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Variability of Prenatal Maternal Investment in the Honey Bee (*Apis mellifera*)

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ABSTRACT

Uneven distribution of food resources to offspring by females plays an important role in adaptation to environmental changes in many taxa. In the honeybee, the differential maternal investment has a potential role in the amplification of intra-colony phenotypic variability which is an important factor in stress resilience. In this study, the repeatability of weight measurements and the optimum stage was determined when the eggs were sampled to minimize the imprecision due to unavoidable intra-sample age differences. Eggs were weighed from fourteen selected colonies and assessed the relative weight variability. A comparison was done between the means and variability of eggs produced in spring and late summer were compared to test the hypothesis that eggs destined to become a mix of summer and winter bees those should be more variable than eggs all destined to become summer bees. The results showed that the optimum age for sampling eggs is 48 h. No systematic difference was found between spring and summer samples but the difference in sample means from the same queens was up to 22%. Whereas, the difference in weight of eggs laid by the same queen within six hours was up to 58%. A comparison with published data on the effects of difference in egg weight from reared adults showed that the level of variability observed was sufficient to expect phenotypic differences at the adult stage. Therefore, it can be concluded that a considerable level of differential maternal investment exists in the honey bee. The research findings provided an opportunity for further studying its consequences and evolutionary significance in social species.

Key words: Maternal investment, egg weight, repeatability, variability, winter bee

INTRODUCTION

Supply of nutrients to eggs by mother is important for the fitness of her offspring. Studies in species of almost all major taxa of egg-laying animals showed that the amount of provisions contained in the propagule, usually approximated by measuring its size or weight, influenced the size, vitality or reproductive success of offspring (Kaplan, 1987; Russell *et al.*, 2007). In situations, where environmental conditions are stable, oviparous females tend to lay eggs which receive similar quantities of nutrients (Crean and Marshall, 2009; Marshall *et al.*, 2008). However, if the environmental conditions are unpredictable, mothers should produce both the small and large eggs as a way of "hedging their bets". If conditions are favourable, a great proportion of offspring both from light and heavy eggs can survive. If they are unfavourable, at least some will likely be able to carry on the genes of their parents (Cooper and Kaplan, 1982; Philippi and Seeger, 1989; Crean and Marshall, 2009).

In the honeybee (*Apis mellifera*), effects of environmental changes on the success of brood rearing are probably less than other species, because the colony and its food stores provide a buffer between exterior influences and the immature stages in their cells. Nevertheless, there are sound reasons why a bet-hatching strategy could be adaptive in this highly social species. Among these, one is that diversity of worker phenotypes provides a security against changing conditions for the colony as a whole. This is thought to be one of the reasons why polyandry is common in highly eusocial insects, because it increases phenotypic variation among workers by increasing genetic variation (Oldroyd and Fewell, 2007; Mattila *et al.*, 2008; Waddington *et al.*, 2010). Besides, differences among workers can also be caused by differences in maternal investment (Borodacheva, 1973) which could add to the variability resulting from the presence of several patriline. A second possible role for differential maternal investment could lie in the fact that female eggs can develop into either queens or workers. It was also found that queen weight and the number of a queen's ovarioles are strongly influenced by the weight of the egg from which she develops (Borodacheva, 1973).

Previously, many studies conducted on egg weight variability in *Apis mellifera* focussed mostly on inter-colony differences and the environmental influence. Taber and Roberts (1963) and Roberts and Taber (1965) observed considerable differences between queens regarding egg weights and found that these differences were partly heritable. Borodacheva (1973) weighed eggs from 200 queens and found that the heaviest egg was more than 2.5 times as heavy as the lightest one. Krol (1996) showed that weather did not show considerable influence on egg weight but found negative correlation between egg weight and the number of eggs produced (Jordan, 1961). Woyke (1998) showed that the size and weight of bee eggs change during development. Therefore, the egg age should be standardized as much as possible if the difference in maternal investment is to be measured.

The aim of this study was to determine the repeatability of egg weight measurements and the age of eggs in order to create a methodological basis for studying the differential prenatal maternal investment in honeybee. In the context of possible effects of differential maternal investment on properties of the offspring, it was mainly differences between eggs laid by the same queen which were of interest. Besides, the study principal aim was to measure variability within samples of eggs laid by the individual queens within a short period of time. Secondly, we studied weight differences between the eggs laid by the same queens either in spring (when the so-called "summer bees" are produced) or in late summer (when both "summer" and "winter bees" are produced). Our hypothesis was that if quantitative differences in maternal investment play a role in the differentiation into "summer" or "winter bees", this should lead to greater variability of egg weights in late summer.

