

Supporting Information

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SI Methods

Experimental Procedures.

Ant hosts. We used the invasive garden ant *L. neglectus* (1, 2), sampled from Jena, Germany (N 50° 55.910 E 11° 35.140), reared in the laboratory as in ref. 3. Ants were kept at a constant temperature of 23 °C with 75% humidity and a day/night cycle of 14 h light/10 h dark. Experiments were performed in Petri dishes with a plastered base and 10% sucrose solution as a food supply for ad libitum consumption. Ants were randomly assigned to the respective treatment groups, described below. *L. neglectus* is an unprotected insect species, and all experiments comply with European laws and IST Austria ethical guidelines.

Fungal pathogens. We used the entomopathogenic fungi *Metarhizium* and *Beauveria*, both of which are natural pathogens of *Lasius* ants (4–7) and occur in high density (up to 5,000 conidiospores per g of soil; ref. 8) and diversity (many sympatrically occurring species and strains; refs. 9 and 10) in the soil, where the ants nest. Natural infection loads of *L. neglectus* populations with individual species of these obligate killing pathogens reach up to 9% prevalence (4), with sporulating cadavers each producing ~12 million new infectious conidiospores (conidia) (11). In *Metarhizium*, topical application of ~30 conidiospores induces 2% mortality in *L. neglectus* workers (3), while application of 300,000 spore constitutes the LD₅₀ (3). We used the strains *M. robertsii* KVL 13-12 and *B. bassiana* KVL 04-004 (obtained from the University of Copenhagen), of which multiple aliquots were kept in long-term storage at –80 °C. Before each experiment conidiospores of both fungi were grown on malt extract agar or sabaroud dextrose agar at 23 °C for 3 wk and harvested by suspending them in 0.05% sterile Triton X-100 (Sigma). Conidiospore suspensions had a mean germination rate of >89%, which was determined directly before each experiment.

Fungal exposure of ants. We exposed individual worker ants by applying a 0.3- μ L droplet of a 1×10^9 conidiospores per mL suspension onto their gaster (abdomen), unless otherwise stated. For the sham control treatment we applied a 0.3- μ L droplet of 0.05% sterile Triton X solution only.

Establishment of low-level infections. To induce socially acquired low-level infections we grouped five naive ants with a single pathogen-exposed individual [distinguishable by paint marking the exposed individual (Edding 780)] in a Petri dish ($\varnothing = 9$ cm, as in ref. 3). The exposed individual was either treated with *Metarhizium* or *Beauveria*. To obtain noninfected control ants five naive ants were grouped with a sham-treated individual. After 5 d of social contact the treated individual was removed and the remaining ants—low-level-infected with either *Metarhizium* or *Beauveria* or noninfected controls—were subjected to further experiments (discussed below). To confirm that this procedure resulted in low-level infections (as it did in ref. 3, where *Metarhizium brunneum* was used) we determined the internal infection load of ants after 5 d of social contact with the treated individual (four replicates of a sham-treated individual and eight replicates each of *Metarhizium*- or *Beauveria*-treated individuals; all five ants housed with the treated ant per replicate analyzed for their infection load; total $n = 100$). To that end, we depleted the poison gland of the ants by harnessing them in a sponge and gently pressing pH-sensitive paper against the tip of their gaster to drain their poison, since it could inhibit pathogen growth if not removed (12). After being cold-immobilized the ants were then placed into tubes (1.5 mL) containing 1 mL 0.05% Triton X, before shaking for 10 min on a vortex mixer at maximum speed (Vortex-Genie 2; Scientific Industries). After washing, the ants

were rinsed with 1 mL 0.05% Triton X and transferred into new tubes (1.5 mL) containing 100 μ L 0.05% TritonX. This washing step removes any conidiospores present on the ants' cuticle, ensuring only internal infections are detected. We then homogenized the ants' bodies in a TissueLyser II (Qiagen) using 2.8-mm ceramic beads (VWR; 2×1 min at 30 Hz) and plated the homogenates after a spin down on selective medium agar plates (containing chloramphenicol 100 mg/L, streptomycin 50 mg/L, and dodin 110 mg/L to inhibit bacterial growth). After cultivation at 23 °C for 2 wk the number of CFUs that developed from viable conidiospores of *Metarhizium* and *Beauveria* present inside the ant's body at time of collection was determined through visual inspection, as in ref. 3. None of the negative controls (20 ants from four independent replicates) showed any growth of entomopathogenic fungi.

