Mate Preference and Disease Risk in *Zootermopsis angusticollis* (Isoptera: Termopsidae)

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ABSTRACT Termites face significant and chronic intranidal selection pressures from parasites and pathogens that colonize their nests. They also encounter microbes outside their nest while foraging and during dispersal of winged primary reproductives to establish new colonies. The latter run the additional risk of becoming infected by a mating partner. Indeed, death of reproductives because of disease is a major cause of incipient colony failure and may favor prescreening prospective mates for signs of illness. To determine the role of disease on mate preference in termites, female primary reproductives of the Pacific dampwood termite *Zootermopsis angusticollis* (Hagen) simultaneously were presented with reproductive males that were either healthy or exhibiting a progression of symptoms associated with infection by the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff Sorokin). We compared duration and frequency of female visits to healthy and infected males. In addition, we determined the physiological consequences for females exposed to fungal conidia, either directly or indirectly through their mate. Females showed no preference for healthy rather than infected males. Moreover, only directly-exposed females experienced negative physiological effects, having a reduced chance of survival, gaining less weight, developing fewer functional ovarioles, and producing significantly fewer vitellogenic oocytes than controls. Although there are important fitness-related costs of direct exposure, the lack of mate selection based on disease risk suggests that more imminent ecological pressures (e.g., predators, desiccation) override the need for a careful and time-consuming assessment of a potential mate's health.

KEY WORDS mate choice, *Metarhizium anisopliae*, reproductive maturation, fungal pathogens, socially transmitted diseases

Diseases transmitted by incidental contact during social interactions between prospective partners before copulation (e.g., courtship, grooming) likely impact the population dynamics and the evolution of a host's mating system to an extent similar to sexually transmitted diseases (STDs; Sheldon 1993, Able 1996, Thrall et al. 1997, Kokko et al. 2002, Knell and Webberley 2004, Lawniczak et al. 2007 and references therein). In insects, both social and sexual disease transmission can result in reduced fertility, reduced egg viability, and also sterility, increased mortality, or both (Thrall et al. 1997, Knell and Webberley 2004). In spite of such negative effects on the host's fitness, there is little empirical evidence that female insects avoid mating with infected males (Abbot and Dill 2001, Webberley et al. 2002, Knell and Webberley 2004, Luong and Kaya 2005). This seems particularly surprising given that the high risks of contracting an infection should influence mate choice to minimize interactions with diseased partners. However, theoretical models have indicated that optimal strategies are not always the ones that minimize risks of infection (Thrall et al. 1997). Instead, coevolutionary outcomes between host mating strategies and disease agents often are not intuitively obvious, and complex host and pathogen interactions with multiple optima can arise (Thrall et al. 1997). Proposed hypotheses explaining why STDs do not necessarily select for preferential mating also may apply to socially transmitted diseases. For example, putative selection pressures for STDs to become cryptic (Knell 1999) also could drive the evolution of masked socially-transmitted diseases. Such "undetectable" pathogens could spread through the population at faster rates. Another possible explanation includes the ability of the pathogenic or parasitic agent to manipulate the host's behavior so as to increase the likelihood of interactions between healthy and infected mates (Abbot and Dill 2001, Luong and Kaya 2005, Thomas et al. 2005).

The evolution of monogamy across different taxonomical groups has been attributed, in part, to the reproductive benefits attained from mate guarding and mate assistance (Thornhill and Alcock 1983, Nalepa and Jones 1991). In addition, high risks of horizontal disease transmission between mates also

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may have influenced the evolution of monogamy (Sheldon 1993, Loehle 1995, but see Thrall et al. 1997). A prime example of a monogamous mating system in insects is found in termites. Although termites exhibit variability in their breeding strategies, swarming, and postflight behavior (Nutting 1969, Grassé 1984), most species have a monogamous mating system that follows a predictable sequence of events after winged male and female sexuals (alates) disperse from their natal colony (Nutting 1969, Hartke and Baer 2011). After a swarming flight, alates land and walk about releasing sex pheromones to attract potential mates (reviewed by Bordereau and Pasteels 2011). Once a potential mate is located, a male follows a female at very close range (forming a tandem pair) until an appropriate nesting site is found. After dropping their wings, the pair sequesters itself in a "nuptial chamber" where they eventually will mate and produce offspring (Nutting 1969, Grassé 1984). Mate choice decisions therefore must occur before the pair sealing itself in the new nest, and ideally before the individuals shed their wings and lose the capacity to relocate readily. Although in the past, mate choice in termites was considered to be nonexistent (Grassé 1984, Nalepa and Jones 1991), more recent studies have shown that termite mate preferences may correlate with a variety of physical and genetic attributes (Shellman-Reeve 1999, 2001; Matsuura and Nishida 2001; Kitade et al. 2004; Husseneder and Simms 2008). It is unknown, however, whether termites can and will select mates on the basis of potential infection risks.

