

# Correlation of queen size and spermathecal contents and effects of miticide exposure during development

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**Abstract** – Normal mating of honey bee (*Apis mellifera* L.) queens and drones results in extreme polyandry. Larger queens are reported to produce more brood, but do they also store more semen? Measurements of queen weight, spermatheca weight and volume, and numbers of sperm in the spermatheca were made on normally reared queens and queens exposed to miticide from early larval stage to emergence. In the normally reared group, larger queens had more sperm in spermathecae of greater volume and weight. The presence of miticide during queen development skewed the relationship of queen weight to spermatheca size and the number of sperm stored such that they were not correlated. This sublethal effect of miticide in the colony has an unknown impact on queen performance.

spermatheca / sperm concentration / extreme polyandry / evolution / queen weight

## 1. INTRODUCTION

The honey bee (*Apis mellifera* L.) is a central figure in discussions on the evolution of mating systems in social insects because of the extreme polyandry that is expressed. Queens have been reported to mate with up to 24 (Neumann et al. 1999), an extreme of 44 (Moritz et al. 1996), 10–28 (Kraus et al. 2005), or more recently 6 to 50 (Delaney et al. 2011) drones over the course of one to four mating flights (Roberts 1944). The exploration of the evolution of sociality, sexual selection, and sperm competition have all gained insights from studies with honey bee queens, drones, and their mating behavior. The inclusive fitness theory of Hamilton (1964) places great importance on the possibilities of sperm competition and genetic relationship

between nest mates. Yet such extremes of multiple mating, which reduce nest mate relatedness in this most highly social species, are hard to fit into kin selection theory.

According to Laidlaw and Page (1984), sexual selection by sperm competition does not operate in honey bees. The proportion of sperm from each drone mated is represented in the proportions of worker offspring. Some more recent studies on sperm competition in honey bees have had conflicting results from in vitro studies (den Boer et al. 2010; Shafir et al. 2009) and paternity studies (Franck et al. 2002; Franck et al. 1999). However, considering the colony-level benefits of increased genetic diversity (Oldroyd and Fewell 2007; Tarpy 2003), sperm competition might not be advantageous for the honey bee male.

Two other recent papers have reported on research designed to throw some light on the multiple mating of honey bee queens and drones. While Page et al. (1984) had found

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basically random distribution of sperm from different fathers in the spermatheca, Schlüns et al. (2004) have added the caveat that the proportion of worker offspring within a patriline depended largely on the volume of semen from each drone, and larger drones produce more sperm (Schlüns et al. 2003). Insemination sequence was not important. Drones normally produce a large excess of sperm as only 3–5 % is actually stored by the queen in the spermatheca (Koeniger and Koeniger 2000).

Tarpy and Page (2000, 2001) surveyed mating frequency and flight duration, as well as manipulating young queens in the process of taking mating flights. They concluded that multiple mating increases a queen's fitness by lowering the probability that she produces diploid males, i.e., eggs fertilized by sperm carrying the same sex locus allele. They proposed that the extreme polyandry that is seen is simply a consequence of normal mating behavior. Queens and drones are taking mating flights in the same places and at the same times, and extreme polyandry can occur by chance alone during times of an overabundance of males. And because of the low risk of mating (calculated by flight time, not mating number) they conclude that the largest constraint to mating frequency is the storage capacity of the queen.

Boch and Jamieson (1960) reported that larger queens did produce significantly larger amounts of brood and Nelson and Gary (1983) attributed greater honey production to colonies with larger queens. More brood means more workers to collect nectar and therefore more honey. Additionally, as one who has dissected a large number of queens (AC) there are visible size differences between spermatheca. We have had several opportunities to collect measurements on queens to address this relationship between queen size and sperm storage.

## 2. MATERIALS AND METHODS

The first data set was from two groups of sister queens that were reared together and mated from the same apiary in Claxton, GA, during 3 weeks at the

end of the summers of 2002 and 2003. These queens were part of a study on the effects of miticide on queen rearing and performance (Pettis et al. 2003; Collins et al. 2004). Once the queens had been reared and mated, they were returned to the Bee Research Laboratory, Beltsville, MD (BRL), for dissection and evaluation of mating success. Queens reared in cups made with wax containing 100 ppm of coumaphos or 1,000 ppm of fluvalinate were significantly lighter in weight than untreated queens or those exposed to only 10 ppm of miticide. This gave us a set of queens with a wide weight range.

The first group of miticide-exposed queens was mated during the hot, dry summer of 2002, and many of the queens did not mate with many drones, based on spermatheca color (Collins and Donoghue 1999) and sperm counts, and oviposition was delayed. Those queens ( $n=30$ ) were removed from the data set reported here, leaving 28 individuals. The 2003 exposed queens had no significant differences in mating success or onset of oviposition between the miticide treatment groups represented. That summer period averaged 10 °F cooler than 2002 and had 60 in. of rain. Again any unmated or poorly mated queens were dropped from the data set. A total of 120 well-mated queens remained.