Survival of low-level-infected ants upon homologous and heterologous pathogen challenge. Ants infected with *Metarhizium* or *Beauveria*, as well as noninfected controls, were exposed to either *Metarhizium* or *Beauveria* (using 5×10^9 conidiospores per mL for *Beauveria* to account for its lower virulence; Fig. S2) and subsequently kept as single ants in new Petri dishes ($\varnothing = 3.5$ cm). Survival of the ants was monitored daily for a period of 12 d. To confirm deaths caused by internal infection of the entomopathogenic fungi ant corpses were surface-sterilized (using bleach and ethanol; ref. 13), kept under humid conditions for 3 wk, and regularly checked for fungal outgrowth and conidiospore formation. Our experiment was performed in four blocks of “low-level infection” and “pathogen challenge” (two homologous and two heterologous combinations), each with its own control group (“noninfected ants” and “pathogen challenge”). Each combination comprised 24 replicates of five ants per rearing group, which were either infected ants or noninfected controls, resulting in 120 ants per combination and 960 ants overall in the experiment; 4.3% of the ants (41/960) died before direct pathogen challenge and were therefore excluded from further statistical analyses. The ants' infection state (i.e., noninfected versus low-level-infected) did not affect mortality before pathogen challenge (χ^2 test: $\chi^2 = 0$, $df = 1$, $P = 1$); 83.1% of dead ants (295/355) showed fungal outgrowth after surface sterilization, thus confirming an internal entomopathogenic infection.

Simultaneous coexposure with *Metarhizium* and *Beauveria*. We studied the effects of pathogen coinfection by exposing naive ants ($n = 120$ per treatment, total of 360 ants) to a mixture of both fungal pathogens (each 50%) or each pathogen alone (as above). Exposed ants were reared singly (Petri dishes: $\varnothing = 3.5$ cm) and survival was monitored daily for 12 d. Dead ants were surface-sterilized (as above); 89.6% (155/173) showed fungal outgrowth (89.1% for *Metarhizium*, 91.3% for *Beauveria*, and 89.5% for coexposure).

Behavioral observations of low-level-infected ants. We observed the behavior of low-level-infected ants toward a newly encountered contaminated nestmate. To this end, we transferred groups of five noninfected or *Metarhizium*- or *Beauveria*-infected ants into Petri dishes ($\varnothing = 3.5$ cm; no food). A color-marked nestmate from their parental colony, with whom the ants had no contact in the previous 5 d, was then introduced after exposure to either *Metarhizium* or *Beauveria*. This led to six combinations of infection state (noninfected or homologous or heterologous prior low-level infection) and nestmate contamination (*Metarhizium* or *Beauveria*), each consisting of 24 replicates ($n = 5$ infected respective to noninfected control ants per Petri dish, i.e., 120 per combination, 720 in total).

After an acclimatization period of 10 min following nestmate introduction we recorded the ants' behavior toward the treated nestmate for 1 h (camera: Di-Li 970-O; software: Debut video capture 1.64; always simultaneously filming one replicate each of the noninfected or *Metarhizium*- or *Beauveria*-infected treatment group with random assignment to the three cameras running in parallel). Videos were analyzed "blind" with regard to treatment, using the software Biologic (<https://sourceforge.net/projects/biologic/>) to record the start and end time point of the ants' behavior. We then calculated (i) the total duration (in seconds) of aggression (comprising aggressive picking/licking/plucking of the body surface, mandible biting into different body parts, and dragging), (ii) the total duration of allogrooming (in seconds), as well as (iii) the number of events of direct poison spraying from the acidopore (the opening of the poison gland at the gaster tip) that the contaminated nestmates received in total from the five nestmates per replicate. All ants in the group typically engaged in allogrooming, often simultaneously, while aggression and poison spraying were rare behaviors, typically being performed by a single ant at a time (see Movie S1 and its still image for examples). We counted the number of ants engaging simultaneously in allogrooming, as well as number of ants engaging in aggression and poison spraying over the course of the observation toward their contaminated nestmate.

