Live Weight of Queen Honey Bees (*Apis Mellifera* L.) Predicts Reproductive Characteristics

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Live Weight of Queen Honey Bees (*Apis mellifera* L.) Predicts Reproductive Characteristics

Ethem Akyol,[1] Halil Yeninar,[2] and Osman Kaftanoglu[3]

ABSTRACT: The study was conducted to determine the effects of queen body weight at emergence on the mating ratio, acceptance of the mated queens by the queenless colonies, onset of oviposition, diameter of spermathecae, the number of spermatozoa in the spermathecae, and the egg-laying rate of the queens.

The queens were categorized in three groups; Heavy (207.63 ± 0.95 mg), Medium (193.47 ± 0.96 mg) and Light (175.00 ± 0. 62 mg) according to weight at the emergence. Acceptance rates in heavy, medium and light queen groups were 93.33%, 86.66% and 66.66%; average mating ratios were 93.3%, 90.0% and 83.3%; and the onset of oviposition was 8.5, 8.8 and 9.8 days after emergence, respectively. The diameters of spermathecae and the numbers of spermatozoa in the spermathecae were 1.25, 1.06, 0.86 mm; and 5.2, 4.8 and 4.2 million respectively. The amounts of brood area, 30 days after the onset of oviposition were 6605.2 ± 63.6, 5571.3 ± 90.3 and 4520.2 ± 58.3 cm^2 in the colonies headed by the heavy, medium and light queens. Statistically important correlations were found between queen weight and the diameter of the spermatheca ($r = 0.98$), the number of spermatozoa in the spermatheca ($r = 0.97$) and egg-laying rate of the queens ($r = 0.90$).

KEY WORDS: *Apis mellifera anatoliaca*, queen weight, queen quality, onset of oviposition

Bal Arilarinda (*Apis mellifera* L) Bazi Üreme Özelliklerinin Belirlenmesinde Ana Ari Ağirliğinin Kullanimi

ÖZET: Bu çalışma, gözden çıkıştaki ana arı ağırlığının ana arıların, çiftleşme oranına, çiftleşmiş ana arıların anasız koloniler tarafından kabul oranına, çiftlşme öncesi süreye, spermateka çapına, spermatekadaki spermatozoa sayısına ve yumurtlama kapasitesine etkisini belirlemek amacıyla yürütülmüştür. Ana arılar gözden çıkıştaki ağırlıklarına göre Ağır (207.63 ± 0.95 mg), Normal (193.47 ± 0.96 mg) ve Hafif (175.00 ± 0. 62 mg) olmak üzere üç gruba ayrılmışlardır. Ağır, normal ve hafif gruplarda ortalama kabul oranları sırasiyla %93.3, %86.66 ve %66.66; ortalama çiftleşme oranları, %93.3, %90.0 ve %83.3; ortalama çiftleşme öncesi süreleri, 8.5 gün, 8.8 gün ve 9.8 gün olarak belirlenmiştir. Ağır, normal ve hafif gruplarda ortalama spermateka çapı, 1.25 mm, 1.06 mm, 0.86 mm ve spermatekadaki spermatozoa sayısı, 5.2 milyon, 4.8 milyon ve 4.2 milyon/ana arı olarak sayılmıştır. Ana arılar yumurtlamaya başladıktan 30 gün sonra ağır, normal ve hafif gruplarda yavrulu alan ortalama 6605.2 ± 63.6 cm^2, 5571.3 ± 90.3 cm^2 ve 4520.2 ± 58.3 cm^2 olarak ölçülmüştür. Ana arı agırlığı ile spermateka çapı arasında $r = 0.98$, spermatozoa sayısı arasında $r = 0.98$, ve ana arının yumurtlama kapasitesi arasında $r = 0.90$ gibi yüksek ve istatistiki olarak önemli korelasyonlar belirlenmiştir.

