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ORIGINAL RESEARCH ARTICLE

Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods.

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Summary

Fifty queens weighed at emergence were allocated into three groups as: light (under 190 mg); moderate (between 190-200 mg); heavy (over 200 mg), and introduced into Kirchainer mating nuclei. The queens were then weighed at ages ranging from three to 40 days and the intervals between emergence, mating and onset of oviposition were determined. The weight of ovaries, size of spermathecae, and number of spermatozoa in the spermathecae were also measured by dissecting queens one month after they started oviposition.

The weight at emergence did not affect behavioural features of reproduction such as the time from emergence to mating flight and to onset of oviposition ($P > 0.05$). The weight of queens at emergence was, however, highly significantly ($P < 0.01$) correlated with the weights three days after emergence ($r = 0.572$), at the onset of oviposition ($r = 0.440$), three days after the onset of oviposition ($r = 0.562$), and one month after the onset of oviposition ($r = 0.808$). The highest correlation was found between the weights at emergence and one month after the onset of oviposition. Highly significant correlations also occurred between the weight at emergence and the diameter ($r = 0.619$), and the volume of spermatheca ($r = 0.607$). No correlation was found between the weight at emergence and the fresh and dry weight of ovaries in queens about 40 days old (one month after the onset of oviposition). This is explained by the number and maturation stage of eggs, which depend upon the colony condition. Discriminant analysis applied to data from all characters reallocated 89.5% of the queens to their pre assigned weight groups.

Peso a la emergencia de reinas de la abeja melífera (*Apis mellifera caucasica*) y su efecto sobre el peso en los periodos de pre y post apareamiento.

Resumen

Se pesaron cincuenta reinas a su emergencia y fueron asignadas en tres grupos como se indica a continuación: ligera (menos de 190 mg); moderada (entre 190-200 mg); pesada (superior a 200 mg), y se introdujeron en núcleos de apareamiento Kirchainer. Posteriormente, las reinas fueron pesadas a partir de los tres a 40 días de edad, determinándose también los intervalos entre emergencias, apareamiento e inicio de oviposición. Un mes después de iniciada la oviposición, las reinas fueron diseccionadas y se registró el peso de los ovarios, el tamaño de la espermateca y el número de espermatozoides en la espermateca.

El peso a la emergencia no afecta a características de comportamiento de reproducción tal como el tiempo desde la emergencia a los vuelos de apareamiento y al inicio de oviposición ($P > 0,05$). El peso de las reinas al emerger fue, sin embargo, altamente significativa ($P < 0,01$), correlacionado con el peso a los tres días de emergencia ($r = 0,572$), al inicio de la oviposición ($r = 0,440$), tres días después de iniciado la oviposición ($r = 0,562$), y un mes después de iniciado la oviposición ($r = 0,808$). Una alta correlación fue encontrada entre

el peso de emergencia y un mes después de iniciada la oviposición. También se observaron correlaciones altamente significativas entre el peso de emergencia y el diámetro (r = 0,619), y el volumen de la espermateca (r = 0,607). No se encontraron correlaciones entre el peso de emergencia y el peso fresco y seco de los ovarios en reinas con cerca de 40 días de edad (un mes después de iniciada la oviposición). Esto se explica por el número y fases de maduración de los huevos, los cuáles dependen de las condiciones de la colonia. El análisis discriminante aplicado a todos los caracteres reasignaron 89,5% de las reinas a su grupo con peso pre-asignado.

Keywords: honey bee, *Apis mellifera caucasica*, queen weight, mating, oviposition, ovary, spermatheca, reproduction

Introduction

It is generally accepted that the quality of honey bee queens is directly linked to the productivity of the colony. The weight is considered as one of the quality criteria of honey bee queens. The weight of queens at emergence shows a wide range because of factors such as the age of larvae, the season, the condition of rearing colonies and racial differences (Mirza *et al*., 1967; Woyke, 1971; Weiss, 1974; Gençer *et al*., 2000; Tarpy *et al*., 2000; Koç and Karacaoglu, 2004; Skowronek *et al*., 2004). Genetically different subfamilies within a group of bee larvae can also contribute in to the variability of emerged queens (Moritz *et al*., 2005). According to Woyke (1971), queen weight at emergence is correlated with the number of ovarioles in the ovaries and the size of the spermatheca.