Termites harbor 1) high cuticular loads of potentially pathogenic bacteria and fungi (Hendee 1933, 1934; Sands 1969; Blackwell and Rossi 1986; Cruse 1998; Rosengaus et al. 2003 and references therein); 2) mites (Wang et al. 2002); and 3) entomopathogenic nematodes (Rouland et al. 1996, Benmoussa-Haichour et al. 1998). The close association of the newly paired reproductives in their nuptial chamber enhances the likelihood of disease transmission, which in turn reduces the probability of successful colony establishment (Rosengaus and Traniello 1993, Rosengaus et al. 2000a, Calleri et al. 2006). Exposure to the novel pathogens carried by a non-nestmate partner can further exacerbate the risks associated with pairing (Rosengaus and Traniello 1993). Hence, there may be substantial selective pressure to quickly evaluate partners for the risk of contagion by using olfactory cues, probably during the period of initial antennation and subsequent tandem running. To better understand the mate decision process at this critical juncture in the life history of a termite colony, we examined whether female reproductives are selective about the health of their prospective mates. Although in species with monogamous breeding systems, both males and females may assess partner quality before mating, in this study we specifically focused on female mate preference because the reproductive consequences of her decisions could be quantified. Specifically, we recorded reproductive development and output after queens were exposed to a fungal entomopathogen either 1) experimentally by directly placing conidia on their cuticle, or 2) socially through their interactions with an experimentally exposed mate. Our findings indicate that although pathogen exposure does exact physiological costs, *Z. angusticollis* females are not particularly choosy about pairing with infected males.

Materials and Methods

Collection and Maintenance of Termite Colonies. Eleven mature colonies of the Pacific dampwood termite *Z. angusticollis* were collected from the Pebble Beach Resort on the Monterey Peninsula, CA and from Huddart Park in San Mateo County, CA. Logs containing colonies were transferred to covered plastic tubs (50 by 30 by 20 cm) lined with moist paper towel. These stock colonies were sprayed periodically with water, given supplemental decayed wood, and maintained at 25°C and 61.5% RH. Fully-pigmented adult winged individuals (alates) were collected, separated by gender (Weesner 1969), and then dewinged by folding anteriorly over the wing suture. Because alates turn dark brown as their cuticle becomes sclerotized after their imaginal molt, choosing alates with similarly dark-brown pigmentation controlled for maturity-based differences in motivational state to both disperse and mate. Choosing alates with intact wings insured that virgin reproductives were used in our experiments (Rosengaus et al. 2000a, Calleri et al. 2006). To prevent mating before experimentation, the de-winged individuals were housed in same gender and same stock colony groups in covered containers lined with moist paper towel and a small piece of natal nest material. The separation of virgin alates from their natal nest does not appear to affect their mating behavior or their rates of successful establishment of incipient colonies (Rosengaus and Traniello 1993; Calleri et al. 2006, 2007).

Preparation of Fungal Conidia Suspensions. *Metarhizium anisopliae* (Metchnikoff Sorokin) is a widely distributed entomopathogenic fungus with loads ranging from 10^3 to 10^6 conidia/g of soil (Hughes et al. 2004, Roberts and St. Leger 2004) and is known to be naturally associated with termites (Sands 1969, Ko et al. 1982, Zoberi 1995, Milner et al. 1998, Calleri et al. 2006). Previous research has demonstrated that *M. anisopliae* influences 1) termite behavior (Rosengaus et al. 1998a, 1999, 2000), 2) the deposition of chemical secretions by these social insects (Rosengaus et al. 1998b, 2000, 2004; Bulmer et al. 2009; Hamilton et al. 2011a,b), and 3) the physiology of larvae and reproductive adults (i.e., immunological responses, Rosengaus et al. 1999, 2007; Calleri et al. 2006, 2007; Hamilton et al. 2011a,b). Hence, this pathogen represents a relevant ecological agent to test whether females preferentially associate with healthy rather than diseased potential mates. Conidia of *M. anisopliae* (originally obtained from American Type Culture Collection, batch 93–09, media 325, ATCC no. 90448) were harvested from cadavers of previously infected termites. Using procedures described in Rosengaus and Traniello (1997) and Rosengaus et al. (1998a), Tween 80 suspensions with concentrations of 6×10^7, 6×10^5,

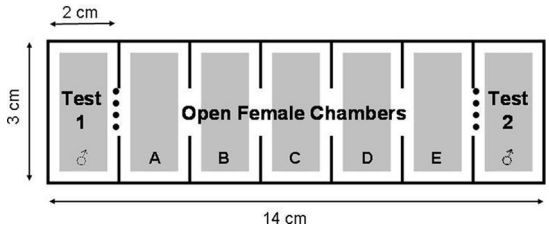

Fig. 1. Diagram of the mate choice arena consisting of a series of five linear chambers where females were allowed to wander freely through all but the terminal compartments. Each inner chamber (A-E) contained moistened filter paper (gray). Individual males were placed in the two terminal compartments that also were lined with treated filter paper (Test compartments 1 and 2 as described in the Methods section). The males were isolated from the inner chambers by wire mesh (●●●●). Females first were introduced into chamber C. See the Methods section for a detailed description.

and 6×10^4 conidia/ml, and control Tween 80 suspensions lacking conidia, were freshly prepared before termite exposures. Conidia viability, measured as the average percent conidia germination (± SD), was 95% ± 3 ($n = 30$ fields of vision).