ANAHTAR KELIMELER: *Apis mellifera anatoliaca*, ana ağırlığı, ana kalitesi, yumurtlama öncesi süre

Introduction

There is a great beekeeping potential in Türkiye and the government is supporting beekeeping projects to increase the number of colonies and honey production. There

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are approximately 5 million colonies in the country (Anonymous, 2005). Even though commercial queen rearing started in the late 1970's and queen production increased from 3000 to over 250,000 per year over the years, queen rearing in Türkiye is not sufficient to supply the demand. The government is subsidizing the price of queens in order to support the beekeepers and offers practical queen rearing courses to develop the queen rearing industry. As a result, hundreds of new queen breeders try to raise queen bees from unknown or untested stocks and thereby sell queens of uneven quality to beekeepers.

The quality of the queens are affected by genotype, nutrition, rearing methods, rearing season, age of larvae at grafting time and the number of larvae grafted per cell builder colony (Kaftanoğlu *et al.*, 1992). Woyke (1971) reported that body weight and ovariole numbers of virgin queens as well as the number of spermatozoa in the spermathecae of the queens could be increased by rearing queens from brood grafted at the earliest possible age. Body size or queen weight can be used as a quality factor for judging the queens since heavier queens have more ovarioles, larger spermathecae and more spermatozoa in the spermathecae (Woyke, 1971; Kaftanoğlu *et al.*, 1992; Kaftanoğlu *et al.*, 2000). Such queens can lay more eggs and can be kept longer in the colonies. Fıratlı (1982) also emphasized that queen weight at emergence could be used as a selection criterion.

The aim of this study is to determine the predictability of quality parameters and reproductive characteristics of queens based on live weights at emergence.

Materials and Methods

This study was conducted in Central Anatolia (Niğde province, 35°04′10″E longitude, 37°56′25″N latitude and 1260 m altitude) in May and June 2005. *Apis mellifera anatoliaca* Maa (Muğla ecotype) colonies were used in the experiment.

One-day-old larvae were grafted into beeswax queen cell cups and they were placed into queenless cell builders (Laidlaw, 1979). The queen cells were harvested from the cell builders 11 days after grafting and then placed into the mating nuclei ($25 \times 15 \times 10$ cm) for emergence.

The weights of the queens were measured with an electronic balance after emergence. A total of 90 queens were divided into three weight groups which were; **Heavy** queens that were 200 mg or heavier ($\bar{x} = 207.63 \pm 0.95$ mg), **Medium** queens between 185 and 199 mg ($\bar{x} = 193.47 \pm 0.96$ mg), and **Light** queens lighter than 185 mg live weight ($\bar{x} = 175.00 \pm 0.62$ mg). The queens were checked daily in order to determine the onset of oviposition and mating ratio (MR). Mating ratio was calculated as the percentage of queens that mated successfully.

Ten queens from each group were dissected 20 days after the onset of oviposition and the diameter of the spermatheca was determined by using an ocular micrometer. The number of spermatozoa in the spermathecae was estimated using a haemocytometer (Mackensen and Tucker, 1970). Fifteen queens from each group were removed from the mating nuclei 30 days after the onset of oviposition and introduced into five framed queenless colonies in order to determine acceptance rates and brood production. The size of sealed brood was measured 30 days after the queen introduction and was calculated by using the Puchta method (Fresnaye and Lensky, 1961). Mating ratios and acceptance rates were analyzed using Chi Square (χ^2) non-parametric tests (Cooley and Lohnes, 1971). Randomized plot design

(ANOVA) was used to test the onset of oviposition, diameter of spermatheca, and number of spermatozoa in the spermathecae and the laying rate of queens. Duncan's Multiple Range Test was used to compare the means between the weights of the groups. Linear Regression analysis was applied to determine the correlations between dependent and independent variables (Little and Hills, 1975; Görgülü and Şahinler, 2006).

Results

The average weights of the queens at emergence, mating ratios and onset of oviposition of queens in each group are summarized in Table 1.

Table 1. The averages weight (mg), mating ratios and onset of oviposition of the queens in each experimental group.

Weight classes of queens (♀)	Average weight(mg) of queens (♀)		Range	Average mating ratios(%)		Average onset of oviposition(days)	
	N	$\bar{x} \pm S\bar{x}$		N	$\bar{x}$ (%)	N	$\bar{x} \pm S\bar{x}$
Heavy	30	207.63 ± 0.95a*	200–219	30	93.33	28	8.50 ± 1.14 b**
Medium	30	193.47 ± 0.96b	185–199	30	90.00	27	8.81 ± 1.21 b
Light	30	175.00 ± 0.62c	168–179	30	83.33	25	9.84 ± 1.18 a

*Different letters indicate significant differences ($P < 0.01$), **Different letters indicate differences ($P < 0.05$).