MATERIALS AND METHODS

Repeatability of egg weight measurements: Ninety eggs (0-6 h-old) were collected from one queen and weighed on a microbalance, Satorius M5P (Fig. 1). Each egg weight was measured three times within a short interval. Eggs were taken from the comb using special forceps (Taber, 1961). Repeatability was calculated using the following equation (Falconer, 1989):

$$r = \frac{V_B}{V_B + V_w}$$

where, r is the repeatability, V_B is the variance between eggs and V_w is the variance within eggs.

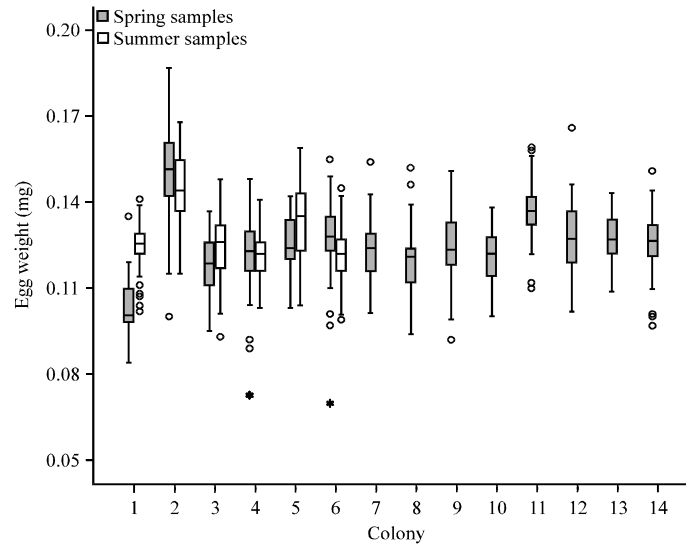


Fig. 1: Variability of egg weight within samples from 14 *Apis mellifera* queens, Grey bars: Egg samples taken late April to mid-June, White bars: Egg samples taken late June to late August, Boxes contain 50% of values, boxes+whiskers enclose all values except outliers and extreme values (circles and stars, respectively), Among samples collected in spring, variability differs significantly (Levene-test, $p < 0.001$), Average weights also differ between colonies (ANOVA on log-transformed data, $p < 0.001$)

REML (Restricted Maximum Likelihood) was used to estimate the components of variance (SAS, 2003).

Determination of weight variability and optimum developmental stage of eggs: Fourteen source colonies were chosen randomly from the stock of the Institute for Bee Research, Germany during late April and mid-June, 2009. To obtain eggs of uniform age, the queen of each colony was confined to a single frame (36×22 cm) and placed in a cage in the center of the colony's brood nest for 6 h. This cage was made of queen-excluder material, which allowed free passage of worker bees except the queen. At the end of the egg-laying period, the queens were released from the cage. The combs containing the eggs were brought to an incubator and maintained the temperature at 34.5°C with a relative humidity of 50±10%. First egg weight measurement was taken the eggs were 24-30 h old and the subsequent weight measurements were taken between 48-54 h and 72-78 h. A total of 50 eggs were weighed individually from each colony. To determine the egg weight variability in the mean egg weight and among eggs destined to become the summer bees or a mixture of summer and winter bees, these measurements were repeated in six out of 14 colonies after 2-2.5 months during mid-July and late August.

Statistical analysis: The software PASW Statistics 18 (SPSS, 2009) was used for data analysis except for the calculation of repeatability. Egg weight variability was compared using the Levene test for equality of variances. Log-transformed data were statistically analyzed by ANOVA for the effects of developmental stage and colony of origin. These factors were analysed using repeated-measurement ANOVA with colony of origin as an additional fixed factor. The effect of season

(spring or summer) on variability and means was investigated by comparing the spring and summer samples of each colony using the Levene-test, followed by t-tests for either equal or unequal variances.

RESULTS AND DISCUSSION

Repeatability: The V_B was estimated to be 0.00021 and V_w to be 0.000013, resulting in a total variance (V_T) of 0.000223. The ratio V_B/V_T , representing the repeatability of egg weighing, was 0.94.