The time interval from emergence until mating and the onset of oviposition is a critical period for honey bee queens. Several factors have been considered to affect mating and the onset of oviposition, such as season (Moritz and Kühnert, 1984; Severson and Erickson, 1989; Kaftanoglu and Kumova, 1990; Koç and Karacaoglu, 2004), climatic conditions (Lensky and Demter, 1985; Szabo *et al*., 1987; Da Silva *et al*., 1995), insemination type (natural or instrumental: Woyke, 1962; Ebadi and Gary, 1980; Kaftanoglu and Peng, 1982), mating apiary type (island, lowland or mountain: Neumann *et al*., 1999), brood status of the colony (Da Silva *et al*., 1995), number of attendant workers in the mating colony (Woyke and Jasinski, 1990) and racial origin of the mating colony (Hellmich II *et al*., 1986). There are conflicting reports about whether the weight of queens at emergence affects the onset of oviposition of queens. Szabo *et al*. (1987) and Medina and Gonçalves (2000) reported that the weight of queens at emergence did not affect the onset of oviposition, but Taranov (1974) found that heavy young queens started oviposition earlier than light ones. In contrast, Skowronek *et al*. (2002) found that heavier queens at emergence initiated oviposition later than moderate and lighter ones when they were instrumentally inseminated.

The live weight of a queen varies at different periods of her adult life as much as does weight at emergence (Shehata *et al*., 1981; Nelson and Gary, 1988; Nelson and Laidlaw, 1988; Skowronek *et al*., 2004). Reproductive status determines the weight of queens, and body weight changes during the sexual maturation process. According to Skowronek *et al*. (2004) and Harano *et al*. (2007), the body weight of virgin queens decreases gradually after emergence. Ovaries of virgin queens are morphologically different from those of laying queens (Patrício and Cruz-Landim, 2002). After the onset of oviposition, the weight recovers to the post emergence level (Skowronek *et al*., 2004). Mating causes remarkable changes in the queen's physiology and behaviour. It stimulates vitellogenesis and egg maturation (Tanaka and Hartfelder, 2004), ovary development and the onset of oviposition (Patrício and Cruz-Landim, 2003). The amino acid content in the haemolymph of queens reaches its highest level with the onset of oviposition (Hrassnigg *et al*., 2003). Ovaries of laying queens are about eight times as large as those of virgins (Shehata *et al*., 1981). Additionally, pheromone profiles of laying queens differ from those of virgins (Apsegaite and Skirkevicius, 1995; Richard *et al*., 2007). The morphology of ovaries also changes with season in laying queens. When queens are laying, they are relatively heavy and have well developed heavier ovaries (Shehata *et al*., 1981).

Since heavier queens at emergence have more ovarioles in the ovaries and larger spermatheca (Woyke, 1971), weight may also affect some other features. We therefore conducted this investigation to determine: 1. the effect of queen weight at emergence on some behavioural features such as the duration of the period of initiation of mating flights, and the onset of oviposition; 2. weight changes of honey bee queens after emergence, live weights at pre and post mating periods and weights and size of some internal reproductive characteristics of queens.

Materials and Methods

Experimental setup

The study was carried out in Ankara, Turkey between May and July 2005 when foraging conditions were good for ensuring a plentiful drone population. The virgin queens were reared from a breeder Caucasian (*Apis mellifera caucasica*) colony by the grafting method (Laidlaw, 1985). Mature queen cells were kept in an incubator at 34°C and were inspected at five minute intervals. Emerging queens were weighed on an electronic balance (Sartorius BP-121S) to the nearest 0.1 mg. Fifty virgins were introduced into newly established Kirchainer mating nuclei immediately after weighing. The queens were allocated into three weight groups: light (under 190 mg, n = 17); moderate (190-198 mg, n = 17); heavy (over 200 mg, n = 16). In order to identify the dates of mating and onset of oviposition of queens, mating nuclei were inspected regularly every day beginning from three days after emergence. The mating flights of queens were observed by inspecting mating nuclei frequently (every 10 minutes) between 14:00–18:00 hours on days when weather conditions were suitable for mating. The queens were inspected in their nuclei for the presence of mating signs when they returned from flights. Orientation flights, when queens returned without a mating sign, were disregarded.