The weights of the Heavy, Medium and the Light queens were statistically significant among the groups (ANOVA $F_{2,89} = 364.3$ $P < 0.01$). The average live weight of the queens was 192.03 ± 0.84 mg and ranged from 168 to 219 mg.

The overall mating ratio (MR) was 88.89% and varied between 83% and 93% (Table 1). There was no significant difference in MR among the groups ($P > 0.05$); however it gradually decreased as the weights of the queens decreased. The Heavy group queens had about 10% higher MR than the Light group queens.

The average onset of oviposition for all queens was found to be 9.02 ± 1.29 days after emergence and varied from 7 to 12 days (Table 1). The Heavy and Medium group queens started oviposition about 1 day earlier than the Light group queens and the difference was statistically significant (ANOVA $F_{2,79} = 9.23$, $P < 0.05$).

The average acceptance rates of the mated queens, diameter of the spermatheca, number of spermatozoa in the spermatheca and brood areas of the colonies, headed by the Heavy, Medium and Light queens are summarized in Table 2. The overall

Table 2. The acceptance rates of the mated queens into queenless colonies, Diameter of Spermathecae, Number of Spermatazoa in the Spermathecae and Amount of Brood area(cm^2)

Weight classes of queens (♀)	Acceptance rates of queens (♀)		Diameter of the spermathecae (mm)		Number of spermatozoa (million)	Amount of brood area (cm^2)	
	N	(%)	N	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	N	$\bar{x} \pm S\bar{x}$
Heavy	15	93.33 a**	10	1.258 ± 0.2 a*	5.19 ± 0.2 a*	10	6605.25 ± 63.66a*
Medium	15	86.66 ab	10	1.061 ± 0.3 b	4.75 ± 0.2 b	10	5571.30 ± 90.33b
Light	15	66.66 b	10	0.861 ± 0.2 c	4.15 ± 0.1 c	10	4520.25 ± 58.36c

*Different letters indicate significant differences ($P < 0.01$), **Different letters indicate significant differences ($P < 0.05$).

acceptance rate was found to be 82.22% and ranged between 66.66% and 93.33%. The acceptance rate was the highest (93.33%) in the Heavy group and the lowest (66.66%) in the Light group ($P < 0.05$). Similar to the MR results, the acceptance rates of the queens by the queenless colonies increased as the weight of the queens increased. Only 7% of the queens were rejected in the Heavy weight group, whereas 33% were killed in the Light weight group.

The overall diameters of the spermathecae of queens in the Heavy, Medium and Light groups were 1.258 ± 0.20 mm, 1.061 ± 0.28 mm, and 0.861 ± 0.18 mm respectively and they all were statistically significant from each other (ANOVA $F_{2,29} = 75.72$, $P < 0.01$). The Heavy queens had 18.6% and 46.1% bigger spermathecal diameter than that of the Medium and Light weight queens respectively.

The average numbers of spermatozoa in the spermathecae of the Heavy, Medium and Light queens were found to be 5.19 ± 0.21 million, 4.75 ± 0.21 million and 4.15 ± 0.16 million respectively and they were all significantly different from each other (ANOVA $F_{2,29} = 70.60$, $P < 0.01$). The Heavy group queens had 9.3% and 25.06% more spermatozoa in the spermathecae than that of the Medium and Light queens respectively.

The average brood production of the Heavy, Medium and Light weight groups were found to be 6605.25 ± 63.66 cm^2, 5571.30 ± 90.33 cm^2, and 4520.25 ± 58.36 cm^2 respectively and they were also significantly different from each other (ANOVA $F_{2,29} = 19.15$, $P < 0.01$). The colonies headed by the Heavy group queens had 18.56% and 46.25% more brood than that of the colonies headed by the Medium and Light weight queens respectively.