To further disentangle which behavioral changes are induced by low-level infections per se and which are only expressed in the presence of a contaminated nestmate, we also observed the behavior of the ants (noninfected or carrying a low-level infection of *Metarhizium* or *Beauveria*) toward a noncontaminated sham-control nestmate (treated with 0.05% Triton X only; 24 replicates of each infection containing five ants each, 360 ants in total).

Conidiospore transfer from the contaminated nestmate to low-level-infected ants. To study if the ants' infection state affected their likelihood of acquiring the homologous versus heterologous pathogen when interacting with a contaminated nestmate, we determined the number of CFUs developing from viable conidiospores on the body surface of noninfected and *Metarhizium*- or *Beauveria*-infected ants after 70 min of social contact with their *Metarhizium*- or *Beauveria*-contaminated nestmate, again in all six possible combinations (same experimental setup as above). Ants (total $n = 820$ individuals, pooled in 164 replicates of five ants each) were transferred into tubes (1.5 mL) containing 100 μL 0.05% Triton X, to wash off and collect conidiospores that these ants may have acquired from their contaminated nestmate. These cuticle washes were treated as the ant homogenates above, to obtain agar plates with CFUs.

We confirmed that this procedure is appropriate to quantify only the conidiospore transfer during contact with the contaminated nestmates but not any conidiospores remaining from the previous low-level infection induction 5 d prior. To this end, we let noninfected and *Metarhizium*- or *Beauveria*-infected ants interact with nestmates that had only received a sham treatment and thus could not transfer pathogen (10 replicates of five ants each; total of 150 ants). None of these washes led to growth of entomopathogenic fungi after 2 wk of cultivation, revealing that the low-level-infected ants did not carry any viable conidiospores on their cuticle from their previous exposure that could still be washed off at this time point. Consequently, all viable conidiospores from our washes can only have been transferred from the contaminated individual within the 70 min of social contact in our experiment.

Relationship between conidiospore transfer and internal fungal load of ants. We determined whether the observed differences in conidiospore transfer between ants displaying the behavioral change, compared with ants that do not, translates into different probabilities and levels of superinfection which establish in the ants after exposure. To this end, we directly exposed *Metarhizium*- or

Beauveria-infected ants to the heterologous pathogen, in an amount they would acquire when expressing the behavioral change (i.e., infected ants interacting with a nestmate contaminated with the heterologous pathogen), and compared their superinfection load with that of *Metarhizium*- or *Beauveria*-infected ants, where we simulated an absence of the behavioral change by exposing them to the heterologous pathogen in an amount of noninfected control ants, which do not express the behavioral change (Fig. 3A).

As the accuracy to determine conidiospore transfer by washing (discussed above) diminishes over time due to increasing conidiospore attachment, yet transfer between ants is possible for ~ 48 h after exposure in this experimental system (14), we had to determine the required application doses by extrapolation from our early observation period (first 70 min of interaction) to the full period of possible pathogen transfer. Within the 48 h of possible transmission the likelihood of conidiospore transfer continuously decreases with time, first because a constantly reducing number of conidiospores can be transferred per interaction due to reduction in number via grooming, or stronger attachment to the cuticle due to germination, and, second because the ants perform fewer grooming interactions per unit of time. In our system, grooming decreases by 40% between 24 and 48 h after exposure (3). We therefore estimated how many conidiospores are transferred within the full infective period of 48 h using an exponential function, taking the number of conidiospores transferred within the first hour (Fig. 3A) as the starting point and a decay rate τ of 10, based on our observations in ref. 3. As experimental topical application further is a "wasteful" procedure and only 10–15% of the applied conidiospores are in fact sticking to the body surface (7), we used the following application doses in our experiment: *Beauveria*-infected: behavioral change present = 460 *Metarhizium* conidiospores, behavioral change absent = 1,850; *Metarhizium*-infected: behavioral change present = 90 *Beauveria* conidiospores, behavioral change absent = 1,480.

After having been kept with a contaminated individual for 5 d to acquire a low-level infection (as above) ants were directly exposed to the heterologous fungus and kept in isolation (Petri dishes: $\varnothing = 3.5$ cm) for another 5 d. After this time ants were freeze-killed and later combined into pools of five individuals each (number of replicates, *Beauveria*-infected at risk for superinfection of *Metarhizium*: behavioral change present = 14, behavioral change absent = 13; *Metarhizium*-infected at risk for superinfection of *Beauveria*: behavioral change present = 12, behavioral change absent = 13; total of 260 ants). To only measure internal infections ant pools were washed to remove any conidiospores still loosely attached to the outer surface of their cuticles. This was done by vortexing them in 500 μL 0.05% Triton X solution for 1 min and subsequently rinsing all ants individually with 100 μL 0.05% Triton X solution.

