

An adhesive drone trap to study the flight altitude preferences of winged ants

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The ability of queens and males of most ant species to disperse by flight has fundamentally contributed to the group's evolutionary and ecological success and is a determining factor to take into account for biogeographic studies (Wagner and Liebherr 1992; Peeters and Ito 2001; Helms 2018). Most of the over 16,000 known ant taxa rely on their flying abilities for dispersal and reproduction (Hölldobler and Wilson 1990; Peeters and Ito 2001; Helms and Kaspari 2015; Bolton 2024). However, they are very different in size, habitat preferences, and most likely in their flying abilities and dispersal strategies. Still, details over the dispersal altitude, range, and flight duration of flying ant males and queens are usually unknown, with rare exceptions (Helms et al. 2016a, 2016b). The main limitation to studying these aspects is technical, as the small size of most flying ants made it difficult to use techniques such as radars or radio-tracking that are employed for other insects (e.g., Minot et al. 2021; Bauer et al. 2024). For example, Helms et al. (2016a, 2016b) obtained data over flying altitudes of ants indirectly by monitoring an insectivorous bird, while laboratory tests were used by Vogt et al. (2000) to deduce flying performances in the red imported fire ant Solenopsis invicta Buren, 1972. The flight of males and queens in ants is generally bound to two aims: mating with a partner and dispersing towards a suitable place to find a new colony (in the case of queens). Two main mating behavioral strategies have been described (Hölldobler and Bartz 1985; Bourke and Franks 1995; Grasso et al. 2003). In the female-calling syndrome, isolated queens remain on the ground near the nests using pheromone release to attract males. This strategy may increase reproductive success as both sexes are at low densities simultaneously on the surface. In the male-aggregation syndrome, numerous colonies simultaneously release flying queens and males over a large area. One consequence of this mechanism is the low probability of inbreeding owing to the large scale and the infrequency of nuptial flights. Furthermore, this strategy grants a better anti-predatory defence in the form of a dilution effect (Hölldobler and Bartz 1985; Bourke and Franks 1995; Grasso et al. 2003). Flight is further influenced by environmental parameters such as temperature, humidity, and wind speed, and is the deadliest phase in the life cycle for

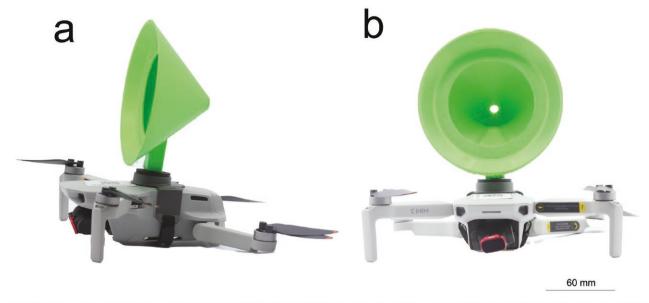
foundress queens, which are exposed to predators and other hazards (Hölldobler and Wilson 1990; Nichols and Sites 1991; Peeters and Ito 2001; Fjerdingstad and Keller 2004; Frederickson 2006; Helms and Kaspari 2015). It is estimated that sometimes the mortality could even be close to 99% during this brief time of their life (Gordon and Kulig 1996; Helms and Kaspari 2015). Commercially available drones offer unprecedented opportunities to monitor insects thanks to their small size, maneuverability, speed, and hovering capabilities (Hassanalian and Abdelkefi 2017; Nowak et al. 2018). Recent studies have successfully tested the use of drones to monitor insects in agricultural contexts as well as natural environments (Kim et al. 2018; Löcken et al. 2020; Kakutani et al. 2021; Madden et al. 2022; Mulero-Pázmány et al. 2022; Almstedt et al. 2023; Giannetti et al. 2024a).

This study aims to develop a monitoring system to sample flying ants and determine their altitude, using a 3D-printed adhesive trap (Figure 1A,B) mounted on a small drone, comparing the flight altitude of ants of different species and sizes.