Measurements not previously reported for these 148 queens include queen weight, spermatheca weight (2002 only;  $n=46$ ), and estimates of the number of sperm present in the spermathecae done by the method of Mackensen (1940). We used Petroff-Hausser sperm counting chambers and serial dilutions of spermathecal contents in a modified Kiev buffer (Moritz 1984). Four people each counted the number of sperm in a counting chamber using separate aliquots of the diluted spermathecal contents. Numbers of sperm were calculated as per instructions with the counting chambers.

The second data set was from a group of 40 normally reared queens harvested from general use colonies managed by the BRL. The queens were from the same commercial queen breeder as the previous group, had been introduced in the spring of 2006 and were sacrificed in August. We considered them to be from the same gene pool. Queen weight, spermatheca weight, and number of spermatozoa in the spermatheca were measured. However, sperm number was estimated using a spectrophotometric technique (Collins 2005) using total spermatheca contents that we believe gives a more accurate measure than with a counting chamber using small subsamples. In addition, spermathe-

ca volume was obtained from digital photographs of the dissected spermathecae with tracheal nets removed using Zeiss AuxioVision digital camera software [Release 4.6] and an appropriately calibrated Zeiss dissecting microscope. Several spermathecae were lost or damaged during dissection giving reduced  $n$  of 37 for those statistics.

### 3. RESULTS

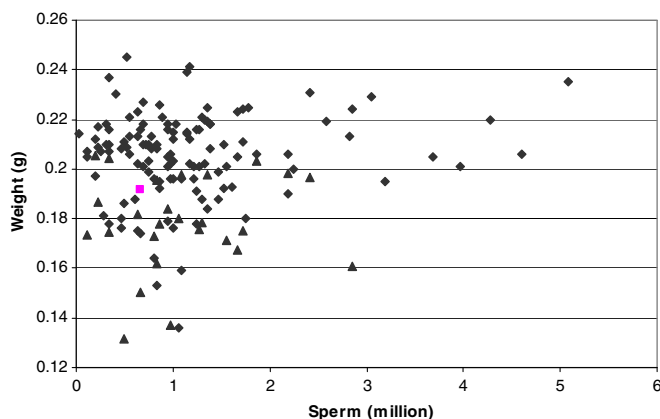
An analysis of variance of queen weight and spermatheca weight determined that the two groups of queens, coumaphos exposed and normally reared, were significantly different for both characters ( $F=213.82$ ,  $df=1$ ,  $P<0.0001$ ;  $F=17.33$ ,  $df=1$ ,  $P<0.0001$ , respectively). The normally reared queens were heavier ( $0.267\pm.005$  vs.  $0.178\pm.003$  g) and had heavier filled spermathecae ( $0.834\pm.031$  vs.  $0.697\pm.016$  g). Because different techniques were used to count sperm number, this value was not compared between the 2002/2003 and 2006 queens.

Figure 1 displays a plot of queen weight (g) against sperm number expressed as millions of sperm in the organ for the miticide-exposed queens only. The correlation coefficients,  $r=0.186$ ,  $n=28$  (2002) and  $r=0.1737$ ,  $n=123$  (2003) were not significant. Figure 2. Shows the same information but for the normal queens.

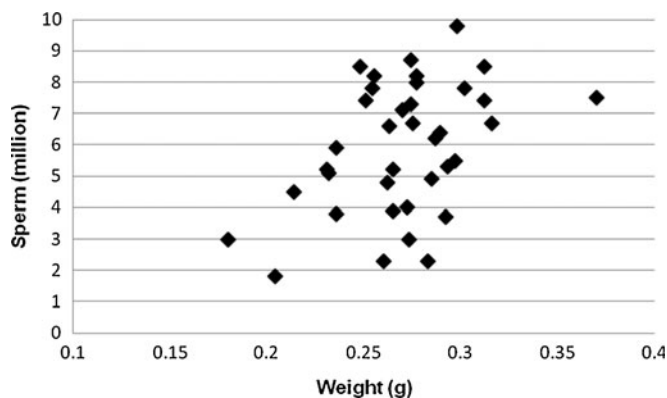
In this case the correlation is highly significant,  $r=0.469$ ,  $n=38$ ,  $P=0.003$ . The correlation of queen weight with spermatheca weight was also not significant  $r=0.365$ ,  $n=26$  (2002 only) for exposed queens yet highly significant for the normal group,  $r=0.523$ ,  $n=38$ ,  $P=0.0007$ . The correlation of queen weight and spermatheca volume (measured for the normal queens only) was  $r=0.548$ ,  $n=38$ ,  $P=0.0004$ , also highly significant. Not surprisingly spermatheca weight correlated well with number of sperm present for both groups of queens,  $r=0.569$ ,  $n=26$ ,  $P<0.01$  for exposed queens and  $r=0.445$ ,  $n=38$ ,  $P=0.005$  for the normal queens. Spermathecal volume (Figure 3; measured only in the normal queens) was highly significantly correlated with the number of sperm,  $r=0.896$ ,  $n=38$ ,  $P<0.0001$ .