Quantification of superinfection load in low-level-infected ants. The fungal load of the heterologous pathogen was determined using quantitative real-time PCR. Before DNA extraction, the samples were homogenized in a TissueLyser II (Qiagen) using a mixture of 2.8-mm ceramic (VWR), 1-mm zirconia (BioSpec Products), and 425- to 600- μm glass beads (Sigma-Aldrich). Homogenization was carried out in two steps (2×2 min at 30 Hz). DNA extractions were performed using Qiagen DNeasy96 Blood and Tissue Kit per the manufacturer's instructions, with a final elution volume of 50 μL Buffer AE.

We then performed a real-time PCR assay to quantify the fungal ITS2 rRNA gene copies. Targeting this multicopy gene ensures a high sensitivity and prevents any cross-amplification of the two fungi present in the ants. Quantification standards were obtained by extracting DNA of pure *Metarhizium* and *Beauveria* conidiospores. Site-specific primers for *B. bassiana* were taken from ref. 15 (forward: 5'-GCCGGCCCTGAAATG G; reverse: 5' - GATTCGAGGT-CAACGTTTCAGAA). Primers for the amplification of *M. robertsii* were designed based on GenBank sequence AY755505.1 (forward:

5'- CCCTGTGGACTTGGTGTG; reverse: 5'- GCTCCTGTT-GCGAGTGT), as in ref. 16. Both primer pairs were shown not to cross-amplify the other fungal species.

Amplification was carried out in 20- μ L reactions using 1 \times KAPA SYBR Fast qPCR master mix (KapaBiosystems), 3 pmol (*Metarhizium*) or 4 pmol (*Beauveria*) of each specific primer (Sigma-Aldrich), and 2- μ L template on a CFX96 real-time PCR instrument (Bio-Rad). Cycling parameters were chosen according to manufacturer's recommendations (annealing temperatures: 64 °C for *Metarhizium* and 60 °C for *Beauveria*). Quantification was based on a standard curve, with standards covering a range from 10⁻² to 10⁻⁵ ng/ μ L fungal DNA for *Metarhizium* and 10⁻² to 10⁻⁴ ng/ μ L fungal DNA for *Beauveria*. The respective lowest standard was determined to be the detection threshold. Each run included a negative control. Specificity was confirmed by performing a melting curve analysis after each run. From our 52 original samples (pools of five ants each) we excluded one outlier that showed exceptionally high fungal multiplication, having a 250-fold higher value than the maximum value of all other samples.

Statistical Analyses. All statistical analyses were carried out in the program R, version 3.4.2 (17), and all reported *P* values are two-sided. We performed a *t* test to compare the low-level infection loads between *Metarhizium*- and *Beauveria*-infected ants (Fig. S1). For all experiments in Figs. 1, 2, and 3A we performed an overall model consisting of pathogen challenge either via direct application or nestmate contamination (containing two levels: *Metarhizium* and *Beauveria*) and infection state (containing three levels: noninfected or homologous or heterologous low-level infection), and an interaction between them. In cases where the interaction was nonsignificant we refitted the model without interaction and tested the significance of the main factors. In cases where the interaction was significant we directly performed post hoc comparisons. To assess the significance of main effects of all models we compared full models to null (intercept only) and reduced models (for those with multiple predictors), using LR. We checked the appropriate diagnostics for all models, including overdispersion, Cook's distance, dfbetas, dffits, leverage, variance inflation factors [package car, version 2.0–19 (18)], distribution of residuals, residuals plotted against fitted values, and Levene's test of equality of error variances, to test for obvious influential cases, outliers, and deviations from the assumptions of normality and homogeneity of residuals. Where post hocs were necessary we performed Tukey post hoc comparisons and adjusted the resulting *P* values using the Benjamini–Hochberg procedure, using the multcomp package [version 1.4-6 (19)].

