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Original article

Effects of age, season and genetics on semen and sperm production in *Apis mellifera* drones*

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Abstract – Adult drone honey bees from 4 Australian breeding lines were reared under similar conditions and examined for semen and sperm production when 14, 21 and 35 days old, during spring, summer and autumn. Almost half (40.5%) of all drones examined did not release any semen when manually everted. For those that released semen, the average volume released per drone was 1.09 μL (range 0.72 (±0.04)–1.12 (±0.04) μL) and the average number of sperms in the semen per drone was 3.63×10^6 (range 1.88 (±0.14)–4.11 (±0.17) $\times 10^6$). The release of semen was dependent on breeding line and age ($P < 0.05$), but not on the rearing season. The volume of semen released per drone was dependent on season, age, and breeding line ($P < 0.05$), while the concentration of sperm in the semen was dependent on season and breeding line ($P < 0.05$). Hence our data indicate that genetics underpins the maturation of drone honey bees as well as the volume of semen they release and the concentration of sperm in that semen.

***Apis mellifera* / drones / semen production / sperm**

1. INTRODUCTION

Mating is the most significant function of an adult drone honey bee (*Apis mellifera*). During mating, semen is transferred from a drone into the genital orifice of a virgin queen on the wing, via the drone's endophallus, which is everted during copulation (Bishop, 1920a; Woyke, 1964). After mating, secretions and parts of the drone reproductive organs remain in the genital tract of the queen and signify a 'mating sign' (Woyke and Ruttner, 1958), which may function to prevent semen from flowing out of the vagina (Bishop, 1920b). The semen consists of sperm, produced in the testes, and mucus, produced by large mucus glands. Sperm production is largely considered completed by the time drones reach sexual maturity, or when they are about 9–12 days old (Bishop, 1920a). Sperm is stored in the seminal vesicles of drones until they mate with virgin queens, at which stage it is ejaculated with mucus (semen) when the endophallus is everted. Normally, several drones will mate with a single virgin queen and copulations during flight follow one another about every two seconds (Koeniger et al., 1979). Each drone ejaculates from 6–12 million sperms and sperm from each mating accumulates in the queen's lateral oviducts (Woyke, 1960;

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Kerr et al., 1962). Most of this sperm is subsequently expelled from the queen (Page, 1986), but about 5–6 million migrate into the spermatheca, where they are stored until released in small quantities when the queen begins laying eggs (Zander, 1916; Bishop, 1920a, b; Page, 1986).

Previous studies in south east Australia found low numbers of sperm in the spermathecae of a high proportion of ovipositing commercially reared queen bees less than 35 days old (Rhodes and Somerville, 2003). This has serious implications for the productivity of colonies headed by such queens, as queen bees are generally superseded and killed by their colonies when they run out of sperm (Winston, 1987). Possible explanations could be that there were low numbers of drones of the right age available to mate with these queens, or sufficient numbers of drones of the right age but problems with their semen production, or a combination of both.

Limited data are available on the proportion of drones that release semen at the tip of the endophallus following manual eversion. Collins and Pettis (2001) reported 60% of 12-day-old and Anderson (2004) 90% of 20-day-old *A. mellifera* drones did not release semen after manual eversion. Several studies have been reported on the volume of semen produced by drones and the concentration of sperm in that semen. Woyke (1960; quoted in Rinderer, 1986) stated that an *A. mellifera* drone produces about 10×10^6 sperm, and 1 μL of semen contains about 7.5×10^6 sperm. Koeniger et al. (2005) compared sperm numbers between *A. dorsata* and *A. mellifera* drones and provided a comparison of sperm counts from *A. mellifera* drones from various authors. Sperm counts ranged from a mean of $4\,(2 \pm 0.1) \times 10^6$ sperms per seminal vesicle for European *A. mellifera* (Rinderer et al., 1999), to $11.9 \pm 1.0 \times 10^6$ sperms per individual drone from an *A. m. carnica* colony (Schlüns et al., 2003). Collins and Pettis (2001) considered that the large variation reported in sperm numbers in semen might be due to using viscous and sticky semen to obtain counts. This may cause sperm cells to clump after being experimentally diluted, resulting in measurement errors. However, Bishop (1920a) reported that sperm release themselves more easily in the ejaculatory duct when mucus is more viscous. Koeniger et al. (2005) also stated that, in general, sperm numbers of individual drones show high variance and also suggested this could be due to errors in the sperm counting method. Nevertheless, they concluded that the cause of differences in sperm concentration reported from individual drones was yet to be determined.

The current study was carried out on *A. mellifera* drones in south east Australia, where studies had previously shown that commercially reared *A. mellifera* queens contained low concentrations of sperm in their spermathecae (Rhodes and Somerville, 2003). The aim was to determine whether particular characteristics of drones in that region might have been a contributing factor. We assessed the effects of age, genetics and season on semen and sperm production by examining 14, 21 and 35-day-old drones reared from four different breeding lines during spring, summer and autumn.

2. MATERIALS AND METHODS

2.1. Obtaining drones and measuring semen and sperm production

Three to five open-mated queen bees were produced by standard queen-rearing methods from each of four instrumentally inseminated breeder queen bees that were similar to *A. mellifera ligustica* and maintained by different commercial queen-rearing operations. The daughter queens were used to provide drones for the current study and each represented dissimilar genetic backgrounds and identified as representing lines 1, 2, 3 and 4. Each of the daughter queens was marked and kept in a double-story hive in a single apiary at Richmond, New South Wales, Australia. To reduce nutritional effects on the drone characteristics being examined, the hives were continuously replenished with pollen and sugar syrup throughout the study. All hives were also free of clinical signs of disease.

Drones were reared from each line during spring 2003 (Oct.–Nov.), summer 2004 (Feb.–Mar.) and autumn 2004 (April). Drone rearing and maintenance involved placing a queen bee in a queen excluder cage on a full frame of drone comb for 24 h.