There were high and significant correlations between queens' weights and brood production ($r = 0.90$, $P < 0.01$, $n = 30$) (Fig. 1); number of spermatozoa in the spermathecae ($r = 0.97$, $P < 0.01$, $n = 30$) (Fig. 2); and diameter of spermathecae ($r = 0.98$, $P < 0.01$, $n = 30$) (Fig. 3). There was also an important correlation between diameter of spermathecae and the number of spermatozoa in spermathecae ($r = 0.97$, $P < 0.01$, $n = 30$).

Discussion

Since the quality of the queen bees depends on genotype and environmental factors, good quality queens can be raised by grafting 1 day old larvae from the superior breeder colonies and rearing them in strong cell builders, during the main nectar flow or swarming season (Kaftanoğlu *et al.*, 1992). The genotype of the larvae is the most important criterion for the quality of the queens. Queen breeders are using selective breeding programs to improve the genetics of the bees for honey production, gentleness, and disease resistance (Rotenbuhler, 1964; Spivak, 1996; Spivak and Gilliam, 1998; Harbo and Harris, 1999; Spivak and Reuter, 2001; Wilkes and Oldroyd, 2002). Without selection and controlled mating it is almost impossible to improve or maintain genetic stocks.

There are several species and subspecies of *Apis mellifera* in different geographical regions and great morphological and physiological variations among the races and ecotypes in Türkiye (Fıratlı and Budak, 1994; Smith *et al.*, 1997; Güler and Kaftanoğlu, 1999; Palmer *et al.*, 2000; Kandemir *et al.*, 2000; Akyol *et al.*, 2006). There is a breeding program for the Caucasian bees (*A. m. caucasica*) by the TEMA Foundation in Artvin province to improve honey production and resistance to

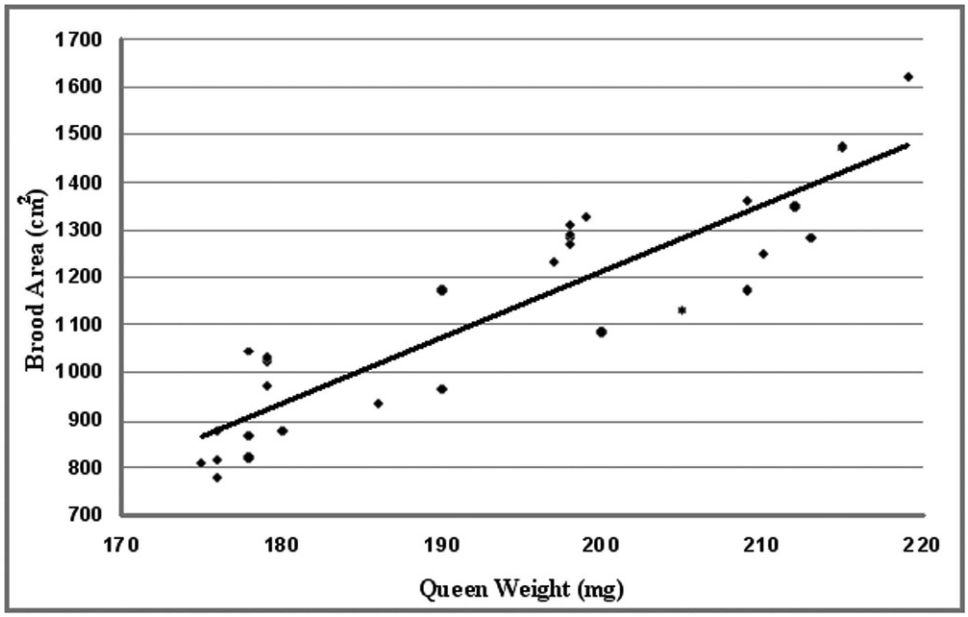

Fig. 1. Correlation between queen weights and brood area (cm^2).

diseases. Similar breeding programs should be applied to the other races and ecotypes to rear better quality and more productive queens.

