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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

A COMPARATIVE STUDY ON THE QUALITY OF HONEY BEE (*Apis mellifera*) QUEENS DEVELOPED FROM LARVAE AFTER THE COLLECTION OF ROYAL JELLY

Bal Arılarında (*Apis mellifera*) Arı Sütü Toplama Sonrası Larvalardan Geliştirilen Ana Arıların Kalitesi Üzerine Karşılaştırmalı bir Çalışma

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ABSTRACT

Rearing bee queens is almost done utilizing grafting young larvae while the effects of grafting using old larvae after the collection of royal jelly on the quality of queens are not known. In fact, the production of royal jelly depends on grafting, then discarding the larvae to collect the royal jelly. This study aimed to investigate this point by grafting old larvae after removing them from their original cells without food. Larvae at age about 2 days were grafted into plastic queen cell cups (selection and grafting method or S&G method) leaving royal jelly behind and then resultant queens were compared with naturally reared ones (or NQ). The study showed the absence of significant variations between the queens reared from the two methods in characteristics of queens and cells. Meanwhile, no significant differences were found in regard to the performance of colonies. The colonies with queens from S&G method had slightly higher performance than those with NQ. The study concluded that grafting using old larvae without their original food does not impair the quality of queens. During the production of royal jelly, larvae may be grafted into new cells to continue their normal development instead of discarding them.

Key Words: *Apis mellifera*, morphology, performance, rearing, cells

ÖZ

Ana arıların yetiştirilmesi genç larvaların aşılanmasıyla neredeyse tamamlanırken, arı sütünün toplanmasından sonra eski larvaların aşılanmasının kraliçe arıların kalitesine etkisi bilinmemektedir. Aslında, arı sütü üretimi aşılamaya ve ardından arı sütünü toplamak için larvaları atmaya bağlıdır. Bu çalışma, eski larvaları yemeksiz olarak orijinal hücrelerinden çıkardıktan sonra aşılayarak bu noktayı araştırmayı amaçlamıştır. Yaklaşık 2 günlük olan larvalar, geride arı sütü bırakarak plastik kraliçe hücre kaplarına (seçme ve aşılama yöntemi veya S&G yöntemi) aşılandı ve ardından ortaya çıkan kraliçeler, doğal olarak yetiştirilenlerle (veya NQ) karşılaştırıldı. Çalışma, iki yöntemden yetiştirilen ana arılar arasında ana arı ve hücre özelliklerinde önemli farklılıkların olmadığını gösterdi. Bu arada, kolonilerin performansı açısından önemli bir fark bulunamadı. S&G yönteminden kraliçeleri olan koloniler, NQ'ya sahip olanlardan biraz daha yüksek performans gösterdi. Çalışma, orijinal besinleri olmadan eski larvaları kullanarak aşılamanın kraliçelerin kalitesini bozmadığı sonucuna varmıştır. Arı sütünün üretimi sırasında larvalar, onları atmak yerine normal gelişimlerini sürdürmek için yeni hücrelere aşılanabilir.

