Seasonal prevalence of queens and males in colonies of tawny crazy ants (Hymenoptera: Formicidae) in Florida

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The tawny crazy ant, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), is an invasive ant from South America that develops overwhelming populations that invade structures, cause electrical malfunctions, damage crops, and reduce biodiversity in natural and agricultural landscapes (Zenner-Polania 1994; Wetterer & Keularts 2008; Wetterer et al. 2014). It has been reported as a problem in Florida since 1990 and in Texas since 2002, and now has spread to several other southern states (Klotz et al. 1995; MacGown & Layton 2010; Gotzek et al. 2012). Eyer et al. (2018) indicated that *N. fulva*, in the USA, is a single supercolony and the individuals within nests in that study constitute colony fragments. The objective of our study was to determine the seasonal prevalence of adult, female and male reproductive castes, and brood of *N. fulva* in north central Florida.

Nylanderia fulva colony fragments (hereafter called colonies) were collected from sites located in Gainesville, Alachua County, Florida, USA, in 2011 and 2012. Monthly collections of 3 to 10 colonies (avg. 7.2 ± 1.6 SD, *n* = 15 mo) were conducted at 3 sites designated as Tumblin' Creek Park (29.6444°N, 82.3316°W), Shands Cabinet Shop (29.6412°N, 82.3297°W), and Petra Design Inc. (29.7001°N, 82.3335°W). Colonies were collected primarily from individual decaying tree branches (about 25 to 51 cm L × about 2.5 to 5 cm diam) found on the ground that usually were well separated (> 5 m apart). Decaying branches typically had loose bark with softening sapwood. Colonies often were found between the bark and sapwood. Whereas other harborages such as tree stumps, rocks, and man-made debris (e.g., boards, cans) provide nesting sites, they may not be conducive for nest collection, are not uniform in size, nor consistently available. Previous collections and observations of *N. fulva* nests from a variety of substrates over several yr and seasons, suggested that fallen, decaying branches were consistent nesting sites and could provide a standardized sampling unit.

Branches were transported to the laboratory where colonies were extracted within 8 d (avg. 2.2 ± 2.1 SD, *n* = 31) of collection. To extract colonies, branches were broken apart and allowed to dry at ambient temperature, upon which colonies moved into nest tubes placed under a dark harborage (Sharma et al. 2019). Nest tubes were adapted from Banks et al. (1981), consisting of 20 mL glass test tubes, half filled with water and plugged with cotton and dental plaster (Castone®, Dentsply International, York, Pennsylvania, USA). The amount of brood per colony was estimated visually by determining the volume occupied by eggs, larvae, and pupae within each nest tube by comparing it with graduated test tubes of the same diam. Workers, male and female reproductives were counted subsequently by tapping out small portions of the contents of the nest tubes directly into plastic containers (118–473 mL) or into a clean, enamel pan where ants were scooped up with index cards and tapped into the containers in countable groups. In some instances, worker numbers were estimated from counting by visual estimates of groups of 10 and 100 ants. All containers and pans had sides coated with Fluon® (PTFE D-210, Daikin America, Inc., Orangeburg, New York, USA) to prevent ants from escaping. If necessary, ants were temporarily immobilized by refrigeration to facilitate counting. Male and female reproductives readily could be distinguished morphologically (MacGown & Layton 2010).

The number of queens (possibly including female dealates), number of males, and volume of brood per colony was determined for each mo, then grouped into 3-mo seasons of winter (Dec–Feb), spring (Mar–May), summer (Jun–Aug), and fall (Sep–Nov). Seasonal categories were based on the average minimum and maximum daily temperature recorded for Alachua County by the Florida Automated Weather Network from Jan 2011 through Mar 2012. The months of Dec to Feb had the lowest average daily minimum and maximum temperatures of 6.6 °C (range: −8.5–18.0) and 20.0 °C (range: 6.2–28.2), respectively. Thus, these mo were designated as the winter season and the succeeding 3 mo intervals comprised the remaining seasons. Using a completely randomized design, number of queens, males, and volume of brood (per colony) were compared among the seasons using Kruskal-Wallis tests and Tukey's HSD test on ranked counts (Proc RANK, Proc GLM, SAS version 9.4, SAS Institute, Cary, North Carolina, USA). The same analysis was used to compare among seasons, each ratio of the number of queens, males, and brood to the number of workers per colony where each ratio was expressed as a quotient. An analysis of variance and Tukey's HSD test were used to compare $\log_{10}(x + 1)$ transformed worker counts among seasons.