Mate Preference Test Chambers. The mate preference arena was constructed using a plastic pillbox (overall 14 cm in length by 2 cm wide by 2 cm in height) with seven linear chambers (each 2 cm wide by 3 cm in depth) connected by 5-mm-diameter holes (Fig. 1). The two end compartments (tests 1 and 2, Fig. 1) were separated from the five inner chambers (A through E) by using wire mesh, sufficiently fine to prevent termites from crossing but allowing mutual antennation and the circulation of volatiles. The five inner chambers were lined with filter paper (Whatman No. 5) and moistened with 50 μl of distilled water. Each end compartment was lined with filter paper squares (Whatman No. 5) and moistened with 50 μl of either 0.1% Tween 80, the suspension medium, or with suspensions detailed in the 5 treatment conditions described below containing the above-mentioned concentrations of *M. anisopliae* conidia. A single de-winged female was placed in the central chamber (chamber C, Fig. 1) and allowed to wander freely through the five interconnected chambers. In each terminal test compartment, a single de-winged male was added. Both males originated from the same colony as the female to control for olfactory cues that could influence female choice. The males on either side of the arena were of similar fresh body mass (average ± SD = 0.0368 ± 0.006 g versus 0.0362 ± 0.007 g, $F = 0.9$, $P = 0.3$), controlling for the potential preference of females to choose heavier males. The five treatment conditions under which females were allowed to make a choice were:

1) Two healthy males, each exposed to control suspension ($n = 29$).

2) One healthy male exposed to control suspension (test compartment 1; Fig. 1), and one male exposed to 50 μl of a 6×10^7 conidia/ml suspension within the previous hour but not yet displaying signs of fungal disease (test compartment 2, Fig. 1; $n = 18$).

3) One healthy male exposed to control suspension (test compartment 1, Fig. 1) and one symptomatic infected male placed on control suspension (test compartment 2, Fig. 1). The infected males were chosen from individuals exhibiting lethargy, an obvious sign of advanced fungal disease (Rosengaus and Traniello 1997), 8 d after exposure to a 6×10^5 conidia/μl suspension (see Traniello et al. 2002 for details of the exposure protocol; $n = 20$).

4) One healthy male (test compartment 1, Fig. 1) and one dead, sporulating male (test compartment 2, Fig. 1) both exposed to control suspension. Sporulating cadavers were retrieved from confirmation plates of previously infected males (see Rosengaus and Traniello 1997 protocol for fostering *M. anisopliae* sporulation; $n = 21$).

5) No males, only filter paper moistened with either control suspension (test compartment 1, Fig. 1) or 6×10^7 conidia/ml (test compartment 2, Fig. 1; $n = 16$).

After placement in the test arena, the termites were allowed to acclimate for 1 hr before observations started. Each arena was videotaped for 30 min at 1-, 12,- and 36-h postintroduction and analyzed separately. Replicates of the same treatment were stacked together inside a covered plastic box lined with moist paper towel to maintain high humidity and reduce the possibility of desiccation. All videotaped sessions were conducted at 25 ± 1°C without any perceptible air currents. Lighting direction was standardized across chambers by using overhead artificial lights. The video recordings were used to determine the frequency and duration with which females visited each compartment. Replicates were included in the analyses only if all living termites remained alive through the 36-h taping period. No individual was used more than once.

Before their reuse, all test arenas first were submerged overnight in 5.2% sodium hypochlorite and then rinsed thoroughly with water. Subsequently, the arenas were rinsed with 70% ethanol, which was allowed to evaporate completely before the introduction of a new set of termites. In addition to sterilizing all compartments, this cleaning process eliminated any potential odor residues that might have influenced the results of subsequent tests.

Female Reproductive Development. We established additional control and experimental incipient colonies to determine how different modes of conidia exposure affect the reproductive physiology of *Z. angusticollis* females. None of these individuals were used in the mate choice experiments described in the Mate Preference Test Chambers section above. Male and female alates were collected from 11 mature stock colonies to establish 165 monogamous pairs. All alates were de-winged, weighed, and while being cold immobilized for 1 hr, they were exposed on their dorsum to either a 3 μl droplet of control Tween 80 suspension media lacking fungal conidia ($n = 96$ males and 96 females) or to a 3 μl droplet containing 6×10^4 ($n = 47$ males and females) or 6×10^5 ($n = 22$ males and females) conidia/ml suspension. The exposure meth-