Survival of low-level-infected ants after challenge. We used COXME [package coxme, version 2.2–5 (20)] with “survival” as the response variable, to test for pathogen challenge (two levels: *Metarhizium* and *Beauveria*) and infection state (three levels: noninfected or homologous or heterologous prior low-level infection) and their interaction as fixed effects (Fig. 1). The model also included two random intercept effects: (i) the four “blocks,” in which the experiment was run, and (ii) “rearing group,” since ants from the same Petri dish are nonindependent. As the interaction was nonsignificant (COXME: overall LR $\chi^2 = 125.01$, df = 6, *P* < 0.0001; interaction: $\chi^2 = 2.65$, df = 2, *P* = 0.27), we refitted the model without the interaction to obtain better estimates of the remaining predictors. As the two pathogens elicited different levels of response we analyzed the effect of infection state separately for *Metarhizium* and *Beauveria* and adjusted the *P* values using the Benjamini–Hochberg procedure to protect against a false discovery rate of 0.05. Similarly, all pairwise post hoc comparisons per pathogen were controlled for multiple testing by adjusting *P* values using the Benjamini–Hochberg procedure.

Survival after coexposure. When testing for the effect of pathogen coinfection after direct coexposure (Fig. S2), survival of naive ants

after exposure with either *Metarhizium* or *Beauveria*, or a simultaneous coexposure with both fungal pathogens, we used a Cox proportional hazards regression [package survival, version 2.38 (21)]. We performed all pairwise post hoc comparisons to test for differences between the three treatments, again using the Benjamini–Hochberg procedure to adjust for multiple testing. **Behavior toward nestmates.** GLMs were used to analyze whether the behavior of ants toward their contaminated nestmates depended on the fixed effects of infection state (three levels: noninfected or homologous or heterologous prior low-level infection) and pathogen challenge via contact with the contaminated nestmate (two levels: *Metarhizium* and *Beauveria*), as well as their interaction (Fig. 2). As we here only obtained a single value per rearing group (i.e., the behavior received by the single treated individual) no random effect had to be included in these models. As the interaction was not significant for any of the behaviors (aggression: GLMs; overall LR $\chi^2 = 16.45$, df = 5, *P* = 0.005; interaction: $\chi^2 = 3.05$, df = 2, *P* = 0.22; allogrooming: overall *F* = 4.86, df = 5, *P* = 0.0004; interaction: *F* = 1.87, df = 2, *P* = 0.16; poison spraying: overall LR $\chi^2 = 23.83$, df = 5, *P* = 0.0002; interaction: $\chi^2 = 0.98$, df = 2, *P* = 0.612) we refitted the models without the interaction to obtain better estimates of the remaining predictors. For the grooming data, we used GLMs with Gaussian errors. Since the aggression and poison spraying behavioral data followed a negative binomial distribution we analyzed this data using GLMs with negative binomial errors [package MASS, version 7.3-47 (22)]. We performed all pairwise post hoc comparisons for infection state and controlled for multiple testing by adjusting *P* values using the Benjamini–Hochberg procedure to protect against a false discovery rate of 0.05.

For each replicate we also defined the maximum number of ants performing allogrooming at a time and aggression or poison spraying over time (Fig. S3). We tested whether the number of ants engaging in these behaviors when they were performed (that is, excluding the replicates where the behavior was never observed, which was 65/144 for aggression, none for allogrooming, and 119/144 for poison spraying) differed between the treatment groups, using logistic regressions (GLMs with binomial errors) that analyzed the proportion of ants engaging in a behavior, out of the total number of nestmates present (always five), using the cbind function. Because poison spraying by noninfected ants was so rare (occurring once in the *Metarhizium* group and being completely absent in the *Beauveria* treatment) we excluded these two control groups from the poison spraying analysis, hence comparing only the homologous and heterologous combinations of both pathogens.

Finally, to analyze the ants' behavior toward noncontaminated nestmates (Fig. S4) we used a Kruskal–Wallis test with pairwise Mann–Whitney *U* tests (Benjamini–Hochberg-adjusted) for aggression, since these data could not be transformed to normality. Allogrooming was normally distributed and hence analyzed using an ANOVA and Tukey post hoc tests. However, because poison spraying occurred only twice statistical analysis was not feasible.