The queens were weighed at: three days after emergence; one day after the last mating flight; the onset of oviposition; and three days after the onset of oviposition. When the queens became about 40 days old (one month after the onset of oviposition), they were weighed and then dissected to remove ovaries and spermatheca. Each pair of fresh ovaries was weighed on an electronic balance to the nearest 0.1 mg. Then each pair of ovaries and the remaining body excluding the abdomen were put in separate Eppendorf tubes to dry out in an oven at 60°C for 24 hours. After weighing they were kept in the oven at 60°C for another 24 hours and weighed again. In order to determine the dry weights of ovaries and bodies, procedures were repeated until weights remained constant. The diameter of spermathecae was measured under a stereomicroscope equipped with an ocular micrometer. The spermathecae were then discharged with a fine insect needle and fine forceps in 1 ml of saline solution (0.9 %). Tap water was added to make 5 ml total volume. Total spermatozoa counts were estimated by a Thoma counting chamber under a light microscope (Leica CM E) by counting six replicate fields (Mackensen and Tucker, 1970).

Statistical analyses

The data were analysed by one-way ANOVA to determine the effect of queen weight at emergence. Duncan's multiple comparison tests were used to compare means and to detect significant differences between queen weight groups ($P < 0.05$). Correlation coefficients between all measured character pairs were also calculated. Discriminant analysis was performed on the 11 character data of 38 queens from light, moderate and heavy groups to reallocate each queen into new groups according to their weights at different ages, and internal characters.

Discriminant analysis was applied to the individual values of each queen using a pooled variance-covariance matrix. Three a priori groups were used to classify all queens. The mating success rates of three groups of queens of different weight were compared by Z-test for two proportions in the independent samples. All statistical analyses were performed using SPSS v. 11.5 for Windows.

Results

Mating flights

Five of the 50 queens (1 from light, 2 from moderate and 2 from heavy group) were lost in the mating nuclei during the pre mating period. All queens flew out of their nuclei for mating in the late afternoon between 16:00–17:30 hours. All 45 queens mated between the ages of six to eight days between 21–24 June 2005. Of the 45 queens, only two (one from moderate and one from heavy group) did not return from their mating flights (mating loss: 4.4%). Twenty one of 43 queens (48.9%) started egg laying after a single mating flight, while 22 queens (51.1%) flew out a second time before the onset of oviposition. The weight at emergence did not affect the number of mating flights performed ($P > 0.05$). The mating success rates of queens (the ratio of queens starting egg laying to queens introduced into mating nuclei) in light, moderate and heavy groups were 94.1%, 82.4% and 81.2%, respectively. The overall mating success rate was 86.0%. The Z-test did not detect statistically significant differences ($P > 0.05$) between the mating success of the three groups of queens.

Behavioural features during reproduction period

The duration of the mean intervals from emergence to first mating flight, from last mating flight to onset of oviposition, and from emergence to onset of oviposition were 6.86 days, 3.05 days and 10.60 days, respectively (Table 1). The weight of queens in the three groups did not affect the time from emergence to mating flight and to onset of oviposition (all differences $P > 0.05$).

Changes in weights during reproductive development

The weight of queens at emergence affected the weights at three days after emergence, at the onset of oviposition, at three days after the onset of oviposition, and at one month after onset of oviposition (Table 2). The differences in the weight at one day after the last mating flight between the weight groups were, however, not found to be statistically significant (Table 2). Statistically significant differences also occurred between weight groups in dry body weight at one month after the onset of oviposition.