ods have been described in detailed by Traniello et al. (2002). The conidia loads used in these experiments represent dosages known for causing intermediate mortality (Rosengaus et al. 1998a, 2000a; Calleri et al. 2006). These de-winged individuals were paired subsequently to form three test groups: nonexposed females with nonexposed males (control), nonexposed females with conidia-treated males (henceforth referred as socially exposed), and conidia-treated females with nonexposed males (henceforth referred as directly-exposed). Pairs were placed inside petri dishes lined with moist filter paper and decayed birch wood. Dishes were stacked inside a closed plastic box lined with moist paper towel and maintained undisturbed at 25°C for 20 d, at which time the surviving termites were weighed again and preserved in 70% alcohol. The decision for preserving females on alcohol 20 posttreatment was based on the fact that in unexposed *Z. angusticollis*, the average time from colony establishment to the onset of oviposition is 22.9 ± 5.5 d (Calleri et al. 2006). In the present experiments, none of the females in any of the three treatments oviposited before their preservation in alcohol. Moreover, because directly-exposed termites succumb to fungal infection within 8- to 10-d postexposure and sporulation of cadavers occurs approximately 4 to 5 d thereafter (Rosengaus and Traniello 1997), it is reasonable to assume that 20-d postpairing provided the reproductive individuals with ample opportunity for both copulation and social infection to take place. Change in fresh mass during the experiment was used to deduce the nutritional state of the female reproductives, which usually is correlated with gonadal development (Brent and Traniello 2001a).

Fitness parameters were recorded for females of incipient colonies in which both the male and female survived 20 d (control females $n = 18$, socially-exposed females $n = 19$, directly-exposed females $n = 29$). By recording reproductive parameters only from females of intact pairs, we controlled for the influence that the male's presence may have on the female's reproductive development (Brent and Traniello 2001b).

The extent of the female's reproductive development was assessed by counting under a dissecting microscope the total number of functional ovarioles and vitellogenic terminal oocytes (see Brent and Traniello 2001a). The number of functional ovarioles comprising an ovary is indicative of a female's investment in her reproductive development, and her potential capacity to produce eggs (Watson 1972), whereas the number of vitellogenic oocytes is indicative of current ovarian activity. Ovarioles were considered functional if they were not filamentous and contained oocytes at some stage of development. Oocytes were considered vitellogenic if yolk protein could be observed and the volume equaled or exceeded 0.01 mm^3 (Hewitt et al. 1972, Brent and Traniello 2001a). All dissections were conducted without prior knowledge of the individual's treatment.

Statistical Analysis. The frequency of visits and the amount of time spent by females next to the test compartments were not normally distributed even after logarithmic transformation (Kolgomorov–Smirnov for log transformed frequency of visits = 0.2, df = 841, $P < 0.0001$; Kolgomorow–Smirnov for log transformed duration of visits = 0.1, df = 841, $P < 0.0001$). Thus, Mann–Whitney (MW) nonparametric tests were used in our analyses. Bonferroni corrections because of multiple comparisons adjusted our significance threshold value to a more conservative $P < 0.01$. Changes in fresh body mass, the number of active ovarioles, and the number of vitellogenic oocytes were distributed normally and their variances were homogeneous. Differences in the reproductive parameters among the three treatments were analyzed by one-way analysis of variance (ANOVA), with pair-wise comparisons carried out after a Bonferroni correction setting a more conservative threshold α-value of 0.02 (Rice 1989). None of the fitness parameters differed significantly between colonies headed by nestmate and non-nestmate reproductives, so data across all colonies were pooled for subsequent analyses. Overall significance levels were set at $P \leq 0.05$. Survival estimates were obtained by Survival Analysis (SPSS 1990).

Results

Mate Preference. Females spent most of their time in the chambers adjacent to the males relative to the time spent traversing between the five inner chambers (Fig. 2). Although the median frequency with which females entered each of the five inner chambers did not differ (Fig. 3), females spent little time in the middle chambers (B-D), using them primarily as a conduit between chambers A and E (both adjacent to the test males) where they appeared to spend most of their time (Fig. 2). If there were no preference, females should have spent equal amounts of time in each of the five intervening chambers.

After controlling for the effect of other variables (such as relatedness and fresh body mass), females did not preferentially associate with healthy rather than diseased males. In general, females did not spend significantly more time next to the compartment of a healthy male (Fig. 2, Treatments 2–5); nor did they differ in the frequency with which they visited healthy males or males exhibiting a progression of disease symptoms (Fig. 3, Treatments 2–5). These results were consistent across most treatments and time periods. Significant differences were observed in the amount of time females spent next to males under the control conditions where females exhibited a preference for one control male over the other at the 12 (MW = 499, $P < 0.01$ following Bonferroni correction) and 36-h (MW = 573, $P = 0.001$) time periods (Fig. 2, Treatment 1). In addition, females given a choice between filter paper moistened with a control Tween 80 suspension lacking fungal conidia and a high concentration of fungal conidia tended to spend more time adjacent to the conidia (at the 1 − [MW = 136, $P = 0.02$] and 36-h [MW = 170, $P = 0.03$]) periods, although these differences were not significant after a Bonferroni correction (Fig. 2, Treatment 5).