The number of queens per colony were significantly different among the seasons (F = 26.06; df = 3, 104; P < 0.0001), with mean winter counts of 15.7 (± 3.6) and spring counts of 16.7 (± 2.3) significantly higher than the summer and fall counts of 2.6 (± 0.7) and 3.6 (± 0.3), respectively (Table 1). A maximum mean count of 35 queens per colony occurred in Jan and Mar 2011 (Fig. 1). The number of males was significantly greater in the fall and winter (F = 18.93; df = 3, 104; P < 0.0001) (Table 1), with most males collected in Nov and Jan (Fig. 1). Numerous dead male alates, evidently attracted to artificial lighting, have been observed in Feb (DHO, personal observation). However, flying males have been trapped throughout the yr near Houston, Texas, USA, with peak densities occurring in late Aug (McDonald & Cook 2015). Brood volume was significantly higher (F = 6.79; df = 3, 104; P < 0.0003) in the spring (1.5 mL ± 0.4) and fall (1.4 mL ± 0.3) when compared to the winter brood level of 0.4 mL (± 0.1) (Table 1). The ratios of queens, males, and brood to workers had similar significant differences among the seasons to the count data. This indicated that the production of reproductives and brood among the seasons relative to colony size (indicated by the number of workers per colony) was consistent with the count data. However, spring queen and winter brood ratios were

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significantly higher and lower, respectively, than the other seasons. In addition, the number of workers per colony was significantly lower in the summer (Table 1).

The seasonal prevalence *N. fulva* reproductives and brood observed in this study resembles the seasonal population fluctuations and nesting behavior observed by Zenner-Polania (1990a) in Colombia. During the dry hot season in Colombia (Dec–Mar), *N. fulva* reside in large permanent nesting sites, such as the base of large trees, where queens, male alates, and brood are found in larger numbers. When the rainy season begins, *N. fulva* move beyond the permanent nest and disperse to reside in small "transitory" nests that occupy transient niches such as under leaves and in soil crevices. The colonies in transient nests were reported to be small, highly mobile, and lacking reproductives.

The *N. fulva* nesting pattern observed in this study also had permanent and transitory nesting. In the summer, higher temperatures and rainfall prevail, with afternoon rain showers common. During this time, transitory nesting was observed, where smaller colonies with fewer workers and queens commonly were collected. In the winter, when cool temperatures occur, *N. fulva* nested at the base of trees with large worker populations, minimal brood, and numerous queens and male alates. It is speculated that the transient colonies coalesced at niches conducive for permanent nest establishment during the winter.

Yearly cycles of dispersed transitory nests in summer coalescing to large more permanent nests in the winter, or seasonal polydomy, have been reported for other pest ants such as the Argentine ant and the odorous house ant (Heller & Gordon 2006; Buczkowski & Bennett 2008). Winter nesting sites often are located at or near the base of woody plants that provide a more stable yr-round microclimate and closer access to food resources such as honeydew producing hemipterans (Heller & Gordon 2006). Similar observations have been made for *N. fulva* in Florida in this study and at other locations (DHO, personal observation).

