The Seasonal Reproductive Status of Tawny Crazy Ant Queens (Hymenoptera: Formicidae) in Florida

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The seasonal reproductive status of tawny crazy ant queens (Hymenoptera: Formicidae) in Florida

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The tawny crazy ant, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), develops extremely large populations that dominate landscapes and invade buildings in states along the Gulf of Mexico (Florida, Alabama, Mississippi, Louisiana, and Texas), Georgia, and the island of St. Croix, US Virgin Islands (Klotz et al. 1995; Wetterer & Keularts 2008; MacGown & Layton 2010; Gotzek et al. 2012; Wetterer et al. 2014; Gochnour et al. 2019). There is evidence that populations of *N. fulva* throughout the USA form a single supercolony (Eyer et al. 2018; LeBrun et al. 2019; Lawson & Oi 2020). Control of invasive ants, such as *N. fulva*, requires a thorough understanding of their biology to guide the development of effective measures of suppression or eradication. For example, phenology and annual population abundance cycles may be used to determine optimal treatment application times (Markin 1970; Hoffmann et al. 2011; Hoffmann 2015). Examining the seasonal prevalence of *N. fulva* reproductives, Oi (2020) reported the presence of numerous queens within nests during the winter, but curiously these nests contained minimal amounts of brood. To further understand these observations, this study examined the seasonal reproductive status of *N. fulva* queens collected in north central Florida.

The reproductive status of *N. fulva* queens was determined by estimating the number of eggs, or large developing eggs, observed in their ovaries and examining spermathecae for sperm. Three *N. fulva* colonies, or more appropriately called colony fragments because of its supercolony status, were collected monthly from 2 sites in Gainesville, Florida, USA (a private residence [29.6302°N, 82.4638°W]; and a wooded area near Petra Design Inc. [29.7001°N, 82.3335°W]), from Sep 2013 to Feb 2015. Colony fragments (hereafter colonies) were extracted from isolated (> 5 m apart) decaying branches found on the ground and from nested plant nursery containers using the methods of Sharma et al. (2019). Ten dealate queens then were separated from each colony and either dissected immediately or frozen live at −80 °C. Ovaries were dissected from 10 queens per colony, and the number of developing and mature eggs in the ovaries of each queen was estimated with a rating scale used for *Solenopsis invicta* Buren (Hymenoptera: Formicidae) where 1 = no eggs; 2 = 1 to 10 eggs; 3 = 11 to 50 eggs; and 4 = > 50 eggs (Valles et al. 2013). Whereas the fecundity of *S. invicta* queens is much higher than *N. fulva* based on oviposition rates (Tschinkel 1988; Arcila et al. 2002; McDonald 2012), this scale could distinguish changes in *N. fulva* fecundity among seasons. In addition, the spermathecae of queens were examined by microscopy for the presence of spermatozoa to determine if queens were inseminated (Glancey & Lofgren 1985) (Fig. 1).

Ovary ratings and percentage of inseminated queens per colony were determined for each mo, then grouped into seasons. Using temperatures recorded by the Florida Automated Weather Network (https://fawn.ifas.ufl.edu/) for Alachua County from Sep 2013 through Feb 2015, seasonal categories were determined from the average minimum and maximum daily temperatures (Oi 2020). The months of Dec to Feb had the lowest average daily minimum and daily maximum temperatures of 6.6 °C (range: −4.6–17.8) and 19.1 °C (range: 3.4–28.3), respectively. These mo were designated as the winter season and the succeeding 3 mo intervals comprised the remaining seasons of spring (Mar–May), summer (Jun–Aug), and fall (Sep–Nov).

The association of the frequency of the 4 ovary ratings per queen with the 4 seasons was determined with a 4 × 4 contingency table and chi-square test. Similarly, the association between the 4 ratings and the number of inseminated and number of uninseminated spermathecae was evaluated by chi-square test of a 4 × 2 contingency table (Proc FREQ, SAS Version 9.4, SAS Institute, Cary, North Carolina, USA). Queens with undetected spermathecae were considered to be uninseminated because previously observed uninseminated spermathecae usually were very difficult to discern.