Anahtar Kelimeler: *Apis mellifera*, morfoloji, performans, yetiştirme, hücreler

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Ana arı yetiştiriciliği genç larvaların aşılanmasıyla neredeyse tamamlanırken, arı sütü toplandıktan sonra eski larvaların aşılanmasının kraliçe arıların kalitesine etkisi bilinmemektedir. Aslında, arı sütü üretimi aşılamaya ve ardından arı sütünü toplamak için larvaları atmaya bağlıdır. Bu çalışma, eski larvaları yemeksiz olarak orijinal hücrelerinden çıkardıktan sonra aşılayarak bu noktayı araştırmayı amaçlamıştır.
Gereçler ve yöntemler: yaklaşık 2 günlük larvalar, geride arı sütü bırakarak plastik kraliçe hücre kaplarına aşılandı (seçme ve aşılama yöntemi veya S&G yöntemi) ve ardından ortaya çıkan kraliçeler, doğal olarak yetiştirilenlerle (veya NQ) karşılaştırıldı. Bu çalışmada kullanılan koloniler, ana arı içermeyen besin peteklerinin yanında yumurta içeren kuluçka peteklerine sahipti. Kraliçe hücrelerinin özellikleri, uzunluk, taban genişliği ve uç genişliği dahil olmak üzere ölçüldü. Ortaya çıkan ana arıların taze ağırlığı, göğüs genişliği, ön kanat uzunluğu ve ön kanat genişliği dahil olmak üzere ana arı özellikleri incelenmiştir. Ayrıca, arılarla kaplı peteklerin sayısı sayılmış ve kapalı kuluçka, depolanmış bal ve depolanmış arı ekmeği alanları ölçülmüştür.
Bulgular: Çalışma, iki yöntemle yetiştirilen ana arılar arasında ana arı ve hücre özelliklerinde önemli farklılıkların olmadığını gösterdi. S&G ve NQ arasındaki fark sırasıyla vücut ağırlığı, ön kanat uzunluğu, ön kanat genişliği ve göğüs genişliği için sadece 1,2 mg, 0,01 mm, 0,02 mm ve 0,1 mm ve hücre tabanı, hücre için 0,56, 0,38 ve 0,04 mm idi. sırasıyla uzunluk ve uç genişliği. Bu arada, kolonilerin performansı açısından iki yöntem arasında anlamlı bir fark bulunamadı. S&G yönteminden ana arılı koloniler, petek sayısı, kapalı kuluçka alanı, depolanan bal alanı ve depolanan arı ekmeği alanı için sırasıyla 0,4 petek, 69,67, 45,17 ve 246,45 cm2 ile NQ'lu kolonilerden biraz daha yüksek ortalamalara sahipti. S&G yönteminden kraliçeleri olan koloniler, NQ'ya sahip olanlardan biraz daha yüksek performans gösterdi. Bu, S&G'den elde edilen kraliçe arıların kalitesinin doğal olarak yetiştirilenlere benzer olduğunu gösterdi.
Sonuç: Çalışma, orijinal besinleri olmadan eski larvaları kullanarak aşılamanın, ortaya çıkan bal arısı kraliçelerinin kalitesini bozmadığı sonucuna varmıştır. Ayrıca, bu yöntemle yetiştirilen ana arılarla yönetilen kolonilerin performansı etkilenmez. Arı sütünün üretimi sırasında larvalar, ekonomik faydaları en üst düzeye çıkarmak için onları atmak yerine normal gelişimlerini sürdürmek için yeni hücrelere aşılanabilir.

INTRODUCTION

Honey bee colonies headed with good young queens are expected to yield better productivity than those headed with older ones (Akyol et al. 2008, Hatjina et al. 2014, Junus 2019). There are various methods that can be employed by beekeepers to produce queens such as grafting (Zawislak and Burns 2012, Büchler et al. 2013, Given 2021). Also, grafting is widely used during the production of royal jelly (Zheng et al. 2011, Al-Kahtani and Taha 2020, Gemeda et al. 2020) and larvae are mostly discarded after the collection of royal jelly from cells. It is not known if these larvae can be used to obtain good queens instead of discarding them. Looking at the literature, comparing queen rearing methods was the main focus of some previous studies (Cengiz et al. 2009, Kumar 2018, Dhaliwal et al. 2019), while other studies focused on age of grafted larvae (Mahbobi et al. 2012, Okuyan and Akyol 2018), queen cell size and numbers (Al-Fattah et al. 2011, Wu et al. 2018, Adgaba et al. 2019), rearing months and seasons (Koç and Karacaoğlu 2011, Kamel et al. 2013, Önk et al. 2016), and grafting methods (Gene et al. 2005, Rafique et al. 2019). There are no studies however on grafting old larvae without their royal jelly.

In fact, queen rearing is regulated by bee workers (Hatch et al. 1999, Tarpy et al. 2004). In queenless colonies, orphan workers build many emergency queen cells (Abou-Shaara et al. 2021). However, the queen cells may be built over old larvae (Fell and Morse 1984, Tofilski and Czekonska 2004), especially bee workers select larvae for emergency queen rearing based on their nutritional status (Sagili et al. 2018). In the present study, to be able to compare naturally reared larvae and those removed from their cells, the age of larvae over which queen cells were built was controlled. Also, grafting was used to move larvae from their queen cells into new plastic cell cups. Then, the quality of the emerged queens was assessed in comparison with the naturally reared ones.