The weight of queens decreased continuously from emergence until mating (Fig. 1). During the maturation period from emergence to mating, the queens from light, moderate and heavy groups lost 15.5 % (29.0 mg), 18.3 % (35.6 mg) and 20.6 % (43.0 mg) of their initial weights, respectively. Heavy queens thus lost 2.3 % and 5.1 % more weight than moderate and light queens, respectively. Although heavy queens were 10.8 % and 6.7 % heavier than the light and moderate ones at emergence (Table 2), at one day after the last mating flight they did not differ significantly from the light and moderate ones and were only 5.1 % and 4.3 % heavier respectively. After mating, queens started to gain weight. At the onset of oviposition, light, moderate and heavy queens were 7.2 % (13.4 mg), 2.8 % (5.5 mg) and 1.6 % (3.3 mg) heavier than their initial weights, respectively. Queens thus started oviposition when they exceeded their initial weights. Light queens gained 4.4% and 5.6% more weight than moderate and heavy queens at the onset of oviposition. At three days after the onset of oviposition the queens continued to gain weight, and were significantly heavier than their weights at the onset of oviposition. When the queens were about 40 days old at one month after the onset of oviposition, they were 10.8 % (20.1 mg), 9.9 % (19.3 mg) and 7.0 % (14.6 mg) heavier than their initial weights in the light, moderate and heavy groups. The heavy queens were 7.6 % and 4.4 % significantly heavier than light and moderate queens (Table 2), respectively. Thus, the differences between queen weight groups at one month after the onset of oviposition were similar to the initial weight differences (Fig. 1).

Internal reproductive characters of queens at one month after onset of oviposition

At 40 days old, no significant differences were found between the queen weight groups in fresh and dry ovary weights and in the number of spermatozoa entering the spermathecae (Table 3). The overall mean fresh weight of ovaries was 53.7 mg, this being about one fourth of the overall weight of queens when dissected. Statistically significant differences occurred between queen weight groups in the diameter and volume of spermathecae. The number of spermatozoa entering the spermatheca did not, however, vary depending on the number of mating flights of queens.

In order to determine whether fresh and dry ovary weights vary depending on queen weight at one month after onset of oviposition, the queens were regrouped as light (under 210 mg), moderate (between 210-220 mg) or heavy (over 220 mg) with respect to their weights at one month after onset of oviposition. ANOVA applied to the new data set of queens showed statistically significant differences in dry and fresh ovary weight. The mean fresh and dry ovary weights of queens in the heavy group (58.3 mg and 9.9 mg, respectively) differed significantly from those of moderate (53.1 mg and 9.4 mg) and light (50.8 mg and 9.0 mg) queen groups, respectively.

Correlations

Significant positive correlations were found between the weight at emergence and all live weights at different periods (Table 4). The weight of queens at emergence was significantly correlated with the weights at three days after emergence (r = 0.572), at the onset of oviposition (r = 0.440) and three days after the onset of oviposition (r = 0.562). The highest correlation was found between the weight at emergence and one month after onset of oviposition (r=0.808). The weight at emergence was significantly correlated with the diameter (r = 0.619) and volume of spermatheca (r = 0.607), respectively. No correlation was found between the weight at emergence and the fresh and dry weight of ovaries in queens about 40 days old.

Discriminant analysis of weight and internal character data

Discriminant analysis was applied to data from all weight and internal characters to reallocate the queens to their pre-assigned weight groups (light, moderate or heavy at emergence). The analysis yielded two canonical discriminant functions. The first and second functions accounted for 83.5 % and 16.5 % of the total variation, respectively. Discriminant analysis reallocated 89.5% of the queens to their pre-assigned weight groups (Table 5, Fig. 2). Reallocation results of light, moderate and heavy queens were 85.7 %, 82.3 %, and 90.9 %, respectively. Two light queens were misclassified as moderate and one moderate and one heavy queen were misclassified as light and moderate respectively. When a stepwise method was applied to the data, 11 measured characters reduced to three; three days and one month after the onset of oviposition and dry body weight. According to the stepwise method, it was possible to correctly classify 83.3 % of the queens to their preassigned groups by using only these three weight characters.