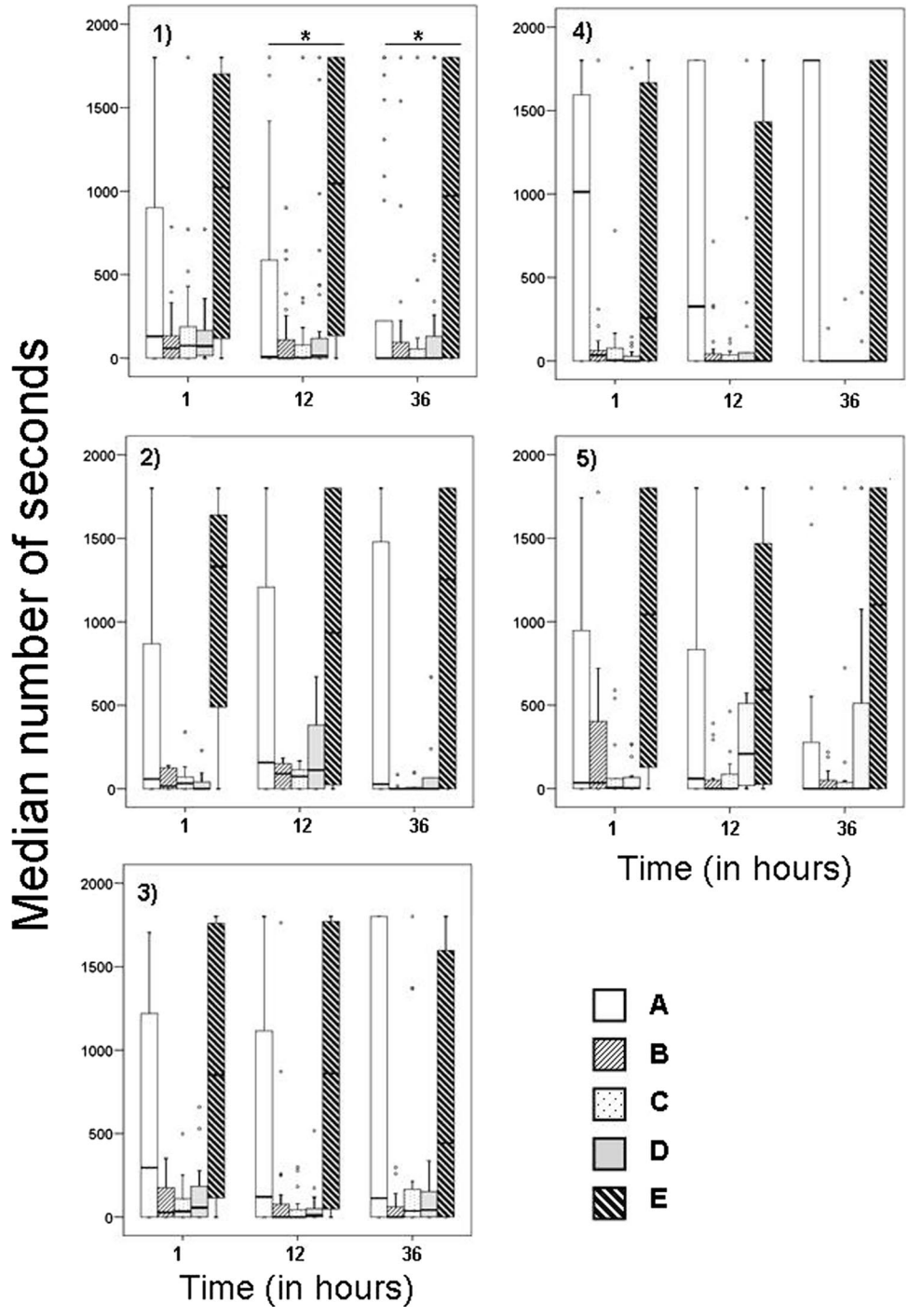

Fig. 2. Median cumulative time (seconds) spent by females in each inner chambers (A-E) of a mate choice arena during 30-min sample periods at 1-, 12-, and 36-h postintroduction. End compartments connected to A and E contained one of five treatments: 1) two healthy males, 2) one newly exposed and one healthy male, 3) one symptomatic and one healthy male, 4) one healthy and one dead sporulating male, and 5) filter paper treated with control medium or *M. anisopliae* conidia. Each boxplot shows the median value and interquartile range. The outliers identified by small circles, included cases with values larger than 1.5 box lengths from the upper edge of the box. Significant differences between compartment A and E (MW *U*-test, $P < 0.01$ after a Bonferroni correction) are indicated by an *. Sample sizes are included in the main text.