Pathogen transfer. The transfer of infectious conidiospores from contaminated nestmates to low-level-infected individuals (Fig. 3A) was analyzed using a GLM with Gaussian errors after data were ln(*x* + 1)-transformed to fulfill the assumption of normality. Again, we tested for pathogen challenge via the contaminated nestmate (two levels: *Metarhizium* and *Beauveria*) and infection state (three levels: noninfected or homologous or heterologous prior low-level infection) and their interaction as fixed effects, without the need to include a random effect, as the five nestmates per rearing group were pooled to increase fungal detection sensitivity. The interaction was nonsignificant (GLM; overall *F* = 5.3, df = 5, *P* = 0.0001; interaction: *F* = 0.13, df = 2, *P* = 0.88); hence, we refitted the model without the interaction to obtain better estimates of the remaining predictors. As the two pathogens elicited different levels of response, we analyzed the

effect of infection state separately for *Metarhizium* and *Beauveria* and adjusted the *P* values using the Benjamini–Hochberg procedure to protect against a false discovery rate of 0.05. Similarly, all pairwise post hoc comparisons per pathogen were controlled for multiple testing by adjusting *P* values using the Benjamini–Hochberg procedure.

Superinfection load. The superinfection load of low-level-infected ants (Fig. 3B) was square root-transformed to achieve normality and analyzed separately for the two pathogen challenges (*Metarhizium* and *Beauveria*), as the ITS2 gene copy numbers cannot be assumed to be equal for the two pathogen species. We performed

LMER, with “presence/absence of behavioral change” (two levels: risk-adjusted and control) as a fixed effect. Since the DNA extraction was carried out over two separate runs, “run” was included as a random intercept effect into all models. Three different experimenters performed the pathogen exposure, so a random intercept was also included for “person.” There was no need to include a rearing group as additional random effect, as the five nestmates per rearing group were pooled to increase fungal detection sensitivity. As we ran two separate analyses, we adjusted the *P* values using the Benjamini–Hochberg procedure to protect against a false discovery rate of 0.05.

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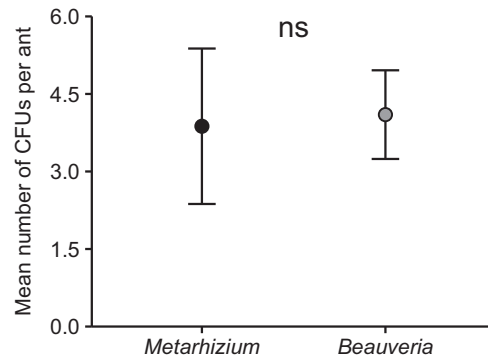


Fig. S1. Low-level infections induced in ants by social contact with a pathogen-exposed individual. Number of CFUs of *Metarhizium* and *Beauveria*, retrieved from conidiospores within the bodies of *Lasius neglectus* ants, after 5 d of social contact with a either a *Metarhizium*- or *Beauveria*-exposed individual. The two pathogens established low-level infections in the ants at equal intensity (t test, $t = 0.130$, $df = 14$, $P = 0.898$; mean variation between ants within rearing group, SEM, *Metarhizium* ± 3.1 , *Beauveria* ± 2.4 CFUs). Sample size $n = 80$ ants in 16 replicates each of five ants. For supporting data see Dataset S1. ns, not significant.