Variability of eggs of individual queen: The weight of eggs laid within 6 h was near-normally distributed in all colonies (Fig. 1). The coefficients of variation ranged between 7.5 and 12.5%. The range, i.e., the difference between the heaviest and the lightest egg, was between 31 and 58% of the respective colony means (Fig. 1).

Effect of developmental stage and colony: Although, the variability in egg weights tended to be higher at the age of 72-78 h but the variance at the three developmental stages was not significantly different ($p = 0.37$). On the other hand, the variance of spring samples from different colonies was significant from each other ($p < 0.001$). This would mean that some queens laid eggs with more variable weight than other queens. The effect of developmental stage and the origin of colony were both highly significant. Also, there was a significant interaction between both factors ($p < 0.001$ in all cases, Table 1). Mean egg weight of colony measured after 48-54 h ranged from 0.102-0.137 mg egg⁻¹. Whereas, the mean egg weight was 0.138, 0.125 and 0.113 mg after 24-32, 48-54 and 72-78 h, respectively (Fig. 2).

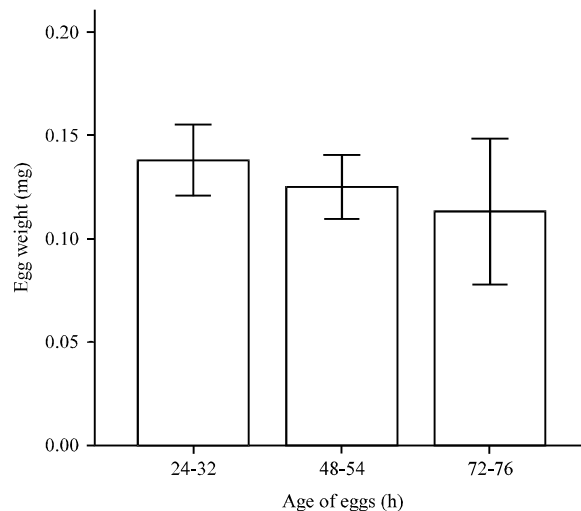


Fig. 2: Variability of egg weight at different stages of development, Error bars: Standard deviations. Pooled data from 14 colonies are shown. Variability of egg weights was not different between the three stages (Levene-test, $p = 0.37$). The effect of the developmental stage on egg weight was highly significant, as was the interaction between the colony of origin and the developmental stage (ANOVA on log-transformed data, $p < 0.001$ in both cases)

Table 1: Analysis of variance of egg weight reduction during incubation and effects of colony

Source of variation	Sum of squares	df	Mean of squares	F-value	p-value
Developmental stage					
Linear	2.868	1	2.868	1371.750	<0.001
Quadratic	0.003	1	0.003	5.036	0.025
Developmental stage×colony					
Linear	0.140	13	0.011	5.159	<0.001
Quadratic	0.012	13	0.001	1.551	0.094
Colony	2.555	13	0.197	34.040	<0.001
Error (developmental stage)					
Linear	1.435	686	0.002		
Quadratic	0.396	686	0.001		
Error (colony)					
	3.961	686	0.006		

Effect of season: There was no significant difference in egg weight among eggs laid in spring or during summer. In the case of six colonies where eggs were sampled in both seasons, four showed similar levels of egg weight variability with only one colony showed higher variance in spring than in summer ($p = 0.04$), while in the last, the variance in egg weight was higher in summer than in spring ($p < 0.001$). The difference in mean egg weight was significant in 4 colonies ($p = 0.001-0.04$) with heavier eggs laid either during spring (1 colony) or during summer (3 colonies) (Fig. 1).

DISCUSSION

Although, egg weight is not necessarily correlated with nutrient content (Wegener *et al.*, 2010) but it is commonly used as a measure of the prenatal maternal investment in oviparous species (Karlsson and Wiklund, 1984; Mappes *et al.*, 1997; Mohaghegh *et al.*, 1998). The high repeatability value of 0.94, among the weights of honeybee eggs, means that the error due to the imprecision of the measurement was negligible, even if compared to the difference among eggs laid by the same queen within the short period of 6 h. Therefore, a single measurement should be sufficient in studies within the context of possible effects of differential maternal investment.