The drone can capture ants only when moving horizontally to the ground (Figure 1D), while the trap does not work when the drone hovers or moves vertically to change altitude (Figure 1C,D). We chose 4 ant species from 3 subfamilies to test the efficiency of the drone, selecting them based on their availability in the study area and the detectability of their nuptial flights (Seifert 2018; Giannetti et al. 2019, 2024b). In total, we selected 9 colonies of *Crematogaster scutellaris* (Olivier 1792), 6 of *Dolichoderus quadripuncatatus* (Linneus 1771), 7 of *Messor ibericus* (Santschi 1931), and 4 of *Colobopsis truncata* (Spinola 1808) (see Table 1). Preliminary tests were conducted using glue bands or spray glue for insects with a 3 cm long adhesive portion on the cone bottom.

In the efficiency test, we detected a statistically significant difference in capture frequency between flight types (Generalized Linear Model binomial logit link function, $\chi^2_{799} = 267.78$, P = 0.01). We captured 49 insects during horizontal flights (19 Diptera, 5 Lepidoptera, 9 Hemiptera, and 16 Hymenoptera), and 6 in vertical flights (5 Diptera, 1 Hemiptera). Captures during vertical flights always occurred in the outer edges of the trap. Based on these results, we

2 Letter to the Editor



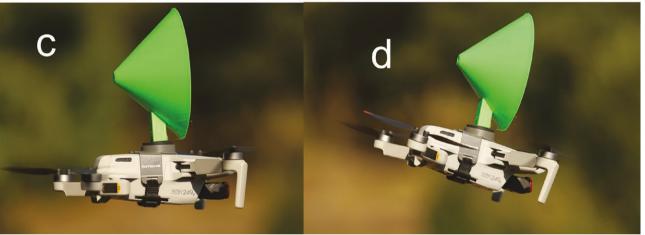


Figure 1 Above, the experimental prototypes of adhesive drone flight trap, seen side and frontal view (A, B). Below are the drone flight positions, showing the position for vertical flight and hovering (C) and the one for forward flight used to capture ants (D).

Table 1. Nuptial flight events during which ants were sampled

Species	Date of flight	Time of flight	T (°C)	W (m/s)
Crematogaster scutellaris	04 September 2022	08:30/09:30 AM	23.5	1.3
Crematogaster scutellaris	05 August 2023	19:00/20:00 PM	24	1.2
Messor ibericus	09 May 2022	11:00/13:00 AM	25	2.1
Messor ibericus	30 April 2023	12:00/14:00 PM	24.3	2.3
Dolichoderus quadripunctatus	07 July 2022	10:00/13:00 AM	28	6.6
Dolichoderus quadripunctatus	29 June 2023	10:30 to 12:00 AM	27	5.2
Dolichoderus quadripunctatus	30 June 2023	12:30/13:30 PM	30	3.6
Dolichoderus quadripunctatus	07 July 2023	11:30/13:30 AM	32.2	3.2
Dolichoderus quadripunctatus	08 July 2023	10:30/2:00 AM	34	3.3
Colobopsis truncata	01 June 2022	18:00/20:00 PM	23	0
Colobopsis truncata	02 July 2022	19:00/19:30 PM	25	0
Colobopsis truncata	02 June 2023	18.30/19:20 PM	25	0

restricted the adhesive portion to the inner sides, ensuring capture occurred exclusively during horizontal flights to keep control of their altitudes. Scan sampling allowed us to

identify the swarming dates for the species monitored, ranging from May to September with different temperatures and wind conditions (m/s).

Letter to the Editor 3

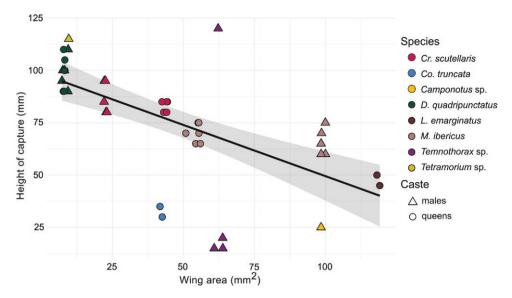


Figure 2 Relationship between the altitude of capture and the wing area surface of ant specimens collected with the drone trap. A maximum of six female and six male individuals were measured per each species.