Parameters commonly considered to compare queens resulting from various methods include cell characteristics, body weight, and morphological characteristics of queens (Al-Ghzawi and Zaitoun 2008, Cengiz et al. 2009, Al-Fattah et al. 2011, Mahbobi et al. 2012, Kamel et al. 2013, Önk et al. 2016, Okuyan and Akyol 2018, Mattiello et al. 2022), and performance of colonies including brood rearing and colony productivity (Gençer et al. 2000, Mahbobi

et al. 2014, Kumar 2018). Such parameters were included in the comparison between queens naturally reared in their cells and those grafted away from their original cells. This study presents also some insights into the development of honey bee queens and provides a method to utilize queen larvae after the collection of royal jelly instead of discarding the larvae.

MATERIALS AND METHODS

Grafting using old larvae

In this study, queenless colonies (Carniolan hybrid bees) were allowed to rear their own queens from combs containing eggs followed by grafting. Firstly, the queens were removed from the colonies. Secondly, queen cells containing larvae at age of about two days were selected. Thirdly, these larvae were moved from their natural cells into plastic queen cell cups. Then, grafted queens were left inside the colonies until the sealing of cells, and then the cells were placed in an incubator (34±1°C, and 80% RH) until hatching. This method was applied in particular to be able to compare queens reared under the same conditions and meanwhile to mimic the process of royal jelly production by removing the larvae without their food. To simplify the study, this method of removing larvae from their cells into new cups was named selection and grafting method (S&G).

Experimental setup.

Queens reared from S&G method were compared with naturally reared queens (NQ) in the colonies. To do this, Carniolan hybrid colonies with the same strength (each with 10 combs covered with bees: 6 combs each with brood and 4 combs each with stored pollen/honey) from an apiary at Damanhour city were used in the study during March – July. There are good sources for nectar/pollen during this period at the study area. The study started with eight colonies but finally, five colonies were considered in the study. Combs with capped brood and old larvae were replaced by new empty combs to ensure the availability of eggs before splitting the colonies. Then, each colony was divided into two small colonies (each with 5 bee combs: 3 brood combs containing mainly eggs and 2 food combs covered completely with bees) placed in 10-frame Langstroth beehives without queens. All colonies were supplied temporarily with 2 combs of capped brood to increase their strength. The first 5 small colonies were allowed to rear queens naturally (NQ) while their sister 5 small colonies were used to obtain queens using S&G method. Each colony was able to rear >13 queen cells, and 10 of them were used in measuring the following parameters.

Cell characteristics

A digital caliper was used to measure the length, base width, and tip width of queen cells for 10 cells from each colony (a total of 50 cells per group).

Queen characteristics

The fresh weight of the emerged queens was determined using an electronic balance. Also, body characteristics related to body size were measured including thorax width, forewing length, and forewing width. The thorax width was measured using a digital caliper while forewing length and width were measured according to Ruttner et al. (1978) using Scan Photo Technique (El-Aw et al., 2012) after scanning the wings at 1200 dpi using a scanner (Canon LiDE 110, k10352, Vietnam). The measurements were taken for 10 queens per colony with a total of 50 queens per group.

Colony performance

The number of combs covered with bees was counted. While a frame divided into grids of cm^2 (Jeffree 1958) was used to measure areas of sealed brood, stored honey, and stored bee bread. These areas were measured after two months from the start of egg laying by the new queens in the 10 colonies (5 per each group) as an indicator for the performance of queens reared by the two methods.

Statistical analysis

The measured parameters for the two groups were compared using t-test. The variations were considered significant when $P \leq 0.05$. The analysis was done using SPSS v. 16 (SPSS for Windows 2007, Chicago, USA).