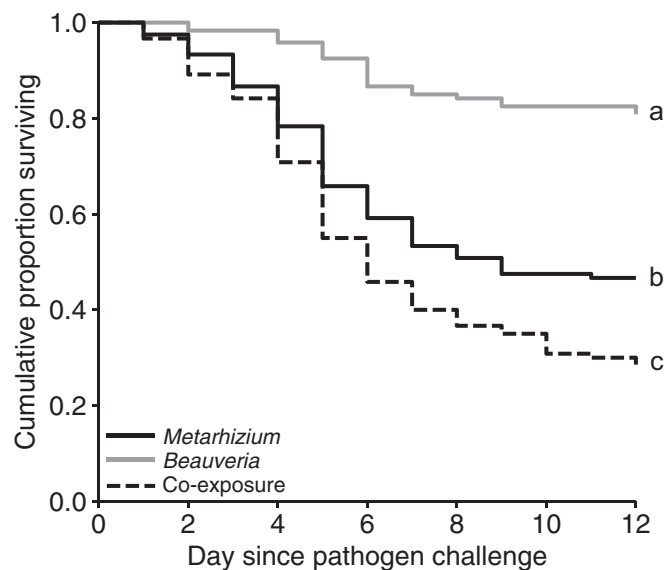


Fig. S2. Ant survival after simultaneous pathogen coexposure. *L. neglectus* workers showed significantly increased mortality after coexposure to a 50–50% mix of *Metarhizium* and *Beauveria* (dashed black line) compared with exposure to either 100% *Metarhizium* (black) or 100% *Beauveria* (gray) alone (Cox proportional hazards regression: overall LR $\chi^2 = 73.82$, $df = 2$, $P < 0.001$; post hoc comparisons: coexposure vs. *Metarhizium* only, $P = 0.006$, HR = 1.6; coexposure vs. *Beauveria* only, $P < 0.001$, HR = 5.8; *Metarhizium* vs. *Beauveria*, $P < 0.001$, HR = 3.7). Different letters indicate statistically significant differences at $\alpha = 0.05$. Sample size $n = 360$ ants. For supporting data see Dataset S2.

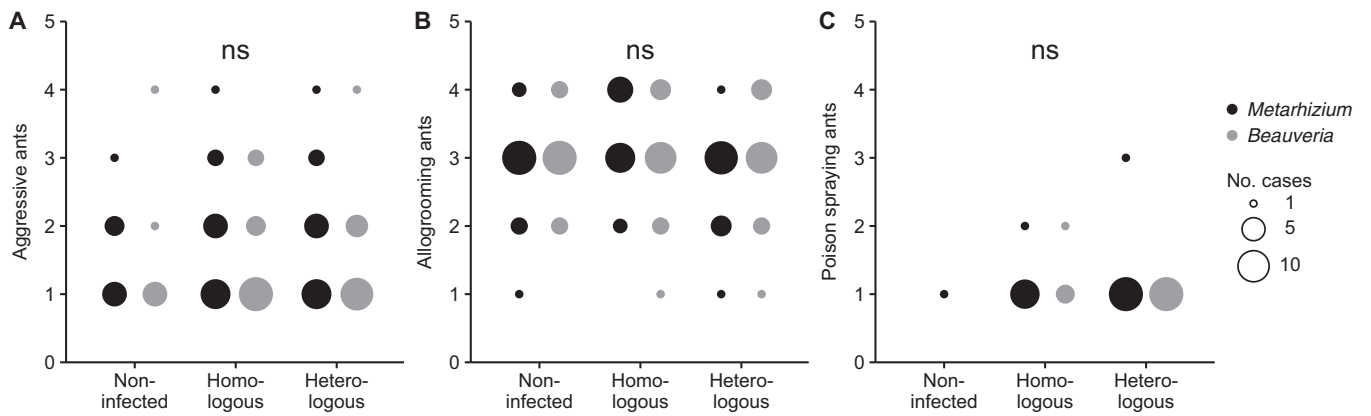


Fig. S3. Number of ants engaging in behaviors toward contaminated nestmates. We observed how many of the five ants per group engaged in (A) aggression, (B) allogrooming, and (C) poison spraying of the contaminated nestmate (Movie S1). (A) Aggression was a rare behavior typically only performed by a single ant at a time, but by up to four ants over the course of the observation, with no effect of the infection state (noninfected ants or ants carrying a homologous or heterologous low-level infection) or whether the nestmate was contaminated with *Metarhizium* (black) or *Beauveria* (gray) (GLM, overall: LR $\chi^2 = 1.32$, df = 5, $P = 0.93$). (B) Allogrooming was typically performed by all five ants of the group, often simultaneously by up to four ants, and there was no effect of infection state or nestmate contamination on the number of ants performing allogrooming at a time (GLM, overall: LR $\chi^2 = 3.26$, df = 5, $P = 0.66$). (C) Poison spraying was rarely performed, usually by one ant at a time but by up to three ants over the course of the experiment. As poison spraying was performed only once by noninfected ants statistical analysis was limited to the low-level-infected ants. Again, there was no significant effect of infection state or nestmate contamination (GLM; overall: LR $\chi^2 = 0.33$, df = 3, $P = 0.96$). Bubble size indicates number of replicates with respective number of ants. Sample size $n = 720$ ants from 144 independent replicates. Allogrooming was performed in all replicates, while only 79 and 25 replicates were included for aggression and poison spraying, respectively, as these rare behaviors were not performed in the remaining replicates. For supporting data see Dataset S3. ns, not significant.