The frequency of the individual ovary ratings was not randomly distributed across seasons (χ^2 = 56.129; df = 9; P < 0.0001), where 73 to 91% (n = 90 and 150, respectively) of the queens had ovary ratings ≥ 3 per season (Fig. 2A). Among the 4 seasons, 81 to 92% of the queens were inseminated, indicating a significant preponderance (χ^2 = 12.166; df = 3; P = 0.0068) of mated female reproductives throughout the yr. The seasonal distribution of uninseminated queens was 19% (n = 155) and 14% (n = 90) for winter and spring, respectively, and 8% each for summer (n = 150) and fall (n = 126). The association between ovary ratings and the inseminated and uninseminated queens also was not random (χ^2 = 81.686; df = 3; P < 0.0001). All 4 ovary ratings were distributed evenly among uninseminated queens, ranging from 20 to 32% (n = 65). In contrast, 89% (n = 456) of the inseminated queens had ovary ratings ≥ 3 (Fig. 2B). Arcila et al. (2002) reared *N. fulva* queens from larvae in queenless colony fragments consisting of larvae and workers, and thus could be a source of uninseminated queens. However, whether these queens were dealates or alate gynes was not specified.

The combination of high numbers of *N. fulva* queens with low brood levels in permanent nest sites, and few, small, ephemeral nests during the winter suggested a winter consolidation of colonies (Zenner-Polania 1990; Oi 2020). Because of the lack of brood in the winter nests despite the presence of many queens, it was hypothesized that queens in the winter were unmated and ovaries would have fewer eggs. The results of this study showed that over 80% of the queens had inseminated spermathecae in each season. In addition, ovary rating frequencies for each season were skewed toward the ratings of 3 and 4 (10–50 eggs and > 50 eggs, respectively) indicating that eggs were present in queens throughout the yr.

The percentages of queens with > 50 eggs increased from 26% in winter to a peak of 68% in summer. The occurrence of queens with 10

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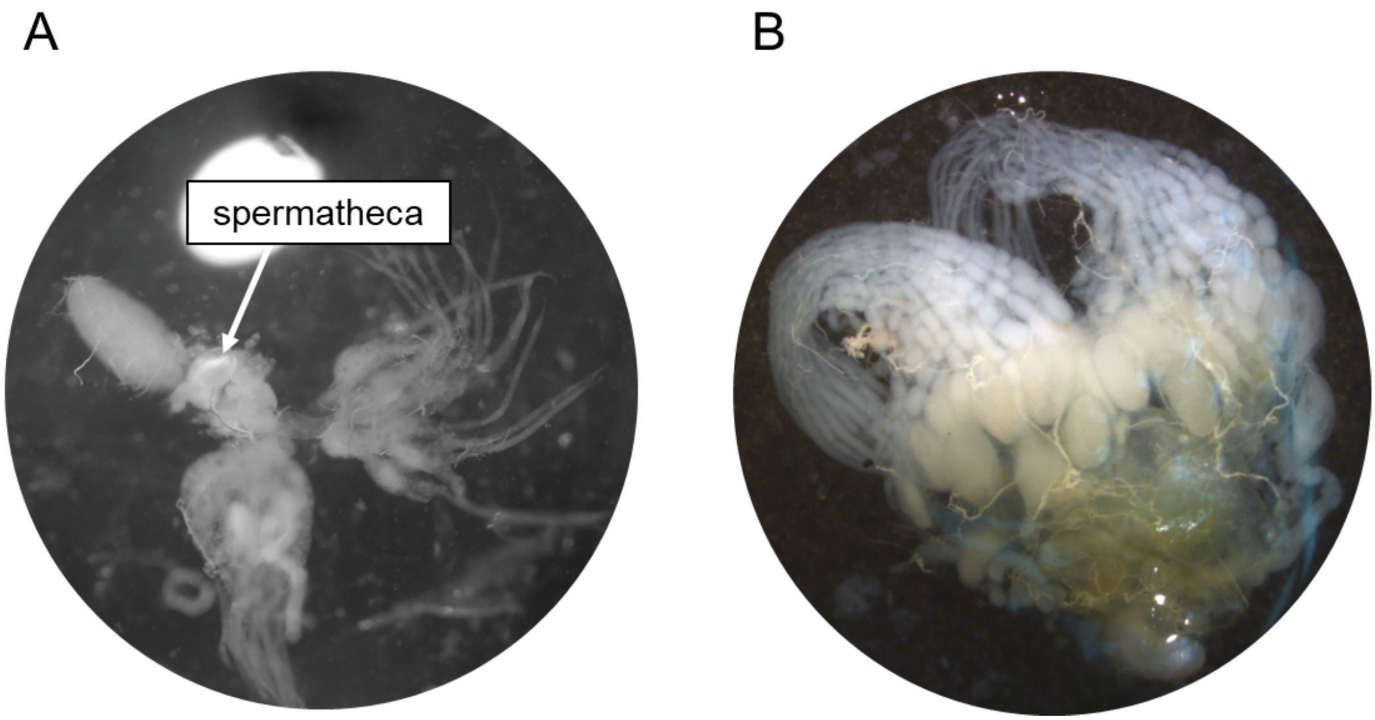