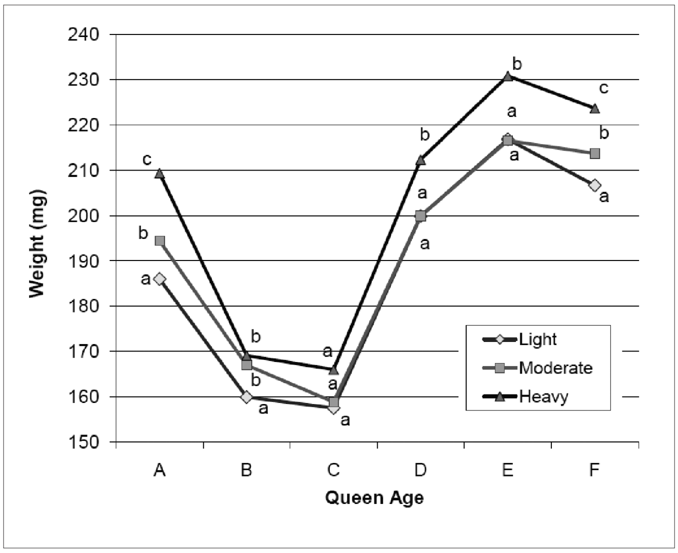

Fig 1. The weight (mg) fluctuations of queens in different weight groups during reproductive development. The weights at: emergence (A); three days after emergence (B); one day after last mating flight (C); onset of oviposition (D); three days after onset of oviposition (E); and one month after onset of oviposition (F). Different small letters denote significant differences between means within an age (P < 0.05).

Table 1. The duration (day, mean ± S.E.) of three reproductive behavioural features in the three groups of queens of different weight (number of queens in parenthesis).

Interval from:	**Light (16)**	**Moderate (14)**	**Heavy (13)**	**Overall (43)**
emergence to first mating flight	6.75 ± 0.250	7.00 ± 0.234	6.85 ± 0.274	6.86 ± 0.143
last mating flight to onset of oviposition	3.13 ± 0.202	3.07 ± 0.127	2.92 ± 0.137	3.05 ± 0.094
emergence to onset of oviposition	10.63 ± 0.221	10.72 ± 0.244	10.46 ± 0.243	10.60 ± 0.134

Differences between the means of the three groups of queens within each behavioural feature were not significant (P > 0.05).

Table 2. Live weights (mg) of queens at different ages: at emergence (A); three days after emergence (B); one day after last mating flight (C); onset of oviposition (D); three days after onset of oviposition (E); one month after onset of oviposition (F); during reproductive development and dry body weights of queens (G).

Queen age	**Light**		**Moderate**		**Heavy**		**Overall**	
	n	**mean±S.E.**	**n**	**mean±S.E.**	**n**	**mean±S.E.**	**n**	**mean±S.E.**
(A)	17	186.5 ± 1.20 a*	17	194.4 ± 0.66 b	16	209.0 ± 1.44 c	50	196.3 ± 1.51 *C*
(B)	17	159.9 ± 1.39 a	16	166.9 ± 1.48 b	15	169.1 ± 1.74 b	48	165.1 ± 1.04 *B*
(C)	16	157.5 ± 2.20 a	14	158.8 ± 2.57 a	13	166.0 ± 3.90 a	43	160.5 ± 1.72 *A*
(D)	16	199.9 ± 2.86 a	14	199.9 ± 3.22 a	13	212.3 ± 2.73 b	43	203.6 ± 1.88 *D*
(E)	16	216.8 ± 2.24 a	14	216.5 ± 2.66 a	13	230.8 ± 3.02 b	43	220.9 ± 1.78 *F*
(F)	16	206.6 ± 1.37 a	13	213.7 ± 1.64 b	13	223.6 ± 1.97 c	42	214.1±1.44 *E*
(G)	16	21.6 ± 0.20 a	13	22.2 ± 0.15 b	13	23.3 ± 0.17 c	42	22.3 ± 0.99

* Different small letters denote significant differences between means within an age, and italic capitals between overall means ($P < 0.05$).

Table 3. Internal reproductive characters (H – L) of different queen weight groups at one month after the onset of oviposition.

Characters	**Weight Groups**	**n**	**mean±S.E.**
(H) Fresh ovary weight, mg	Light	15	52.0 ± 1.22 a*
	Moderate	13	54.8 ± 2.31 a
	Heavy	12	54.5 ± 1.22 a
	Overall	40	53.7 ± 6.00
(I) Dry ovary weight, mg	Light	16	9.2 ± 0.21 a
	Moderate	13	9.3 ± 0.18 a
	Heavy	13	9.7 ± 0.20 a
	Overall	42	9.4 ± 0.75
(J) Spermatheca diameter, mm	Light	15	1.06 ± 0.009 a
	Moderate	13	1.09 ± 0.010 b
	Heavy	12	1.11 ± 0.012 c
	Overall	40	1.10 ± 0.007
(K) Spermatheca volume, mm^3	Light	15	0.62 ± 0.015 a
	Moderate	13	0.69 ± 0.019 b
	Heavy	12	0.73 ± 0.024 b
	Overall	40	0.70 ± 0.013
(L) Spermatozoa number (x 1000)	Light	15	4 821 ± 146 a
	Moderate	13	4 927 ± 102 a
	Heavy	13	4 892 ± 159 a
	Overall	41	4 877 ± 78

* Different letters denote significant differences between the means ($P < 0.05$).