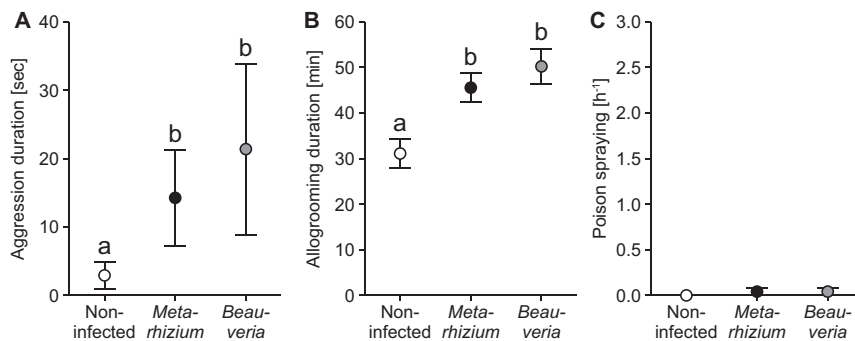


Fig. S4. Behavior toward noncontaminated nestmates. Noninfected control ants (white) or ants carrying a low-level infection with either *Metarhizium* (black) and *Beauveria* (gray) were observed for the levels of (A) aggression, (B) allogrooming, and (C) poison spraying they directed toward noncontaminated, sham-treated nestmates. (A) Aggression was essentially absent in noninfected ants but was present in ants with low-level infections of *Metarhizium* or *Beauveria*, which did not differ (Kruskal–Wallis test, $H = 6.24$, df = 2, $P = 0.044$; post hoc comparisons: noninfected vs. *Metarhizium*, $P = 0.047$, noninfected vs. *Beauveria*, $P = 0.047$, *Metarhizium* vs. *Beauveria*, $P = 0.834$). (B) Allogrooming duration was shortest when performed by noninfected ants and increased significantly when ants were infected, with no difference between the two pathogens (ANOVA, $F = 8.32$, df = 2.69, $P = 0.00059$; post hoc comparisons: noninfected vs. *Metarhizium*, $P = 0.006$, noninfected vs. *Beauveria*, $P = 0.0006$, *Metarhizium* vs. *Beauveria*, $P = 0.34$). (C) Poison spraying was performed only once each by two ants, rendering statistical analysis nonfeasible. Mean \pm SEM displayed. Different letters indicate statistically significant differences at $\alpha = 0.05$. Sample size $n = 360$ ants. For supporting data see Dataset S4.



Movie S1. Behavioral repertoire of low-level-infected ants displayed toward a contaminated nestmate. *L. neglectus* workers that had previously acquired low-level infections by social contact with an exposed individual were observed for their behavior toward a newly encountered, contaminated nestmate (red color marking), from which they could contract either the homologous or the detrimental, heterologous pathogen. Displayed behaviors: (i) four low-level-infected ants removing infectious particles from the body surface of their contaminated nestmate by grooming, (ii) a low-level-infected ant disinfecting a contaminated nestmate by antimicrobial poison spraying (two examples), and (iii) a low-level-infected ant performing aggressive behavior (dragging) against a contaminated nestmate (two examples). Still image from Movie S1: In the upper right a low-level-infected *L. neglectus* worker disinfects its pathogen-contaminated nestmate by antimicrobial poison spraying [i.e., by bending its rear end (gaster tip) with the poison gland exit toward the contaminated nestmate].

[Movie S1](#)

Dataset S1. Supporting data for low-level infection intensity

[Dataset S1](#)

Data supporting Fig. S1.

Dataset S2. Supporting data for survival analyses

[Dataset S2](#)

Data supporting Fig. 1 and Fig. S2.

Dataset S3. Supporting data for behavioral analyses toward contaminated nestmates

[Dataset S3](#)

Data supporting Fig. 2 and Fig. S3.

Dataset S4. Supporting data for behavioral analyses toward noncontaminated control nestmates

[Dataset S4](#)

Data supporting Fig. S4.

Dataset S5. Supporting data for pathogen transfer

[Dataset S5](#)

Data supporting Fig. 3.