Fig. 1. Ovaries dissected from *Nylanderia fulva* queens. (A) Ovary rated as a "2" (1–10 eggs) where the spermatheca is present. (B) Ovary rating of "4" (> 50 eggs).

to 50 eggs declined from 55% in winter to a low of 28% in summer, while queens with < 10 eggs was below 20% for all seasons (Fig. 2A). These trends suggested that egg production increased in queens as the seasons progressed toward the summer. More dramatic changes in ovary status have been reported in other ants such as *Dolichoderus mariae* Forel (Hymenoptera: Formicidae) where queen ovaries were inactive in Jan but had increasing numbers of eggs in Apr and Jun (Laskis & Tschinkel 2008).

Ovaries of *N. fulva* queens were primed with eggs in the winter, but this did not reflect the absence of brood in nests examined in the winter (Oi 2020). It is likely that the warmer temperatures during spring and summer and the associated increase in food availability, for example, honeydew producing insects (Sharma et al. 2013), will initiate and sustain the production and oviposition of eggs as seen in other invasive ants (Markin 1970; Tschinkel 1993; Abril et al. 2007; Abril & Gomez 2014; Hoffmann 2015). If queens are not actively producing and laying eggs during the winter, their ingestion of food and bait could be minimal and thus limit the impact of the bait on the queens (Collins et al. 1992; Hoffmann 2015). In Argentine ants, the dominant reproductive queens ingested more bait than non-dominant queens allowing the latter queens to survive and potentially allowing colonies to recover (Hooper-Bui et. al. 2015).

Targeting bait applications to winter nesting sites where *N. fulva* queens have coalesced represents an enticing control strategy. Indeed, removal of winter nests has resulted in temporary suppression of Argentine ants (Diaz et al. 2014), but bait applications are logistically more feasible than excavating nests over large areas. However, given the potential for limited bait ingestion by queens in the winter, it would be prudent to specifically examine seasonal queen development and empirically determine when and how *N. fulva* queens could be most efficiently controlled.

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Summary

The tawny crazy ant, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), is an invasive ant that is spreading in the southern USA. The control of invasive ants requires an understanding of their biology to implement measures of suppression such as strategically applying ant baits to eliminate queens. *Nylanderia fulva* queens were collected monthly in north central Florida and dissected to determine their seasonal reproductive status. The percentages of queens with > 50 eggs increased from 26% in winter to a peak of 68% in summer, while queens with < 10 eggs was below 20% per season. Thus, eggs were present in queens throughout the yr. Likewise, mated queens were present in each season, with 81 to 92% of the queens inseminated. While queens were fecund yr-round, the lack of brood production in winter may indicate a curtailment of colony foraging to feed queens and larvae which could impede the strategy of baiting queens consolidated in winter nest sites.