Table 4. Correlation matrix of 12 measured characters: weights at emergence (A); three days after emergence (B); one day after last mating flight (C); onset of oviposition (D); three days after onset of oviposition (E); and one month after onset of oviposition (F): and measurements one month after onset of oviposition: dry body weight (G); fresh ovary weight (H); dry ovary weight (I); spermatheca diameter (J); spermatheca volume (K); and the number of spermatozoa in spermatheca (L) ($^{\alpha}P < 0.05$, $^{\beta}P < 0.01$).

	A	B	C	D	E	F	G	H	I	J	K
B	0.572$^{\beta}$										
C	0.371$^{\alpha}$	0.227									
D	0.440$^{\beta}$	0.176	0.320$^{\alpha}$								
E	0.562$^{\beta}$	0.167	0.324$^{\alpha}$	0.441$^{\beta}$							
F	0.808$^{\beta}$	0.598$^{\beta}$	0.375$^{\alpha}$	0.545$^{\beta}$	0.658$^{\beta}$						
G	0.774$^{\beta}$	0.608$^{\beta}$	0.361$^{\alpha}$	0.451$^{\beta}$	0.588$^{\beta}$	0.688$^{\beta}$					
H	0.156	0.401$^{\beta}$	-0.028	0.269	0.141	0.460$^{\beta}$	0.276				
I	0.221	0.133	0.037	0.153	0.285	0.458$^{\beta}$	0.193	0.462$^{\beta}$			
J	0.619$^{\beta}$	0.434$^{\beta}$	0.096	0.054	0.304	0.422$^{\beta}$	0.541$^{\beta}$	0.145	0.305		
K	0.607$^{\beta}$	0.427$^{\beta}$	0.084	0.059	0.308	0.415$^{\beta}$	0.537$^{\beta}$	0.154	0.299	0.998$^{\beta}$	
L	0.120	0.075	-0.023	0.031	0.259	0.121	0.212	0.023	0.134	0.350$^{\alpha}$	0.347$^{\alpha}$

Table 5. Classification results (n (%)) of discriminant analysis applied to 11 characters of 38 queens.

Pre-assigned groups	**Classified as**			**Total**
	Light	**Moderate**	**Heavy**	
Light	12 (85.7)	2 (14.3)	0 (0.0)	14
Moderate	1 (7.7)	12 (82.3)	0 (0.0)	13
Heavy	0 (0.0)	1 (9.1)	10 (90.9)	11

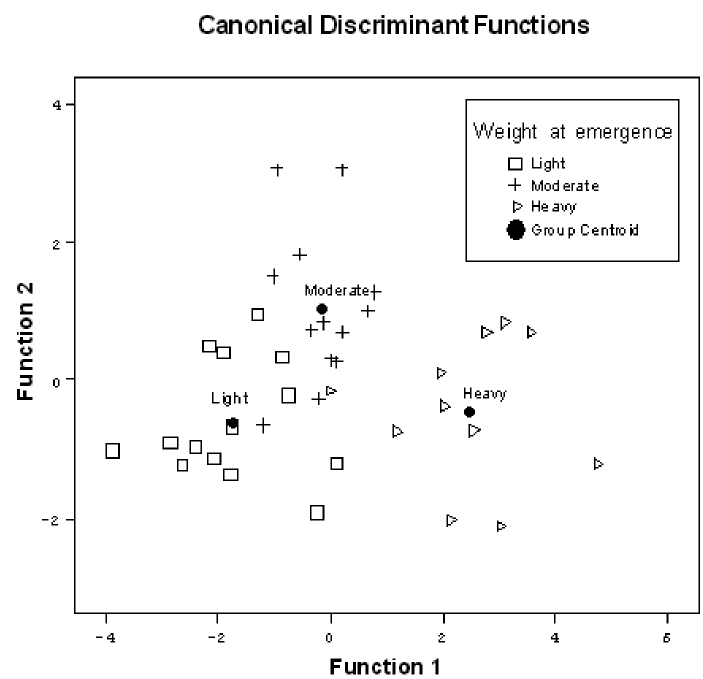

Fig 2. Distribution of 38 queens from light, moderate and heavy weight (at emergence) groups on the plane of discriminant functions 1 and 2 derived from discriminant analysis of 11 weight and internal characters. Each mark on the plane corresponds to a queen.