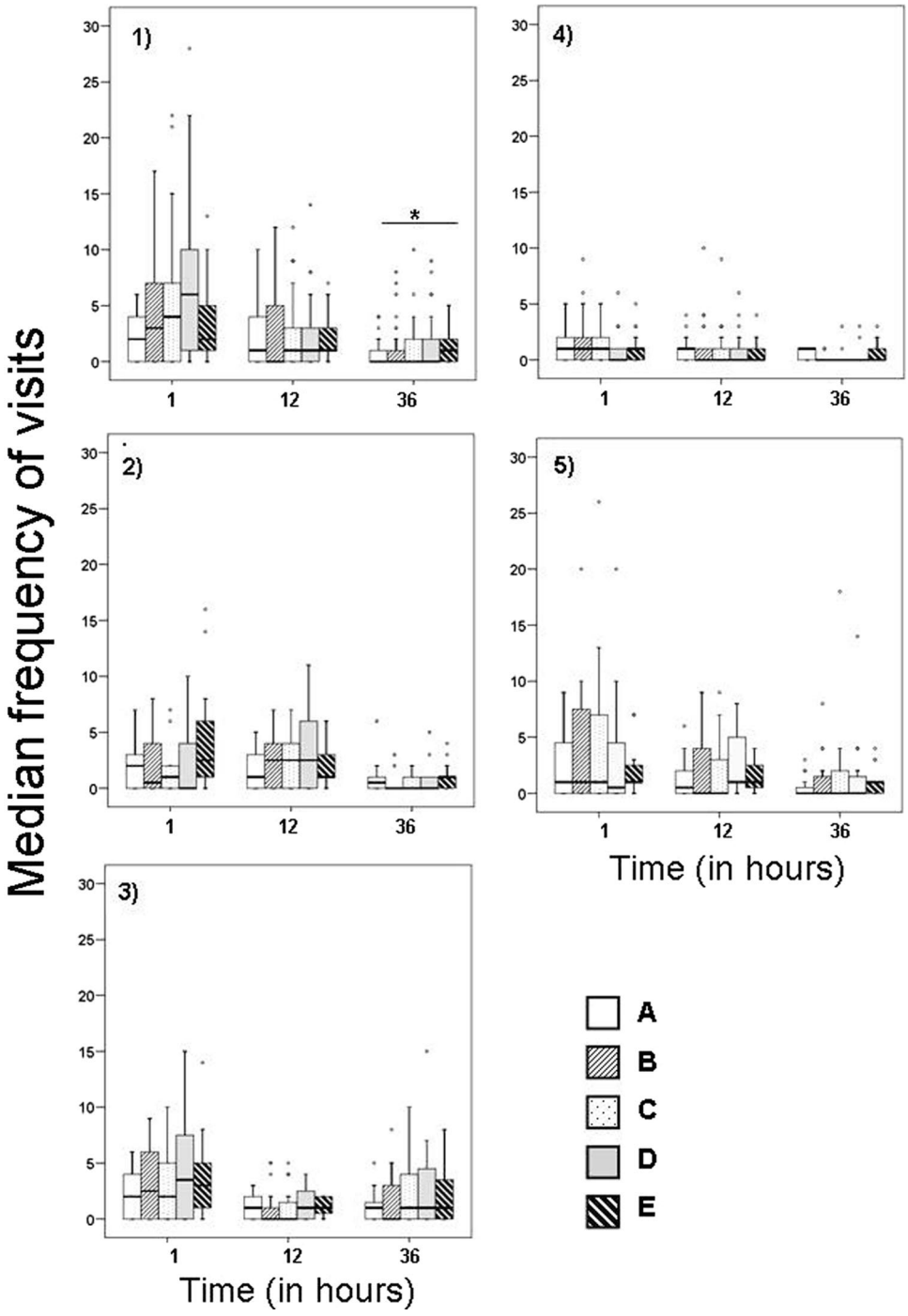

Fig. 3. Median number of visits by females to the five chambers (A-E) of a mate choice arena in which the end compartments received one of five experimental treatments (1–5, as described in Fig. 2) measured at 1-, 12-, and 36-h postintroduction. Each boxplot shows the median value and interquartile range. The outliers, identified by small circles, included cases with values larger than 1.5 box lengths from the upper edge of the box. With the exception of the control treatment (1) at 36 h, none of the pairwise comparisons were significantly different (MW *U*-test).