Knowledge of seasonal polydomy patterns of *N. fulva* may contribute to the development of control strategies for this invasive ant. Zenner-Polania (1990b) described how timing bait applications after the rainy season improved efficacy because natural food sources of *N. fulva* that compete with bait were less abundant during the dry season, and noted reductions in colony populations at baited permanent nest sites. Monitoring in Feb and baiting *N. fulva* infestations "early in the season" was recommended as part of an integrated pest management strategy to control this ant (Oi et al. 2016). Results of this study support this recommendation because baits can be applied to permanent nests at tree bases to more efficiently target *N. fulva* queens that are more prevalent in these nests during winter and spring (Jan–Apr).

Summary

The tawny crazy ant, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), is an invasive ant from South America with overwhelming populations that invade structures and overrun landscapes. To contribute to the development of biologically based control strategies for this ant, the seasonal prevalence of queens, males, and brood within colonies of *N. fulva* was determined from monthly collections of colonies located in north central Florida. The average number of queens per colony was significantly greater in the winter and spring than summer and fall. The fall and winter male counts were significantly higher than the other 2 seasons. Brood was most prevalent in the spring and fall and significantly greater than the brood collected in winter. Comparisons of queen, male, and brood to worker ratios generally had similar results to the average counts of queens and males and brood volume per season. The seasonal fluctuations in levels of queens, males, and brood reflect the winter coalescing of colonies into larger, permanent nest sites, and the summer dispersal of smaller colonies into transient nest sites located throughout landscapes.

Key Words: *Nylanderia fulva*; queens; seasonal polydomy; colony movement; seasonal phenology

Sumario

La hormiga loca, *Nylanderia fulva* Mayr (Hymenoptera: Formicidae), es una hormiga invasora de América del Sur con poblaciones abrumadoras que invaden toda clase de estructuras y de campos. Para contribuir al desarrollo de estrategias de control biológicas para esta hormiga, se determinó la prevalencia estacional de las reinas, machos y crías dentro de las colonias de *N. fulva* a partir de colecciones mensuales de colonias ubicadas en el centro norte de la Florida. El número promedio de reinas por colonia fue significativamente mayor en el invierno y la primavera que en el verano y otoño. El conteo de los machos en el otoño e invierno fue significativamente más alto que

Table 1. Mean (± SE) number of *Nylanderia fulva* queens, males, workers, and volume of brood per colony and the mean (± SE) ratio of the number of queens, males, and brood volume to number of workers per colony among seasons.

Season	No. colonies	No. queens	No. males	No. workers	Brood (mL)	Queen/worker ratio[c]	Male/worker ratio	Brood/worker ratio
Winter (Dec–Feb)	36	15.7 a[a] (± 3.6)	67.0 a[a] (± 15.9)	1933 a[b] (± 245.3)	0.4 a[a] (± 0.1)	1.09 b (± 0.26)	3.53 a (± 0.64)	0.03 a (± 0.01)
Spring (Mar–May)	30	16.7 a (± 2.3)	0.0 b (± 0.0)	1327 a (± 248.8)	1.5 b (± 0.4)	2.57 a (± 0.49)	0.00 b (± 0.00)	0.13 b (± 0.03)
Summer (Jun–Aug)	20	2.6 b (± 0.7)	1.1 b (± 0.4)	394 b (± 82.3)	0.8 ab (± 0.2)	0.67 b (± 0.13)	0.63 b (± 0.25)	0.23 b (± 0.07)
Fall (Sep–Nov)	22	3.6 b (± 0.3)	54.6 a (± 35.9)	1109 a (± 198.7)	1.4 b (± 0.3)	0.74 b (± 0.23)	4.41 a (± 1.81)	0.22 b (± 0.06)

[a]Means in a column followed by the same letters are not significantly different (*P* > 0.05; Kruskal-Wallis test and Tukey's HSD test on ranked data; non-transformed means (± SE) are presented.

[b]Means of the number of workers within a column followed by the same letters are not significantly different (*P* > 0.05; Analysis of Variance and Tukey's HSD test on log10(× + 1) transformed data; non-transformed means (± SE) are presented).

