Supplemental Methods

*Species identification*

Ants in the subfamily Dolichoderinae are distinguished from those in other subfamilies by the presence of a single petiole and a small, slit-shaped opening at the tip of the gaster. *D. bicolor* workers were identified as being distinctly bicolored with an orange head and thorax and a black gaster. *F. mccooki* workers were distinguished from the closely related *Forelius* *pruinosus* by the presence of erect hairs on the antennal scape [Fisher, and Cover, 2007]. The taxonomy of *D. insanus* is in need of revision [Fisher, and Cover, 2007; Snelling, 1995] and the species used in this study is classified provisionally as such based on guidance in Fisher and Cover (2007). *D. insanus* used in this study were various shades of brown and not bicolored. *D. pustulatus* collected by the authors were distinguished from the closely-related *D. plagiatus* by their uniform black color and the small number of hairs on the antennal scape [Ellison et al., 2012].

*Habitat & collection locations*

In choosing these species, we first observed differences in foraging behavior that match observations from Beckers et al. (1989), with *F. mccooki* using trunk trails, *D. bicolor* using mass foraging without distinct trunk trails, and *D. insanus* observed using primarily individual and small group foraging. Colonies of all three species are polydomous and often occur in the same 50 - 100 m2 site (personal observation). *D. insanus* colonies had 1 to 3 active nest entrances spanning distances less than 5 meters; individual ants from nest entrances greater than 10 meters apart generally showed aggressive behavior toward each other and therefore nests greater than 20 meters apart were classified as different colonies for this study. *D. bicolor* colonies used in this study had 3 – 9 nest entrances spanning distances less than 5 meters with entrances greater than 25 meters apart classified as different colonies. *F. mccooki* colonies used in this study usually had one or two operational nest entrances but trails connecting such nest entrances could extend for 50 meters, so we chose nest entrances greater than 50 meters from one another.

*D. pustulatus* inhabit bogs and marshy areas, building shallow nests in cavities formed by thickets of dead leaves, stems, and soil [Kannowski, 1967; Ellison et al., 2012]. *D. pustulatus* workers used for neuromorphological study were collected from Forest County, Pennsylvania by RKG and provided from Door County, Michigan by Dr. James Traeger. *D. pustulatus* collected in Pennsylvania were found foraging individually or in pairs in tall grasses along the edge of a pond.

*Colony size*

Estimates for *D. pustulatus* do exist; colonies are monogynous and with a mean colony size of 445 individuals (range: 41-1299; mean worker population: 203; Kannowski, 1967]. Full colony sampling data for *D. bicolor*, and *F. mccooki* are not published. *D. insanus* colonies from a study in southeastern Arizona are reported as having 103 – 104 individuals [Valone, and Kaspari, 2005], but we could not find an empirical study to support these estimates. During peak foraging times we have observed hundreds of *D. bicolor* and more than a thousand *F. mccooki* workers outside their nests, but only tens of *D. insanus*.

*Exploration behavior*

Percent area explored was quantified by converting videos to .avi format using FFmpeg [FFmpeg Developers, 2019] and then into greyscale in FIJI [Schindelin et al., 2012]. From the greyscale stacks, the Z Project function in ImageJ was used to create maximum (lightest) and minimum (darkest) projections of greyscale values from all frames after the first ant exited the center dish. The minimum value image was then subtracted from the maximum value image and the resulting difference in pixel values converted into a binary image using the IsoData threshold function (based on Ridler and Calvard 1978). This binary image was treated as presence/absence data.

Because species vary in size, a body size adjusted percent area explored was calculated by scaling the occupied pixels to the average pixel size of an ant. Average ant pixel size was measured from 20-25 ants from single frames from one video per species for each recording session. Ant pixel size varied by session because of small variations in camera positioning across recording sessions, but measured pixel sizes for each species differed significantly from one another (ANOVA followed by Tukey’s post hoc comparisons, p < 0.05 all contrasts) and match size rankings from morphometric data (fig. 1b, c).

*Brain investment*

Tissue processed for autofluorescence was fixed in a 2% paraformaldehyde, 2% glutaraldehyde in phosphate buffered saline (PBS; tablets, Sigma, St. Louis, Missouri) for 24 hours, rinsed 3X in PBS, and stored in 0.1 M cacodylate buffer (pH 6.8) until processing for imaging. Brains prepared for glomeruli counts and tracing were fixed in 4% paraformaldehyde in PBS for 12 hours at room temperature following microwave fixation (low power and 18°C under vacuum for two cycles of 2 minutes). Monoclonal *Drosophila* synapsin I antibody (SYNORF1, AB\_2315426) purchased from the Developmental Studies Hybridoma Bank (catalog 3C11) was used as the primary antibody to label synapsin. Following blocking with goat serum, brains were permeabilized with 1% Triton X-100 in PBS (Electron Microscopy Supply, Fort Washington, PA; PBS-TX ), rinsed with 0.1% PBS-TX, and incubated on a shaker at 25°C for two nights in primary antibody (1:500 in 2% goat serum in 0.2% PBS-TX). Subsequently, brains were washed in 0.1% PBS-TX and incubated for an additional night in Alexa Fluor 568 (ThermoFisher) goat anti-mouse secondary antibody (1:100 in PBS) wrapped in foil at 25°C on a shaker. After secondary incubation, brains were washed in 0.1% PBS-TX and rinsed with distilled water. Brains were scanned on a Zeiss LSM880 inverted confocal microscope. Brains prepared with synapsin were optically sectioned in the horizontal plane at 1 micron intervals and corrected by a factor of 1.64 (adjusted section thickness = 1.64 microns) to account for the refractive index mismatch between air and methyl salicylate [Bucher et al., 2000]. Autofluorescence brains were optically sectioned in the horizonal plane at 3 micron intervals (adjusted section thickness = 4.92 microns).

*Statistical Analyses*

For non-normally distributed variables, model selection proceeded via comparison of AIC values and residual plots of selected models were inspected to confirm random dispersion over values of the independent variable (see figure legends for models used in each analysis). In general, count data were analyzed using a glm with a Poisson distribution, non-normal continuous data were analyzed using a glm with a gamma distribution and log link function, and percent data were analyzed using beta regression [Cribari-Neto, and Zeileis, 2015]. Tukey-adjusted least square means comparisons were used for post-hoc comparisons.

Supplemental references

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Supplemental figures

Fig. S1. Measures of body size in a sample of *D. bicolor*. Differences in total body mass driven by gaster mass *(a)*. Head width correlates better with the combined head and thorax mass, than with total body mass *(b)*.

Fig. S2. Exploration behavior of three dolichoderine species that vary in colony size. *(a)* Number of seconds it took the first ant to exit center dish for all species but excluding single ant trials because of a lack of *F. mccooki* exits *(a)* and for trials of all group sizes for only *D. bicolor* and *D. insanus* *(b)*. Inset in *(b)* depicts significant species by group size interaction. Generalized linear model (GLM) with Poisson distribution and a log link function used for comparisons. Percent area explored *(c)* and body-size adjusted percent area explored *(d)* for each species. *(e)* Beta regression with log link function for raw percent area explored (not adjusted for body size) and *(f)* exploration rate for trials when a single ant exited the center dish. Significant species contrasts indicted by asterisks (\*\* for p < 0.01, \*\*\* for p < 0.001). Letters denote significant contrasts in Tukey-adjusted least square means comparisons (α = 0.05).

Fig. S3. Brain scaling in desert species. ANOVA of brain neuropil volumes scaled to body mass. Tukey-adjusted least square means comparisons followed by false discover rate correction denoted with letters (α = 0.05).