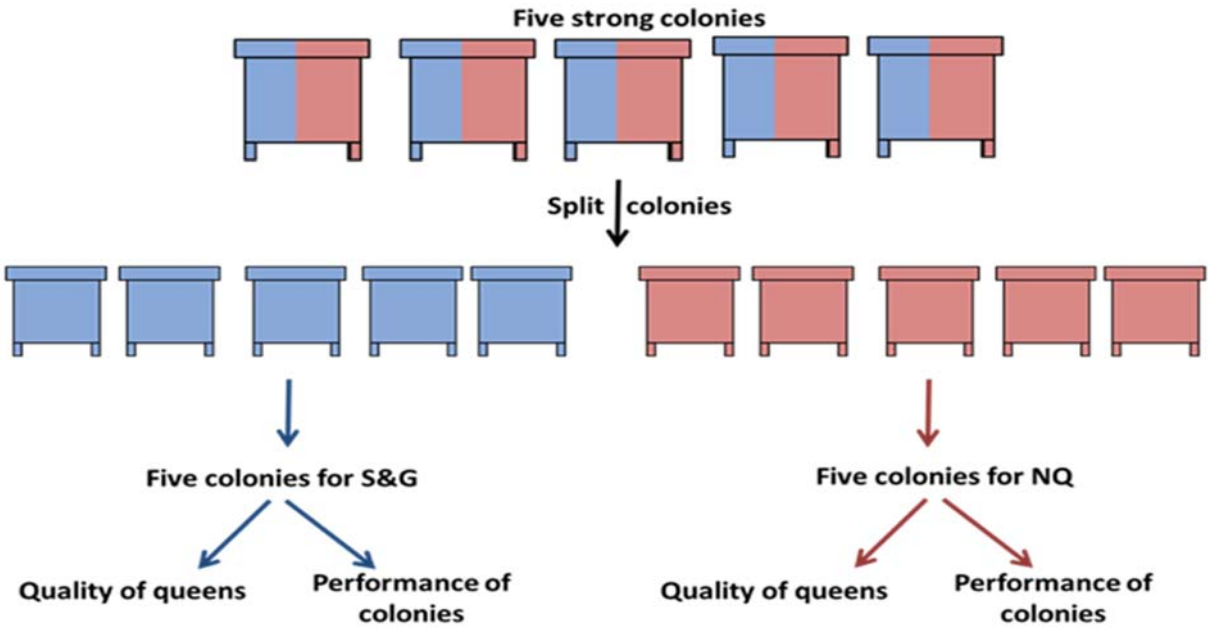

Fig.1: The experimental setup of the study to compare between naturally reared queens (NQ) and queens reared by selection and grafting method (S&G).

RESULTS

Cell characteristics

No significant differences (P>0.05) were found between the two groups in cell length and tip width while base width differed significantly (P<0.05) between them as shown in Table 1. The measured characteristics of cells were slightly higher in S&G method than NQ, except base width. The difference between the two groups was 0.56, 0.38, and 0.04 mm for base width, cell length, and tip width, respectively.

Table 1: Cell characteristics (Mean±SE) of natural queens (NQ) and queens from selection and grafting (S&G) method.

Characteristics	Mean±SE (mm)		t-test
	NQ	S&G	
Base width	11.56±0.16	11.00±0.00	(t=3.35, P=0.001)
Length	17.56±0.25	17.94±0.21	(t=1.1, P=0.25)
Tip width	6.42±0.11	6.46±0.12	(t=0.22, P=0.81)

Queen characteristics.

No significant differences (P>0.05) were found between the two groups in all characteristics (Table 2). Naturally reared queens had slightly higher values in the measured characteristics than S&G queens. The difference between queen characteristics of the two groups was 1.2 mg, 0.01 mm, 0.02 mm, and 0.1 mm for body weight, forewing length, forewing width, and thorax width, respectively.

Table 2: Queen characteristics (Mean±SE) of natural queens (NQ) and queens from selection and grafting (S&G) method.

	Mean±SE		
Characteristics	**NQ**	**S&G**	**t-test**
Weight (mg)	198.34±1.35	197.14±1.27	(t=0.64, P=0.51)
Forewing length (mm)	9.11±0.007	9.10±0.008	(t=1.42, P=0.15)
Forewing width (mm)	3.01±0.010	2.99±0.011	(t=0.99, P=0.32)
Thorax width (mm)	4.82±0.05	4.72±0.06	(t=1.18, P=0.23)

Colony performance.

No significant differences (P>0.05) were found between them in measured parameters (Tables 3). The colonies headed with NQ or queens from S&G method showed approximately the same performance level. The number of combs covered with bees, after two months, ranged from 3 to 5 combs for the both groups. The colonies with queens from S&G method had slightly higher means than colonies with NQ by 0.4 comb, 69.67, 45.17, and 246.45 cm^2 for the number of combs covered with bees, sealed brood area, stored honey area, and stored bee bread area, respectively.

Table 3: Parameters (Mean±SE) of colonies headed with natural queens (NQ) and queens reared from selection and grafting (S&G) method.

