REARING HONEY BEES IN VITRO: EFFECTS OF FOOD QUANTITY ON SURVIVAL AND DEVELOPMENT

Osman Kaftanoglu,
School of Life Sciences
Arizona State University
Tempe, AZ. 85287-4501

Summary

In vitro honeybee rearing technique was simplified and sophisticated by monitoring the amount of food given to each larva. Honeybees (*Apis mellifera* L) were reared in individual queen cell cups by feeding them 100 mg, 150 mg, 200 mg, 250 mg and 300 mg basic larval diet. The survival rates were lower in 300 mg food (12.7%) due to drowning of larvae at the later larval stages and at the 100 mg food (32.7 %) due to insufficient food. The survival rates of the larvae and adults were 61.8% and 55.5% in the 150 mg food, 83.6% and 75.5% in the 200 mg food, and 77.3% and 63.6% in the 250 mg food groups respectively. The adult weights and the ovariole numbers were 78.3±7.09 mg and 15.0±8.86 ovarioles in the 100 mg food , 101.8±18.35 mg and 21.8±18.59 ovarioles in the 150 mg food, 125.5±19.45 mg and 29.9±35.44 ovarioles in the 200 mg food and 147.7±14.28 mg and 39.8±36.31 ovarioles in the 250 mg food groups. This new technique reduces the labor *in vitro* rearing of honeybees and enables to rear any size workers for behavioral studies.

Introduction

Rhein (1933) was the first who tried the rear adult bees in an incubator by feeding the larvae with the fresh larval food taken from the cells of the same aged larvae. He failed to rear worker bees by feeding them fresh worker larval food. He could not rear any perfect queens but he reared large workers with many ovarioles. Later on many attempts have been made to rear honey bees in vitro for over 80 years (Michael and Abromovitz, 1955; Weaver, 1955, 1958, 1962, 1970, and 1974; Smith, 1959, Hoffmann, 1960; Mitsui et al., 1964; Rembold, 1965; Jay, 1965; Rembold et al., 1974; Asencot and Lensky, 1976; Schuel, and Dixon, 1968 and 1986; Schuel et al., 1978; Hanser, 1983; Vandenberg and Shimanuki, 1987; Peng et al., 1992)

Aupinel et al.,(2005) further improved the technique by feeding different instar larvae with different sugar, protein and yeast extract concentrations and Brodshnider et al.,(2009), tested the flight performance of in vitro reared bees.

Recently a new technique of honeybee rearing *in vitro* was developed by feeding the larvae once instead of everyday or twice a day (Kaftanoglu et.al, 2010). This technique reduced the labor greatly and made the mass production of honeybees *in vitro*. However, the larvae were fed on a common basic larval diet and the competition for the food resulted in big variation on the larval weights, adult weights and ovariole numbers (Kaftanoglu et al 2010, 2011). Crailsheim (2013) summarized the in vitro rearing techniques for standard toxicological studies. Hladun et al (2013) used the mass feeding technique and studied the effects of selenium on the development of larvae in vitro.

Since honeybees could be reared by mass provisioning we developed a technique to reduce the variation by monitoring the quantity of the food given to individual larvae and tested the effects of food quantity on the development of bees reared *in vitro*.

Materials and Methods

Collection of honey bee larvae

A healthy open mated Italian honey bee colony at the Bee Research Facility of the Arizona State University was used as the larval source. The queen was confined to a fully drawn comb in an excluder cage to obtain 1 day old larvae (Peng et al., 1992).

Individual brown queen cell cups (Mann Lake QC-110) were placed in 60x15 mm polystyrene disposable Petri dishes. Queen cell cups were filled with 100 mg, 150 mg, 200 mg, 250 mg, and 300 mg of larval diet (53% RJ, 6% glucose, 6% fructose, 1% YE and 34% distilled water) by a variable volume pipettor. There were 5 replicates of each group and 22 larvae in each replicates. The larvae were fed one time as described by Kaftanoglu et al., (2010). The bees were reared in an incubator at 34 ^{0}C and 90 % RH. The brood comb that larvae were grafted removed from the hive newly emerged adult bees were weighed and dissected as control.