Female Reproductive Development. Of the 165 established colonies, 66 reproductive pairs survived the first 20-d postpairing, a typical survival rate for this species (Rosengaus and Traniello 1993, Rosengaus et al. 2000a). In the majority of surviving pairs, male and female reproductives originated from the same colony

(nestmate pairs, $n = 57$; non-nestmate pairs, $n = 9$). Not surprisingly, females directly-exposed to conidia had lower survival probabilities than either control Tween 80-exposed females or females paired with exposed males. None of the 6×10^5 conidia/ml suspension-treated females (translating into 300 conidia) survived 20 d. Control females and socially-exposed females had 53 and 55% survival at the end of the census period, whereas that of directly-exposed females (exposed to ≈30 conidia) was 48% (2×3 $\chi^2 = 0.6$, df = 2, $P = 0.7$). Although these differences were not statistically significant, after controlling for the independent effects of colony of origin (Wald Statistic [WS] = 17.7, df = 10, $P = 0.06$), relatedness between reproductives (WS = 3.3 df = 1, $P = 0.07$) and conidia dosage (WS = 3.8, df = 1, $P = 0.05$), treatment was a significant and independent predictor of female survival (WS = 7.1, df = 2, $P = 0.028$; Cox proportional regression). Relative to control females and socially-exposed females, directly-exposed females had twice the hazard ratio of dead (WS = 4.7, df = 1, $P = 0.03$). Moreover, the median survival time (LT_{50}) estimates for control females, socially-exposed females (whether 10^4 or 10^5 conidia/ml dosage) and females directly-exposed to 10^4 conidia/ml would have likely extended past 20 d had they not been preserved for dissection. In contrast, LT_{50} for females directly-exposed to a 10^5 conidia/ml was only 6 ± 0.7 (±SE) days (Kaplan–Meier Survival Analysis, SPSS).

Overall comparisons across all three treatments showed that fresh body mass gain ($F = 5.8$; df = 2, 63; $P = 0.005$), number of active ovarioles ($F = 3.3$; df = 2, 63; $P = 0.04$), and number of vitellogenic oocytes ($F = 8.5$; df = 2, 63; $P = 0.001$) differed significantly, although within-treatment pairwise comparisons were not necessarily significant following a Bonferroni correction (Fig. 4). Pairwise comparisons between treatments indicated that such differences mainly were driven by the negative impact of direct conidia exposure on female fitness parameters relative to controls. Compared with control females, those that were directly-exposed gained as much mass (Fig. 4A; mean difference 0.002 ± 0.001, $P = 0.2$), however, they exhibited a trend toward developing fewer functional ovarioles (Fig. 4B; mean difference $= 3.3 \pm 1.3$, $P = 0.05$), and had significantly fewer vitellogenic oocytes (Fig. 4C; mean difference $= 2.0 \pm 0.6$, $P = 0.007$). No significant differences in the reproductive output parameters or change in fresh body mass were observed between control and socially-exposed females (Fig. 4A–C).

Discussion

Termites live under constant risk of exposure to pathogens. Inside the colony, the spread of these pathogens is kept in check by the production of antimicrobial secretions (Rosengaus et al. 1998b, 2000b, 2004; Bulmer et al. 2009; Hamilton et al. 2011a,b and references therein), as well as behavioral (Logan et al. 1990; Milner and Staples 1996; Rosengaus et al. 1998a, 1999b, 2000a; Myles 2002; Wilson-Rich et al. 2007 and references therein) and immunological (Rosengaus et al. 1999a, 2007, 2011; Chouvenc et al. 2011) adaptations. Of particular interest is the ability of some termite species to externalize their induced immune-related proteins and incorporate them into their nest material (Bulmer et al. 2009; Hamilton et al. 2011a,b), thus lowering the risks of infection posed by the abundant and diverse microbial communities within nests. Alates leaving the protective environment of their natal nest during an annual swarm substantially increase their risk of infection. In addition to the cuticular microbial loads carried from their natal nest as they disperse, alates likely encounter novel pathogens on the ground, in the new nesting site, and on their mates (Rosengaus and Traniello 1993, Rosengaus et al. 2000a). In our experience, such cuticular microbes may become pathogenic, particularly during stressful events such as swarming. The alates' infection risk is exacerbated further by being deprived of the communal amplification of disease resistance responses that are normally found within an established colony (Traniello et al. 2002). Even if a female should survive infection, the ensuing immunological responses (Rosengaus et al. 1999a, 2007) can significantly reduce mass gain and oviposition rates (Calleri et al. 2006). Choosing a healthy male would reduce her risk of exposure, and would also provide a partner capable of aiding in pathogen defense (Traniello et al. 2002); nest guarding (Shellman-Reeve 1994); and brood care (Rosengaus and Traniello 1991; Shellman-Reeve 1990, 1999). Despite the potential benefits of being selective, our laboratory-based study provides no evidence that female mate choice in *Z. angusticollis* is influenced by disease risk, although size, nutritional status, and degree of heterozygosity appear to be important criteria for mate selection in other termite species (Shellman-Reeve 1999, 2001; Matsuura and Nishida 2001; Kitade et al. 2004; Husseneder and Simms 2008). The detection of infection in a mate may be less noticeable than size, pheromonal chemistry, or both (likely indirect measures of nutritional status and levels of heterozygosity, and sexual maturation (Liebig et al. 2009), particularly when the mate is asymptomatic.