As the rearing conditions (age of the larvae, temperature, nutrition, strength of the cell builder etc) improved, the live weight and the quality of the queens increased regardless of the genetics of the breeder. It is clear that live weight of queens at

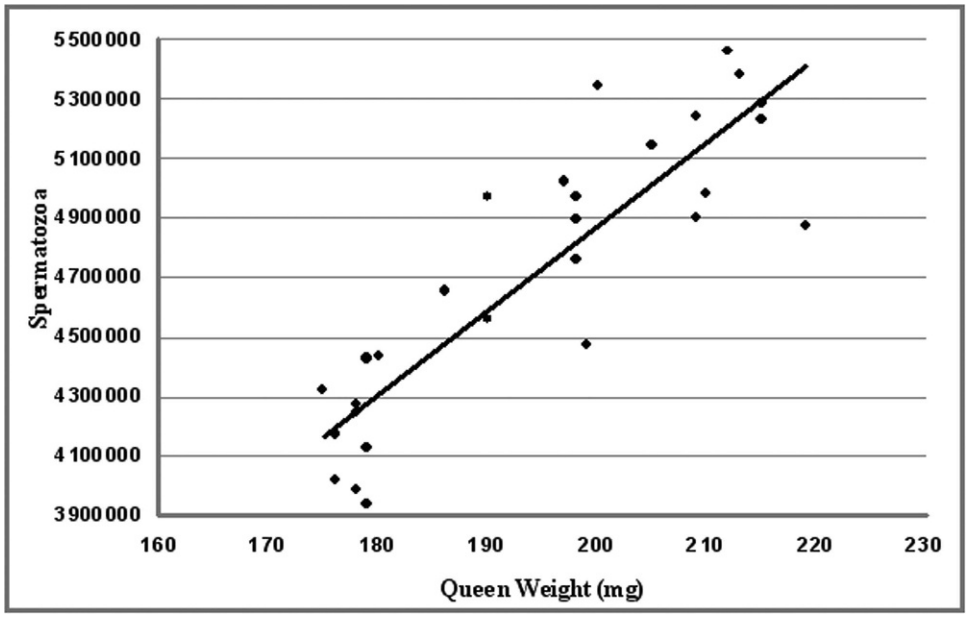

Fig. 2. Correlation between queen weights and spermatazoa numbers.

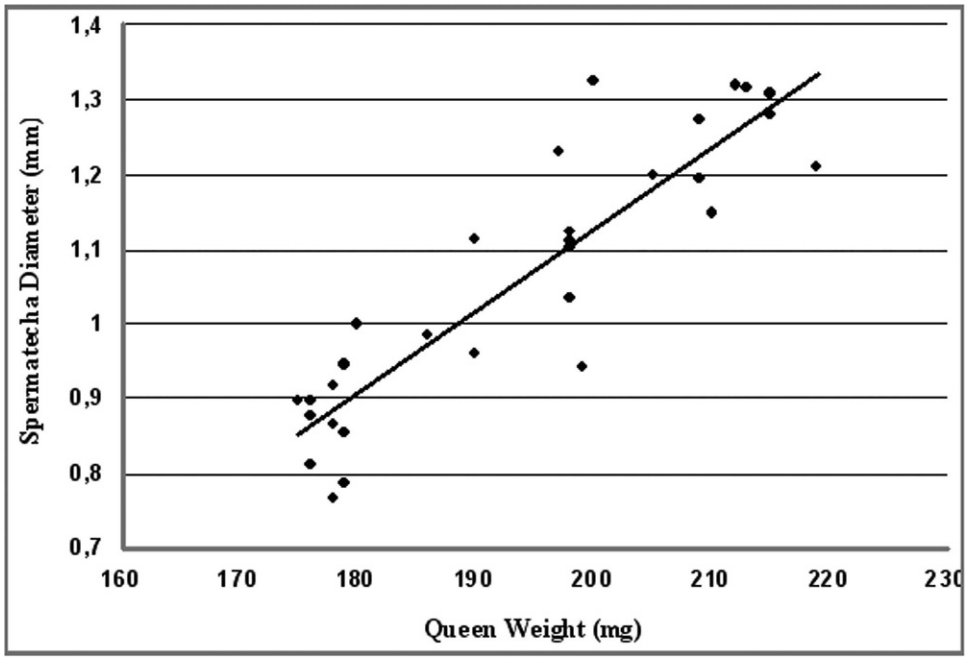

Fig. 3. Correlation between queen weights and diameter of spermathecae (mm).

emergence is a good criterion for determining queen quality. Even though all larvae were grafted from the same breeder colony, there might be a huge paternal genetic influence because queens mate multiply. Therefore the weight differences at emergence may be the result of genetic variance coming from drones, age of the larvae and differences in cell builder colonies (strength, number of nurse bees, number of queen cells etc.).