Results and Discussion

Effects of food quantity on survival

The quantity of the food affected the larval survival *in vitro* (Table 2). The larval survival was very low (12.7%) on 300 mg food. Most of the larvae drowned in the food especially on the late larval stages. On the other hand insufficient quantity (100 mg) of food also decreased the larval survival to 32.7 %. The best larval survival (83.6 %) was obtained when the larvae were fed 200 mg of food. Similarly, the adult survival rate was the highest (75.5) in this group and 83 out of 110 larvae became adults. When the food was increased to 250 mg some larvae were drowned, the larval and adult survival rates decreased to 77.3% and 63.6% respectively.

Table 2: The effects of food quantity on survival

	Larvae			Adults		Queens/Intermediates	
Groups	Grafted	Survived	% Survival	N	%	N	%
100 mg	110	36	32.7	15	13.6	0	0
150 mg	110	68	61.8	61	55.5	0	0
200 mg	110	92	83.6	83	75.5	4	4.6
250 mg	110	85	77.3	70	63.6	7	9.1
300 mg	110	14	12.7	1	0.9	1	100
Total/Average	550	295	53.6	229		12	

Feeding the larvae 150 mg, 200 mg or 250 mg food was satisfactory for the survival of *in vitro* reared bees that were mass fed only once during larval development. The larval and adult survival rates were similar or higher than the results of Vandenberg and Shimanuki (1987), Peng et al., 1992; Aupinel et al., (2005).

There were no queen or intermediate developments in the 100 mg and 150 mg diet groups. All the bees developed into worker bees. As the amount of food increased the number of queens and intermediates also increased, and 4.6% of the 200 mg diet group and 9.1 % of the 250 mg diet groups developed into queens or intermediates respectively. There was only 1 adult bee in the 300 mg diet group weighing 201 mg and

had 200 ovarioles. Since there was only one bee in this group it was not included in the data analysis.

Table 2. Effects of food quantity on adult weights and ovariole numbers

Food		Adult Weights (mg)					Ovariole numbers		
Quantity	N	X±SD	Min	Max	% CV	N	X±SD	Min	Max
100 mg	15	78.3±7.09a*	62	87	9.05	8	15.0±8.86 ab	7	35
150 mg	61	101.8±18.35b	64	138	18.02	59	21.8±18.59 ab	6	95
200 mg	87	125.5±19.45d	92	172	15.49	75	29.9±35.44 bc	9	240
250 mg	77	147.7±14.28e	112	183	9.67	75	39.8±36.31c	7	165
Control	39	114.3±7.15 c	95	131	6.26	39	9.1±3.53 a	3	17

*P<0.0001

Food quantity in mass feeding affected the adult weighs significantly (ANOVA: $F_{4,278}$= 104.113, P<0.0001). The weights of the bees increased as the amount of larval diets increased. The bees that were fed with 100 mg larval diet were the smallest and the bees that were fed 250 mg larval diet were the biggest. The average weights of the hive reared control bees (114.3±7.15 mg) were between the 150 mg and 200 mg larval diet groups.

Similarly food quantity affected the ovariole numbers, bees that were reared by feeding 150 mg larval food had the lowest and 250 mg larval food had the highest number of ovarioles (ANOVA: $F_{4,255}$= 8.315, P<0.0001).

Aupinel et al. (2005) and Crailsheim et al (2013) recommend feeding the larvae 160 mg diet which is sufficient for normal development. Quality of royal jelly is very important for the development of worker bees reared in vitro. Even 150 mg diet is sufficient for the development of bees; however if royal jelly has high moisture content and/or is low quality survival rate is lower and it might be necessary to use a new batch of royal jelly or increase the food quantity up to 200 mg.

This new technique of rearing honey bees by feeding them once in individual cell cups with the known amount of larval food is a practical and useful tool for the evolution and development research as well as honey bee genome projects. Normal size, smaller or

larger size bees with the predicted ovariole numbers can be reared *in vitro* and their development and/or behavior can be studied. It is very simple and does not require much labor except preparing food and grafting.

