigalk, M., Bernard, S.J., Sylvester, H.A. and Rinderer, T.E. 2003. Commercial Management of ARS Russian Honey Bees. *American Bee Journal.* 143(10): 819-820.

Tucker, K.W. 1958. Automictic parthenogenesis in the honey bee. *Genetics* 43: 299-316.

Velthuis, H.H.W. 1970. Queen substances from the abdomen of the honey bee queen. *Z Vgl Physiol* 70: 210-222.