[c]Ratios are expressed as a quotient of queens and male counts, and brood volume to worker counts per colony. Ratios within a column followed by the same letters are not significantly different (*P* > 0.05; Kruskal-Wallis test and Tukey's HSD test on ranked data; non-transformed means (± SE) are presented).
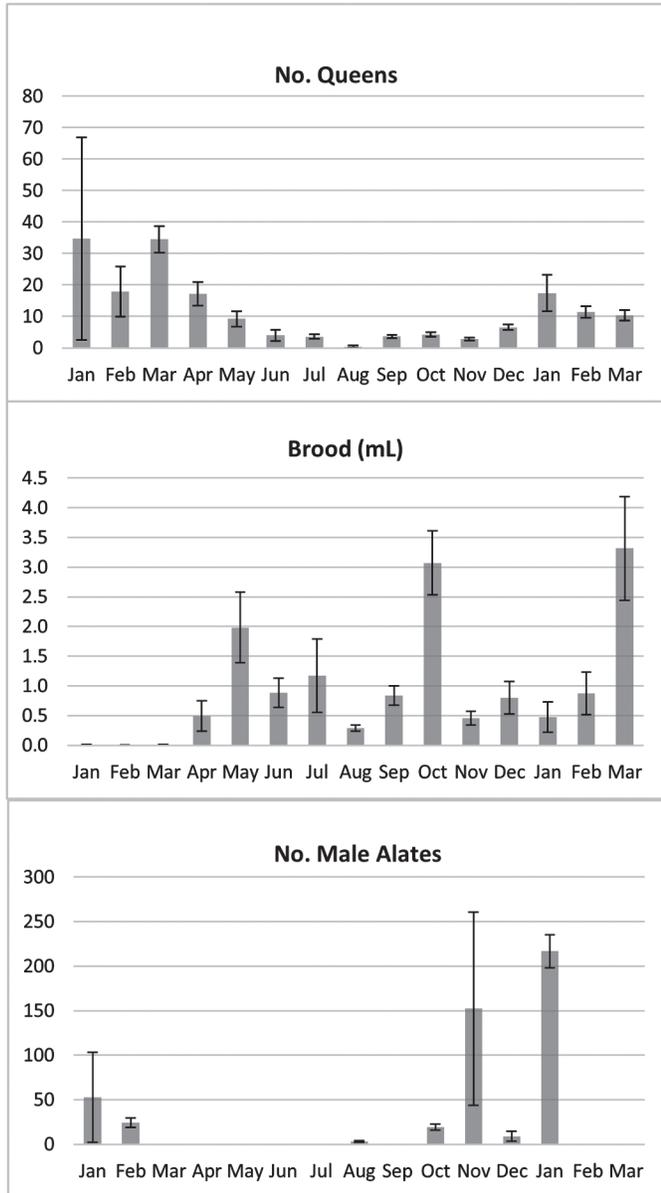
Fig. 1. Mean ± SE (*n* = 3–11) number of queens (including female dealates), volume of brood (mL), and number of male alates per colony, collected monthly in Gainesville (Alachua County), Florida, USA, to show monthly fluctuations within seasons designated as winter (Dec–Feb), spring (Mar–May), summer (Jun–Aug), and fall (Sep–Nov).

las otras 2 temporadas. La cría fue más prevalente en la primavera y el otoño y significativamente mayor que la cría recolectada en el invierno. Las comparaciones de las proporciones de reinas, machos y cría por trabajador en general tuvieron resultados similares al promedio del conteo de las reinas y machos y el volumen de cría por temporada. Las fluctuaciones estacionales en los niveles de reinas, machos y crías

reflejan la fusión invernal de las colonias en sitios de nido más grandes y permanentes, y la dispersión en verano de colonias más pequeñas en sitios de nido transitorios ubicados a lo largo del campo.

Palabras Clave: *Nylanderia fulva*; reinas; polidomia estacional; movimiento de colonias; fenología estacional

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