The average live weight of the queens in our study (192.03 ± 1.49 mg) is similar to the results of Akyol and Kaftanoğlu (1995) which was 197.38 mg; higher than Gül and Kaftanoğlu (1986) and Kale (1992)' which were 181.13 mg, and 169.9 mg respectively; and lower than Güler and Kaftanoğlu (1999) and Kaftanoğlu *et al.* (2000) which were 205.83 mg and 204.0 mg respectively.

The average mating ratios of the current experiment were found to be similar with the results of Kaftanoğlu *et al.* (1988 and 1993)′ which were 89.3%, and 83.3%; higher than Kale (1992), Öztürk (1994), Güler and Kaftanoğlu's (1999) results which were 71.4%, 76.66%, and 79.64% respectively. Mating ratio can be increased by painting the nuclei in different colors and placing them in a sigmoid shape facing different directions (Kaftanoğlu *et al.*, 1992).

The average onset of oviposition of this experiment was similar to the results of Kaftanoğlu *et al.* (2000) which was 9.4 ± 0.733 days after emergence. The average onsets of oviposition of the Heavy group was nearly one day shorter than the Light group and, Heavy group queens were accepted 40% better than Light queens by queenless colonies.

The acceptance rate of queens by queenless colonies is very important for colony survival. Beekeepers buy queens, and re-queen the colonies in the spring or in the fall. If the queens are not accepted, worker bees raise new queens from existing

larvae. These natural queens will mate with unknown local drones and the beekeepers may end up having aggressive colonies. If the colony cannot raise a new queen, some worker bees start laying unfertilized eggs and soon become a laying worker colony. Laying worker colonies die within a couple of months if not requeened again. Lots of inexperienced beekeepers end up loosing their colonies in an effort to re-queen them personally.

There are many reasons for the rejection of an introduced queen. The presence of old or virgin queens in the colony will always cause the rejection. Beekeepers must inspect the colonies 7–8 days after the introduction of new queens and destroy all the natural queen cells. Queen quality is also very important for successful queen introduction. Good quality queens probably produce more pheromones, start laying sooner and become accepted. Moreover, the genetics of the colonies, climatic conditions, nectar and pollen flow, and queen introduction methods are also important factors for the successful queen introduction.

The diameter of the spermathecae and number of spermatozoa are good indicators of the queen quality (Kaftanoğlu *et al.*, 1992). If queens can store more spermatozoa they have the potential of laying more fertilized eggs and have a better chance of living longer. If queens have fewer spermatozoa they are most likely to be superseded within one year. The diameter of the spermathecae in Heavy and Medium group queens were similar to the results of Kaftanoğlu *et al.* (2000) which was 1.168 $\pm$ 0.155 mm but the average number of spermatozoa of the Heavy and Medium groups in this experiment was found to be higher than their results which was 4.015 $\pm$ 0.377 millions per queen.

The average amount of brood area (5565.60 cm^2) of current study, in June, was similar to the results of Akyol *et al.* (1999) and Akyol and Kaftanoğlu (2001), Şahinler and Kaya (2001) which were 6052.0 cm^2, 5738.18 cm^2, 6201.0 cm^2 respectively; and higher than Şahinler and Gül (2004) which was 3904.80 cm^2. Heavy queens probably have more ovarioles in the ovaries than the lighter queens as Fıratlı (1982) and Kaftanoğlu *et al.* (2000) stated.

Results of these experiments show that queen weight has a significant effect on the onset of oviposition, acceptance ratio of queens, diameter of spermathecae, number of spermatozoa in the spermathecae and laying rate of queens. Performance and productivity of honeybee colonies can be increased by using heavier queens.

Queen breeders should try to raise good quality, heavier queens and kill the smaller size queens when they inspect the mating nuclei 2–3 days after queen cell introduction. Beekeepers should use heavy queens in their colonies in order to get better acceptance, have populous and more productive colonies.

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