Given the preposition that egg weight drops by over 30% from egg deposition to hatching (Woyke, 1998), it was considered necessary to determine the developmental stage at which potential difference in prenatal maternal investment can be measured with the smallest possible error due to age difference within each egg sampling. However, it is known that handling young (<24 h) honeybee eggs with forceps or a brush strongly reduces their hatching success (Collins, 2002). Therefore, the best compromise between damage to eggs and variation due to unavoidable differences of age should be sought. Figure 2 shows that after 48-54 h, the variability of egg weights was hardly greater than that found after 24-32 h. Whereas it tended to increase thereafter. However, after 48 h, the loss in egg weight through handling of eggs with specialized forceps declined markedly (Collins, 2002). It was, therefore, concluded that the optimum age for measuring prenatal maternal investment in honeybee eggs is about 50 h.

Several studies reported that phenotypic variations of honeybee workers reared simultaneously within a colony have important implications for the functioning of the colony, especially for task-sharing between its members (Kerr and Hebling, 1964; Waddington, 1989; Makert *et al.*, 2006). A relationship between egg weight and offspring phenotype was found in many species (Dzialowski and Sotherland, 2004; Bonato *et al.*, 2009). In the honeybee, Bilash *et al.* (1985) compared morphological traits of workers reared within the same colony but stemming from

two different mothers that produced eggs with average weights of either 0.131 or 0.118 mg. They found significant difference regarding the length of the proboscis and wings. In the present study, egg weight varied from 31-58% (0.03 to 0.09 mg) and the coefficient of variation (CVs) was 7.5-12.5% within samples produced by the same queen within six hours. As the weight reduction through developmental processes averaged only 9% over 24 h (age of eggs 24-32 h to 48-54 h), most of this variability can be attributed to weight differences "at laying". Krol (1996) studied the variability of egg weight in five colonies headed by sister queens. She found the ranges rarely below 20% and reached up to 66% of the respective means in samples produced by individual queens within a time span of 16 h. Together, these results showed that honeybee queens regularly produce eggs with considerable variability in weight. A comparison of results with the findings of Bilash *et al.* (1985) showed that the level of these variations is often sufficient to expect measurable effects on the phenotype of the offspring.

In honey bees living under temperate climates, one of the most important forms of phenotypic variation is the differentiation between so-called "summer" and "winter bees". The two phenotypes differ strongly regarding their longevity, hypopharyngeal gland morphology and important biochemical characteristics (Fluri *et al.*, 1982). Besides, if the maternal supply to eggs plays a role in this differentiation, this should be reflected in the differences between samples of eggs laid in spring (when exclusively summer bees are reared) and late summer (when a mixture of summer and winter bees are produced), with more variable egg samples expected in late summer. Seasonal influences on egg weight were revealed several times, with lighter eggs generally reared during times of strong colony growth in late spring (Jordan, 1961; Taber and Roberts, 1963; Bilash *et al.*, 1985). In the present study, no systematic difference was observed between spring and summer samples. This could be attributable to the fact, such as egg laying rate of queens and foraging conditions, were not accounted for. However the highest difference between spring and summer samples produced by the same queen amounted to 0.02 mg (22% of the mean of the lighter sample), a value higher than that shown by Bilash *et al.* (1985) to produce morphometric effects in workers. This demonstrated that there is sufficient variation of egg weights to justify further studies on the interactions between prenatal maternal investment and medium-term environmental changes in the honeybee.

Comparative data on within-clutch egg weight variability from other animal taxa are extremely scarce. In birds, coefficient of variation of 1.2-5.8% have been reported (Yosefa and Zduniak, 2008). Fox and Czesak (2000) reported that within-clutch variation in egg size can be quite high in some arthropods but suggested that most of it was due to physiological limitations to repeatability rather than to adaptations through evolutionary processes. On the other hand, several studies predicted a fitness gain from within-clutch variation of maternal investments on theoretical grounds (Cooper and Kaplan, 1982; Philippi and Seeger, 1989; Crean and Marshall, 2009). Assuming that nutrients contained in the egg only represent a small fraction of total nutrients received by immature honeybees, it may seem unlikely that the difference in nutrient supplies to eggs should not be compensated during the later developmental stages. However, the mechanism of female caste determination (Rembold, 1974; Kucharski *et al.*, 2008) is an impressive example of the sensitivity of honeybee developmental pathways to nutritional influences early in development. Also, differences at early stages could potentially be enhanced rather than compensated if they function as signals to nurse bees, leading to differential treatment of larvae. The present study showed that prenatal maternal investment in *Apis mellifera* is highly variable. Therefore, studying the role of this variability for colony functioning and fitness could become an exciting and challenging area for future investigation.

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