References

Asencot, M., Lensky, Y. (1976) The effects of sugars and juvenile hormone on the differentiation of the female honeybee larvae to queens. Life Sci. 18:693-699

Aupinel, P., Fortini, D., Dufour, H., Tasei, J-N., Michaud, B., Odoux, J-F., Delegue, M-H. P. (2005) Improvement of artificial feeding in a standard *in vitro* method for rearing *Apis mellifera* larvae. Bulletin of Insectology 58(2):107-111

Brodshneider R, Riessberger-Galle U, Crailsheim K. 2009. Flight performance of artificially reared honeybees (*Apis melliferra*), Apidologie 40(4):441-449

Crailsheim K., Brodschneider R., Aupinel P., Behrens D., Genersch E., Vollmann J., RiessbergerGallé U. (2013): Standard methods for artificial rearing of *Apis mellifera* larvae. Journal of Apicultural Research, 52, 1

Dietz, A., (1967) The influence of environmental and nutritional factors on growth and caste determination of female honey bees. Diss. Abst. 27(12): pt 1:4599-4600

_____ (1973) Longevity and survival of honey bee larvae on artificial diets. J. Ga. Entomol. Soc. 8:59-63

Dietz, A., Haydak, M.H., (1971) Caste determination in honey bees. I. The significance of moisture in larval food. J. Exp. Zool. 177:353-358

Dixon, S.E., Schuel, R.W. (1963) Studies on the mode of action of royel jelly in honeybee development III. The effect of experimental variation in diet on growth and metabolism of honeybee larvae. Can. J. Zool. 41:733-739

Hanser, G., (1983). Rearing queen bees in the laboratory. Pp:63-81. In Queen rearing: Biological basis and technical instruction. Edited by F. Ruttner. Apimondia Publishing House, Bucharest.

Hanser, G., Rembold, H., (1960) Über den Weiselzellenfuttersaft der Honigbiene IV. Jahreszeitliche Veränderungen im Biopteringehalt des Arbeiterinnenfuttersaftes. Hoppe Seyler's Z. Physiol. Chem. 319:200-205

Haydak, M.H., (1970) Honey bee nutrition. Ann. Rev. Entom. 15:143-156

Hladun, K.R., O. Kaftanoglu, D. Parker, K. Tran, and J. T. Trumble. 2013. Effects of selenium on development, survival and accumulation in the honey bee (*Apis mellifera* L.). Environmental Toxicology and Chemistry. (In Press).

Hoffmann, I., (1960) Rearing worker honey bee larvae in an incubator. Bee World 41:10-11

Jay, S.C., (1965) Laboratory rearing studies of the postcapping stages of the honey bee (Apis mellifera L.) I. Rearing brood outside cells. Can.J. Zool. 43:541-552

Jung-Hoffmann, I., (1966) Die determination von Königin und Arbeiterin der Honigbiene. Z. Bienenforsch. 8:296-322

Kaftanoglu, O., Linksvayer, T., Page R.E. Jr. (2010) Rearing honey bees (*Apis mellifera* L.) *in vitro*: effects of feeding intervals on survival and development. *Journal of Apicultural Research* 49: 311-317.

Kaftanoglu, O., Linksvayer, T., Page R.E. (2011) Rearing honeybee larvae in vitro. 1: Effects of sugar concentrations on survival and development. Journal of Insect Science 11: 96. available online: insectscience.org/11.96

Lindauer, M., (1952) Ein Beitrag zur frage der Arbeitsteilung im Bienenstaat. Z. vergl. Physiol. 34:299-345

Michael, A. S., Abramovitz, M. (1955) A new method of rearing honey bee larvae in vitro. J. Econ. Ent. 48:43-44

Mitsui, T., Sagawa, T., Sano, H., (1964) Dtudies on rearing honey bee larvae in the laboratory. I. The effects of royal jelly taken from different ages of queen cells on queen differentiation. J. econ. Ent. 57:518-521

Peng, Y.S.C., Mussen, E., Fong, A., Montague, M.A., Tyler, T. (1992) Effects of chlortetracycline on honey bee worker larvae reared in vitro. J. Invertebrate Pathology. 60:127-133

Rembold, H., (1965). Biologically active substances in royal jelly. Vitamines and Hormones. 23:359-382

Rembold, H., Hanser, G., (1964) Über den Weiselzellenfuttersaft der Honigbiene VIII. Nachweis des determinierenden Prinzips im Futtersaft der Königinnenlarve. Hoppe Seyler's Z. Physiol. Chem. 339:251-254