Appendix S1 Methods and Materials. Section 1: Fungal inoculation experiments. Section 2: RNA extraction protocol.

1. Fungal infection experiments

Colonies used for fungal infection experiments were similar in size, with ca. 2 liter of fungus garden and 100-200 major garden workers being easily available at each of our consecutive bouts of sampling. The colonies were kept under standardized conditions in a climate room at 25˚C and 70% RH in a 12 h light:dark cycle, with a diet of fresh bramble leaves (*Rubus fruticosus*), apple and dry rice. To avoid age- or caste-specific variation, we used only major garden workers of approximately the same intermediate age class, *i.e.* we avoided both light-colored callows and very dark old foraging workers. This implied that the ants varied very little in the amount of cuticular actinomycete bacteria, i.e. score 0 – 3 on the scale of Poulsen *et al.* (2003), typical for large workers that are about to start their forager careers.

The entomopathogenic fungus *Metarhizium brunneum* (Bischoff *et al.* 2009; formerly *M. anisopliae*) was chosen as disease agent that has direct negative impact on *A. echinatior* workers (Baer *et al.* 2005; Hughes *et al.* 2002; Hughes & Boomsma 2004), whereas *Escovopsis weberi*, the specialized parasite that attacks the fungal cultivar of the ants, was chosen as disease agent that has direct negative impact on gardens but not on *A. echinatior* workers (Reynolds & Currie 2004; Yek *et al.* 2012). *M. brunneum* (KVL 04-57) was obtained from the stock collection of the Department of Agriculture and Ecology, University of Copenhagen, whereas the *E. weberi* strain was isolated from *Acromyrmex*

nests in Gamboa, Panama by Hermogenes Fernández-Marín in early 2010, and identified based on morphological characters (Muchovej & Della Lucia 1990).

For each colony, we established five sub-colonies in transparent plastic boxes (7.4 X 7.4 X 3.1 cm^3) that each contained 10 major garden workers and 1 g of fungus garden. We used a fine paint brush to transfer fungal conidia (asexual spores) either to the ants or onto the fungus garden. For each infection, we used conidia taken from a 1 cm^2 piece of pure fungal culture, grown on potato dextrose agar (PDA) medium, which corresponds to 2.1 X 10^8 conidia/ml for *M. brunneum* and 9.2 X 10^7 conidia/ml for *E. weberi*. The viability of conidia was checked by measuring their germination on plates with the same medium, which produced germination rates of >90% for *M. brunneum* and *E. weberi*.

After fungal infections, we recorded the grooming behaviors of infected ants for two consecutive days. They were characterized by a sequence of behaviors starting with scraping antennae and legs with the forelegs and licking the forelegs with the mouth parts (Bot *et al.* 2001), upon which the ants started to remove fungal conidia from either their own body (self-grooming) or the body of a nestmate (allo-grooming). In contrast, "garden-grooming" started with antennating a garden fragment, followed by extending the maxillae and labium to grasp the piece, and lifting it from the fungus garden matrix to pull it through the mouth parts (Bot *et al.* 2001). Ants were either active or stayed motionless for long periods of time hidden under garden fragments or just sitting on the fungus garden, which we categorized as "immobility inside the fungus garden".

2. RNA extraction protocol (modified from RNeasy Plant Mini Kit, Qiagen)

Each sample (10 major *A. echinatior* workers, approximately 100 µg) was transferred to a 2 ml extraction tube that contained 750 µl RLT buffer, 7.5 µl β-mercaptoethanol and 1 ceramic bead. Tubes with ants were disrupted in a Fastprep instrument at level 6 for 30 seconds, followed by centrifugation at 13.500g for 1 minute. The homogenized samples were then transferred to Qiashredder tubes, followed by centrifugation at 13.500 for 2 minutes. The liquid without any precipitate was transferred to a phase lock tube that had been centrifuged at 13.500g for 30 seconds. 700 µl phenol/$CHCl_3$/iso-amy-alcohol (25:24:1) pH 8 was added to the phase lock tube (5 Prime), and the resulting liquid was gently mixed by turning the tubes upside down for 1 minute. The mixtures in the phase lock tube were incubated at room temperature for 3 minutes, after which the tubes were centrifuged at 20.000g for 30 minutes in a cooling centrifuge at 20˚C. The upper phase was poured into a clean tube and half the volume 96% ethanol was added, thoroughly mixed and immediately transferred to a Qiagen column. The column was centrifuged at 8000g for 30 seconds and the flow through discarded. 450 µl RW1 buffer was added to the column, centrifuged at 8000g for 30 seconds and flow through discarded. Then, 80 µl DNAseI (10 µl DNAse diluted in 70 µl buffer) was added to the column and incubated at room temperature for 15 minutes, after which 450 µl RW1 buffer was added followed by centrifugation at 8000g for 30 seconds and removal of the flow through. 500 µl RPE buffer was then added, centrifuged at 8000g for 30 seconds and flow through discarded. Finally, 500 µl RPE buffer was added, centrifuged at 8000g for 2 minutes and the collecting tube with flow through discarded. The Qiagen column was then refitted with a new collection tube and centrifuged at 20.000g for 1 minute, after which the new

collection tube was discarded. The Qiagen column was then refitted with a clean Eppendorf tube, 50 µl RNase free H_2O was added onto the column and left to incubate for 1 minute, after which the column was centrifuged at 8000g for 1 minute. The flow through was then transferred back into the column and incubated for 1 minute. Finally, the Qiagen column was centrifuged again at 8000g for 1 minute. The integrity of the RNA sample (collected now in the Eppendorf tube) was confirmed by agarose gel electrophoresis and total RNA quantity and purity were determined spectrophotometrically.

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