	Mean±SE		
Parameters	**NQ**	**S&G**	**t-test**
Number of combs	3.80±0.37	4.20±0.49	(t=0.64, P=0.53)
Sealed brood area (cm^2)	1406.45±119.22	1476.12±225.24	(t=0.27, P=0.79)
Stored honey area (cm^2)	709.67±145.34	754.84±338.87	(t=0.12, P=0.91)
Stored bee bread area (cm^2)	529.03±120.95	775.48±177.92	(t=1.14, P=0.28)

DISCUSSION

The best quality of queens was found when queens were reared from 1 to 2 days old larvae (Gençer et al. 2000, Mahbobi et al. 2012, Okuyan and Akyol 2018, Dhaliwal et al. 2019). In the present study, workers in queenless colonies accepted the grafted old larvae when moved into new plastic queen cell cups. Accordingly, Staron et al. (2019) recorded low death rates when grafting old larvae, suggesting the good survival of old larvae after grafting. The two methods used in this study had approximately similar cell characteristics without significant differences in cell length and tip width. This indicates the lack of any negative impacts of the S&G method on the ability of bees to construct normal cells on older grafted larvae. The significant differences between the two methods in cell base can be explained by using plastic cups with fixed width in S&G method than the naturally built queen cells.

The measured queen characteristics proved that queens from S&G method were not different from those reared naturally. This supports the idea that grafting old queen larvae into new cells did not affect the subsequent development of queens. Based on bee subspecies, the queen weight of 190-200 mg can be considered as moderate queens (Kahya et al. 2008) or heavy queens (Al-Fattah et al. 2011, Dhaliwal et al. 2019); therefore, queens developed from the two rearing methods can be considered at least as moderate queens. This indicates the good quality of queens; especially queen weight is a good indicator for colony productivity (De Souza et al. 2013) and queen quality (Wilkinson and Brown 2002, Kahya et al. 2008, Hatjina et al. 2014), and large queens have large spermatheca and can store more sperms (Collins and Pettis 2013). In line with the obtained results, the weight of queens from grafting method (189.80 mg) was higher than naturally reared queens in queenless colonies (Kumar 2018). The measurements of forewing length and width recorded in this study for queens from the two groups were similar to those recorded by Kamel et al. (2013) for bee queens in Egypt reared during different months.

Despite its importance, queen weight has no role in the acceptance of queens by bee workers as well as the beginning of oviposition (Medina and Goncalves 2001). The commencement of egg laying after mating is impacted by many factors (Woyke and Jasinski 1990, Schlüns et al. 2005), and can range from a few days to one month (Moritz and Kuhnert 1984, Cobey 2007). In the present study, all queens developed from the two groups were observed to lay eggs within the first two weeks after emergence and had similar performance. The insignificant variations between performance parameters reflected that queens from the two methods were able to naturally mate in a similar way. It is known that poor queen mating can impair its egg laying ability, and subsequently colony development (Abou-Shaara et al. 2021). But in this study colonies were developed over two months in a similar way.

The obtained results are somewhat supported by a previous study, wherein the queens from the grafting method were better than naturally reared queens in the studied parameters including areas of brood, pollen, and honey (Kumar, 2018). The higher brood area in S&G group can be explained by the higher area of stored food than NQ group. Accordingly, a relationship was found between stored pollen area and brood rearing activity (Abou-Shaara et al. 2013). Gençer et al. (2000) recorded significant differences in brood rearing activity and the number of combs covered with bees in colonies headed with heavy and light queens. On the contrary, all queens from the two groups in this study were approximately with the same weight, and thus had no variations in their performances.

Conclusion

This study tested the effects of grafting old larvae without their food (S&G method) on the quality of queens in comparison with naturally reared ones. To mimic the situation during royal jelly production as larvae are discarded after royal jelly collection, but here the larvae were grafted again into new plastic cups. This method yields queens with similar quality to those reared naturally inside hives. The results proved that the transportation of old larvae into new cells (i.e. plastic queen cell cups) did not affect negatively on the quality of queens and colony performance. The comparison between naturally reared queens and queens reared using S&G showed the absence of high variations in queen cell characteristics and queen morphology. Also, the performance of colonies headed with queens from the two methods showed insignificant variations. On the beekeeping scale, royal jelly producers may plan to utilize the larvae in queen rearing instead of discarding them but accelerate the process of royal jelly collection to be done with younger larvae (< 3 days). This study also shows that the interruption in the feeding of larvae may not pose serious effects on their development and quality. More studies using different honey bee subspecies are advised.

Author contribution: The author designed, performed, analyzed the data, wrote and revised the manuscript.

Conflict of Interest: No conflict of interests to be reported.

Ethical issue: Not applicable because this study on honey bees and not animals or humans.

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