In total, we captured a total of 24 male and 22 female ants, including 4 additional bycatch species, out of 264 flights in 12 sampling days with a 0.17 capture frequency (number of capture/total flights). The capture altitude ranged from 80 to 85 m for *Cr. scutellaris* females (mean = 85 m, n = 7) and from 80 to 95 m for their males (mean = 85 m, n = 15), from 90 to 110 m for *D. quadripunctatus* females (mean = 97, n = 9) and from 90 to 115 m for their males (mean = 102, n = 10), 65 to 75 m for *Messor ibericus* females (mean = 72, n = 10) and from 60 to 75 for their males (mean = 66, n = 8), and 30 to 35 m for *Co. truncata* females (n = 2). As for bycatch species, we captured 2 *Lasius emarginatus* females (from 45 to 50 m), a male of *Camponotus* sp. (25 m), 4 males of *Tetramorium* sp. (2 at 15 m, 1 at 20 m, and 1 at 120 m), and a male of *Temnothorax* sp. (115 m).

A linear mixed model was used to study the relationship between flight altitude, absolute wing surface area across sampled ants (measuring up to 6 individuals per sex of the same species), and wind speed while setting the ant species' identity as a random factor. The wing area is an easily measurable indicator of overall size in flying ants, although its relationship with body mass may vary across species. We found wing surface area to have a significant negative effect on ants' flight altitude (P = 0.007; slope = -0.376, SE = 0.139), while wind speed did not have a significant influence (P = 0.115; slope = -1.553, SE = 0.986) (Figure 2).

The newly tested adhesive drone trap we designed proved its ability to allow for sampling flying ants while collecting information about their flight altitude. The sampling protocol allowed for a rapid sampling time, and the scheme can be easily adapted to different environmental conditions, and mounting the trap on the upper part of the done protected it from the turbulence generated by the propellers, ensuring maneuverability and stability (Kim et al. 2018; Löcken et al. 2020). Meteorological conditions, including wind speed, temperature, and precipitations may influence ants' flight patterns (Boomsma and Leusink 1981; Helms 2023). We did not observe precipitations coinciding with any of the flights observed, something that is however mainly reported for Mediterranean species that fly at the end of summer and

early fall as soon as the dry season ends. Furthermore, wind was not the deciding factor determining ants' flight altitude according to our data, although it is likely to have a strong effect on the average dispersal distance for the species that reach the higher altitudes. Instead, the size of flying ants had a significant role in explaining their flight altitude. Smallersize species flew at the higher altitudes, with D. quadripuncatus, Temnothorax, Tetramorium attaining the highest records, coherently with what was reported by Helms et al. (2016a, 2016b). However, for Temnothorax most of the few specimens caught were found at low altitudes. Camponotus, Co. truncata, and L. emarginatus, were only detected near the lowest altitudes. Despite size differences between the two sexes being sometimes pronounced, males and queens of the same species were caught flying within the same altitudinal range (see Cr. scutellaris and M. ibericus), which is expectedly related to the mating function of flights. At the current stage, our knowledge of ants' dispersal abilities is deficient for most species. New and accessible technological solutions, such as the drone-mounted trap we tested, can represent an important tool to fill this important gap. Furthermore, finding a solid link between size or other morphological features and the maximum or more usual altitudinal flight ranges may represent a significant advantage, allowing predictions on a wide variety of species whose sexual castes are described but unknown from a behavioral standpoint. Sampling more species and obtaining more data with the protocol we designed and tested in this study is a promising way to uncover this secretive – yet crucial – aspect of ants' biology. The adhesive drone trap showed a good capturing ability in horizontal flights and allowed to determine the altitude of the different captures. The capture frequency is influenced by the size and loading capacity of small drones. Future tests could allow the evaluation of larger traps, applicable to drones with large load capacities and orientable in different positions.

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4 Letter to the Editor

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Conflict of Interest

There are no conflict of interest to declare.

Authors' Contributions

D.G, E.S., and D.A.G. conceived and designed the study; D.G. conceptualized and realized the trap; D.G. performed the field experiment; E.S. analyzed the data; and D.G., E.S., and D.A.G. wrote the manuscript.

Data Availability

The data that support the findings of this study can be found in Supplementary materials

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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