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SCIENTIFIC NOTE

**COLONY CHARACTERISTICS AND *NOSEMA*
INFECTION OF *BOMBUS AURICOMUS*
(HYMENOPTERA: APIDAE)¹**Charles A. E. Dean,² Michelle A. Duennes,² Chia-Ching Chu,³ Sydney A. Cameron²

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The black and gold bumble bee, *Bombus auricomus* (Robertson, 1903) is native to North America east of the Rocky Mountains, encompassing much of the Northeastern United States and Southeastern Canada (Hobbs, 1965; Williams et al., 2014). This widespread species is commonly found in open grasslands, prairies and woodlands. Along with *B. nevadensis* and *B. confusus*, *B. auricomus* belongs to the early-diverging subgenus, *Bombias* (Cameron et al., 2007, Williams et al., 2008), a taxon with only sparse natural history reports in the *Bombus* literature.

A *B. auricomus* colony was obtained from the crawl space beneath a home near Meadowbrook Park in Urbana, IL, on 2 August 2014. Despite major disturbance to the colony during excavation, adults remained docile and made no attempts to attack or escape the nesting area. This is consistent with earlier reports from Frison (1917). The colony was relocated to a 20 x 30 x 18 cm Plexiglas nest box and maintained in captivity in the laboratory at 24°C in constant darkness, except during periods of feeding and observation when red light was used to minimize colony activity. The colony was fed a 50:50 (by vol) honey:water solution and fresh frozen pollen *ad libitum*. Workers defended the nest and foraged for food within the nest box, and both gynes and workers participated in incubating and provisioning the brood. Males occasionally assisted in incubating the pupal cocoons, but to a lesser degree than females and for a shorter period of time. At the time of excavation, the queen showed signs of age in appearance only, with slightly frayed wings and worn pile. In other regards she was vigorous during observations in the laboratory.

We counted egg cells, larval clusters and pupal cocoons every 2-3 days over a 31-day period. We distinguished adult workers (mean = 4.15 mm) from gynes (mean = 5.4 mm) by size. On average, one egg cell was laid atop a cocoon per day, resulting in a distinct linearly arranged cluster of egg cells appearing

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approximately every 3-5 days. Daily observations over the course of 40 days showed that ~20 pupal cocoons were typically present in the brood comb. Meanwhile, ~1-3 adults emerged per day and the proportion of workers, gynes and males produced changed over the observation period. While Frison (1917) recorded the development times and behavior of *B. auricomus* castes, which he defined as workers, drones and queens, we present new data regarding the abundances of workers, males and gynes in the nest and how they fluctuate over time. Throughout the observational period, one worker was produced for every two males or gynes. During the first week of observation, there were equal numbers of males and gynes produced on a daily basis. However, throughout the second and third weeks, two gynes were produced for every male. An average of 50 ± 10 adults were present in the nest at any given time: mean number of workers = 12 ± 3 , mean number of gynes = 17 ± 10 and mean number of males = 13 ± 7 .

Both Frison (1917) and Hobbs (1965) made observations regarding the size of *B. auricomus* adults. Here, we add to these observations with empirical measurements of the intertegular distance of all adult bees in the colony, which is used as a proxy for body size (Cane, 1987). Intertegular distances were taken of all 61 living (13 workers, 19 males, 28 gynes, 1 queen) and 7 deceased (5 workers, 2 gynes, 2 males) adults in the nest 27 days after excavation. The average body size for workers, queen/gynes, and males were significantly different from each other, with the reproductive females being larger, as expected (Table 1).

Table 1. Average body size and standard deviation of each caste in the *B. auricomus* colony.

Caste	Avg. Size (mm)	STDEV	Indiv. Alive	Indiv. Dead
Worker	4.15	0.48	13	5
Male	4.78	0.32	19	2
Gyne	5.4	0.22	28	0
Parental Queen	5.4		1	0

The abbreviation “STDEV” indicates standard deviation and the abbreviation “Indiv.” indicates number of *B. auricomus* individuals measured.

We also examined the presence of *Nosema bombi*, an obligate fungal parasite that damages host tissues in the gut wall and Malpighian tubules (Fries et al., 2001), thereby decreasing host lifespan and colony growth (Rutrecht and Brown, 2009). Three workers, three gynes, three males, and the parental queen were tested for *N. bombi* infection via DNA extraction. The complete digestive tract was excised from each bee, pulverized with micro-pestles and used for DNA extrac-

tion with the PowerFecal DNA Isolation Kit (MO BIO Laboratories, Inc.). Gut DNA samples (10 ng) were amplified via PCR, using *N. bombi*-specific rRNA primers: NbombiSSUJf1/NbombiSSUJr1 (Klee et al., 2006). The PCR conditions were 94°C for 2 min, 40 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 45 s, and 72°C for 5 min. A field-collected *B. auricomus* sample infected with *N. bombi* (previously verified by PCR and sequencing) was used as a positive control in screening the bees. The PCR assay amplified a targeted 323 bp fragment in every *B. auricomus* sample tested, indicating the presence of *N. bombi* in all castes.

There is little research on the conservation status of *B. auricomus* and the prevalence of *N. bombi* within this species, unlike for other species found to be in decline in North America (e.g., Cameron et al., 2011). Some studies suggest *B. auricomus* may be regionally vulnerable (Colla et al., 2012) or locally rare (Malfi and Roulston 2014). Colla et al. (2012) classified *B. auricomus* as “vulnerable” because the species persisted through less than 50% of its range across a 100-year period. Furthermore, Malfi and Roulston (2014) in Virginia and Tripodi et al. (2014) in Kansas reported significantly higher *N. bombi* prevalence in *B. auricomus* populations relative to putatively non-rare species surveyed. Given these data, it is possible that *B. auricomus* may be vulnerable locally in certain regions of the eastern and central U.S. However, other evidence suggests that *B. auricomus* is stable throughout some parts of its overall range. Grixti et al. (2009) found that this species had a stable abundance in Illinois across a 100-year survey of museum collections, and it is currently one of the most abundant species in prairie areas of at least Illinois and Missouri (Cameron, unpublished data). It is possible that while *B. auricomus* is susceptible to *N. bombi* infection, it may persist through the infection, thus maintaining healthy populations (possibly even expanding populations) in Illinois and Missouri. This requires further study.

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