### 4. DISCUSSION/CONCLUSION

Our conclusion from this study is that under normal rearing conditions, larger queens have larger spermathecae, store greater numbers of spermatozoa and the full spermathecae are heavier. Some other recent studies (Delaney et al. 2011; Tapy et al. 2011) had conflicting results; in the first they state that the queen's weight was not correlated with the number of spermatozoa, and the second that queen body size influences the



**Figure 1.** Body weight and number (million) of sperm in the spermatheca measured on two groups of sister honey bee queens reared with exposure to miticide and mated from the same apiary at the same time *triangle*, 2002; *squares*, 2003.

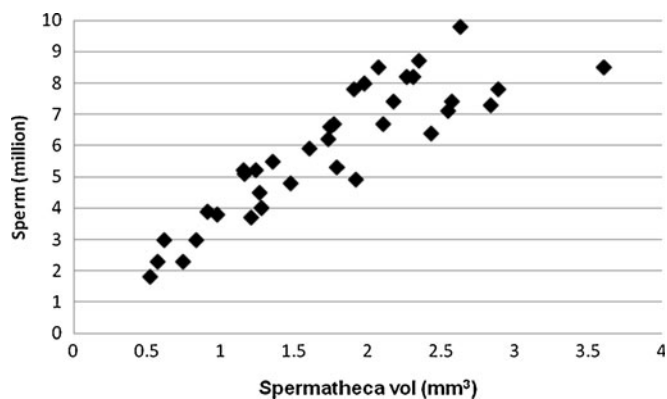


**Figure 2.** Body weight and number (million) of sperm in the spermatheca measured on sister honey bee queens reared under normal conditions and mated from the same apiary (2006).

number of sperm stored but has little effect on the number of matings.

Queen weights for the normally reared queens from this study are similar to the weights reported by Tarpy et al. (2011, 2012), 206.6 and 218.7 mg. Interestingly, the weights reported for the smaller coumaphos-exposed queens average about the same as queens Tarpy et al. (2011) reared from 2-day-old larvae (181 mg). The mean number of sperm stored in normal queens was  $5.76 \pm 2.26$  million, again similar to the queens in the 2011 study (5.06 million). Almost all coumaphos-exposed queens had less than two million sperms, comparable to the queens grafted from 2-day-old larvae (2.8 million).

However the presence of miticide, coumaphos, has an additional sublethal effect on queen development to those we reported earlier (Pettis et al. 2003). The normal relationship between body size and the size of the spermatheca is distorted in random ways in each individual. It is likely that this distortion affects other organs as well with currently unknown consequences for the performance of the queens. Our initial choice to use the large group of queens available from the miticide impact experiment to evaluate the relationship of queen size and sperm storage capacity was unfortunate. This group of queens was also poorly mated based on number of sperm stored.



**Figure 3.** Spermatheca volume vs. number of sperm in the spermatheca from the group of normally reared queens (2006).

If we calculate the sperm concentration by dividing the number of sperm by the volume of the corresponding spermatheca (values from the normally reared queens only), we get a very narrow range of values (mean = 3.534, standard deviation = 0.613). Does this mean that there is an optimum, or maybe maximum, concentration for sperm moving into the spermatheca? However, our mean concentration is only about half of the approximately six million sperm reported in Laidlaw and Page (1984). What would be interesting to know is the sperm concentration in queens who mate with extreme numbers of drones. Do they store a relatively larger number of sperm per volume or do they simply benefit from greater genetic diversity among the mates? A logarithmic correlation of the number of mates with the number of stored spermatozoa is reported by Schlüns et al. (2005), Delaney et al. (2011), and Tarpy et al. (2012). As sperm are considered to be metabolically quiescent during storage (Verma and Shuel 1973) there is probably little cost to storing larger quantities. But if only so many sperm can pack themselves into the storage organ, then actual numbers of sperm stored would not be greater, only the number of resulting patrines. Tarpy et al. (2011, 2012) made estimates of the capacity of a spermatheca in terms of number of sperm based on their volumes and reported that the filled capacity in their experimental queens averaged only 42 or 47 % of maximum. Do these values also reflect an optimum concentration?

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**Corrélation entre la taille de la reine et le contenu de la spermatheque et les effets de l'exposition à un miticide durant le développement**

**Spermatheque / concentration du sperme / polyandrie extrême / évolution / poids de la reine**

**Korrelation zwischen der Körpergröße der Königinnen und der Füllung der Spermatheka sowie der Effekt von Akariziden während der Entwicklung der Königin.**

**Spermatheka / Spermienkonzentration / extreme Polyandrie / Evolution / Königinnengewicht**

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