Key Words: *Nylanderia fulva*; oviposition; reproduction; ovary; spermatheca; reproductive phenology

Sumario

La hormiga loca de color marrón amarillento, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), es una hormiga invasora que se está extendiendo por el sur de los Estados Unidos. El control de las hormigas invasoras requiere una comprensión de su biología para implemen-

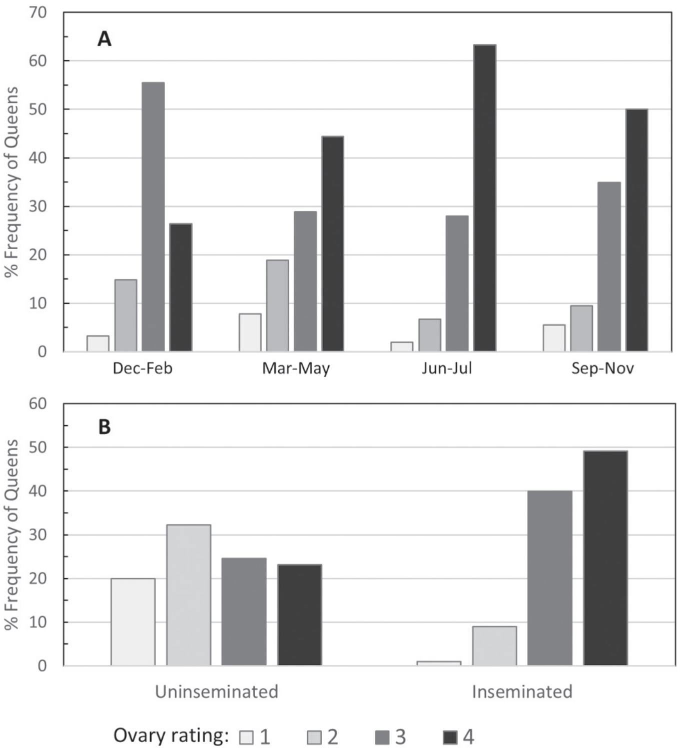

Fig. 2. Percent frequency of *Nylanderia fulva* queens with specified ovary ratings: 1 = 0 eggs; 2 = 1 to 10 eggs; 3 = 10 to 50 eggs; 4 = > 50 eggs. (A) among seasons (winter: Dec–Feb, *n* = 155; spring: Mar–May, *n* = 90; summer: Jun–Aug, *n* = 150; fall: Sep–Nov, *n* = 126), and (B) between uninseminated (*n* = 65) and inseminated (*n* = 456) queens over all seasons.

tar medidas de supresión, como la aplicación estratégica de cebos para eliminar a sus reinas. Se recolectaron las reinas de *Nylanderia fulva* mensualmente en el centro norte de Florida y se disecaron para determinar su estado reproductivo estacional. El porcentaje de reinas con > 50 huevos aumentaron del 26% en invierno a un máximo del 68% en verano, mientras que las reinas con < 10 huevos estuvieron por debajo del 20% por temporada. Por lo tanto, los huevos estuvieron presentes en las reinas durante todo el año. Asimismo, las reinas apareadas estuvieron presentes en cada temporada, con un 81 a 92% reinas inseminadas. Mientras que las reinas fueron fecundas durante todo el año, la falta de producción de crías en invierno puede indicar una reducción de la búsqueda de alimento en las colonias para alimentar a las reinas y larvas, lo que podría impedir la estrategia de cebo para las reinas consolidadas en los sitios de anidación de invierno.

Palabras Claves: *Nylanderia fulva*; oviposición; reproducción; ovario; espermateca; fenología reproductiva

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