Discussion

Besides natural variation and environmental queen rearing conditions, heritable genetic characters (Moritz *et al*., 2005) could also result in queens of different weight at emergence. Our results showed, however, that the behavioural features such as the interval from emergence to first mating flight and to the onset of oviposition were not affected by the weight of queens at emergence. Thus, queens weighing 186.5, 194.4, 209.0 mg all behave similarly in the first post emergence period. Our findings conform to the findings of Szabo *et al*. (1987) and Medina and Gonçalves (2000). We found that 51.1 % of queens flew for a second time before the onset of oviposition. This is similar to the 63% found by Woyke (1964). We also showed that the weight at emergence did not affect the number of mating flights taken. Fresnaye (1966) demonstrated that rearing season and meteorological conditions influence the rate of development and the age at which the queens reach sexual maturity. According to our results, the age at which the queens reach sexual maturity is not affected by queen weight at emergence.

In this study, fluctuations in body weight of queens during the pre and post mating periods were observed. The weight of queens decreased continuously from emergence until mating. As stated by Harano *et al*. (2007), weight loss of virgin queens due to body water loss seems reasonable, because the mating flight probably requires a lighter body. During the maturation period from emergence to mating, all queens lost weight and had closer weights at the time of mating, despite differences in weight at emergence. After mating, queens started to gain weight and to oviposit when they exceeded their initial weights. During the pre mating period, heavier queens at emergence lost proportionally more weight than the lighter queens. In contrast, light queens gained more weight than heavy queens during the three days from the onset of oviposition. The weights at different reproductive periods were correlated with the weight at emergence, the highest significant correlation ($r = 0.808$) being between the weights at emergence and at one month after the onset of oviposition.

Surprisingly, the weight of ovaries at one month after the onset of oviposition was not correlated with queen weight at emergence. At one month after the onset of oviposition, the weight of ovaries does not depend upon the number of ovarioles, but upon the egg laying activity and the number of mature eggs in the ovaries. In our case, one month after the onset of oviposition, the combs in the mating nuclei were filled with brood and egg laying activity began to slow down due to brood congestion. The weight of queens one month after the onset of oviposition was thus relatively lighter than at three days after the onset of oviposition. In such conditions, differences in ovary weight due to different number of ovarioles could therefore not be expressed. Ovary weight of queens kept for one month in small nuclei cannot therefore suggest the potential egg laying activity of the queens. If the queens were kept in normal strong colonies, they would probably be heavier one month after the onset of oviposition than three days after.

Statistically significant differences did occur in fresh and dry ovary weight when the queens were regrouped with respect to their weights at one month after the onset of oviposition. We found that queen body weight at one month after the onset of oviposition was correlated with fresh and dry ovary weights. We did not count the number of ovarioles in the ovaries, so do not know whether some ovaries were heavier because they had more ovarioles, or because they contained more mature eggs, despite having the same or fewer ovarioles.

In our investigation we found the size of spermatheca to be correlated with the weight at emergence, as reported by Woyke (1971). We found that the number of spermatozoa entering the spermatheca did not vary depending on the frequency of mating of queens. This is in agreement with Woyke (1964), who found that queens that have a low number of spermatozoa in the spermatheca after the first mating flight, fly out again to mate for the second time to collect additional sperms.

Woyke (1971) found a highly significant correlation ($r = 0.75$, $P < 0.01$) between the queen weight at emergence and the number of ovarioles in the ovaries, but as explained above, the weight of ovaries in one month old queens does not only depend upon the number of ovarioles, but also upon the number and development stage of eggs in them. It may therefore be that a queen with many ovarioles has fewer mature eggs in her ovaries than a queen with fewer ovarioles, so the weight of ovaries of a queen with many ovarioles may be similar or lighter than that those of a queen with fewer ovarioles. The ovary weight at one month after the onset of oviposition can not therefore reliably be used for estimating the egg laying potential and quality of reared queens kept in mating nuclei.

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