There are several potential reasons why *Z. angusticollis* alates failed to avoid males that represented a risk of infection. First, alates may rely on the process of swarming itself for the elimination of diseased individuals from the breeding population. Sick individuals may be less capable of dispersing from their natal nest, eluding predators, or keeping up with a female during the period of tandem running while searching for a nest site. This would result in a passive screening process in which only relatively healthy mates remain in the breeding population. Second, de-winged individuals inevitably will encounter pathogens and parasites wherever they land, and exhibiting a strong aversive behavior would result in a certain degree of "paralysis" that would prevent both normal dispersal and the rapid location of suitable nesting sites. Third, dispersing reproductives may have little time to deal with the potential threat of contracting an infectious disease, particularly when they face certain and more

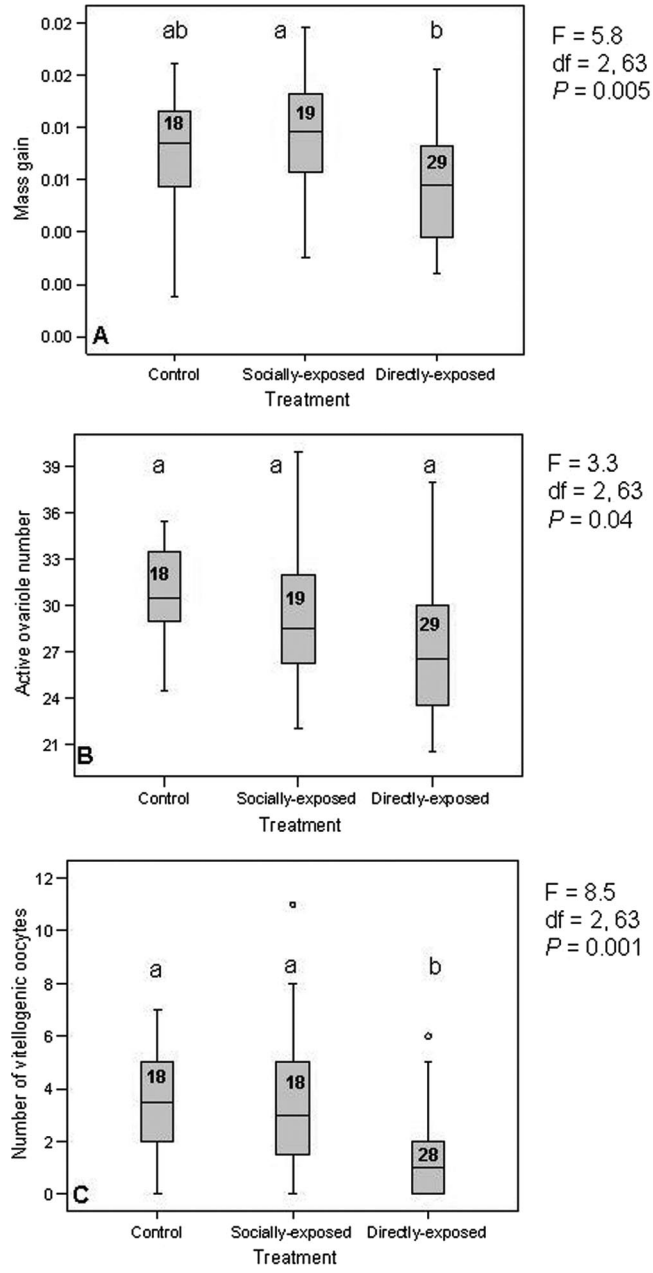

Fig. 4. Mass gain (A), number of active ovarioles (B), and number of vitellogenic oocytes (C) of female reproductives 20 d after treatment (unexposed, socially, or directly exposed to conidia). Each boxplot shows the median value and interquartile range. Outliers, identified by small circles, included cases with values between 1.5 and 3 box lengths from the upper edge of the box. The statistical analyses to the right of each figure represent the overall significance across the three treatments. Bars with the same letter are not significantly different ($P \leq 0.02$) in pairwise comparisons (by one-way ANOVA adjusted with Bonferroni correction, SPSS). Numbers inside bars represent sample sizes.

imminent dangers such as high rates of predation (Basalingappa 1970, Sheppe 1970, Deligne et al. 1981, Dial and Vaughan 1987, Myles and Nutting 1988, Lepage 1991, Shellman-Reeve 1997, Korb and Salewski 2000, Matsuura and Nishida 2002) and desiccation (Collins 1969). Finally, because potential mates and nesting sites have scattered and unpredictable distributions (Hamilton 1978, Nalepa and Jones 1991), the added cost of an extended selection process can be significant, especially after the alates drop their wings and become less mobile (Hamilton 1978, Nalepa and Jones 1991, Rosengaus and Traniello 1991). Therefore, the limited reproductive opportunities, the high costs of searching for a mate, and the need for expediency

may select against risking a single mating opportunity by being too choosy.

One unexpected finding was that often females spent more time in the proximity of compartments containing fungal conidia (Fig. 2, Treatment 5). Although not significant, this tendency suggests that *Z. angusticollis* reproductives may be attracted to the fungus in a manner similar to that reported for the termites *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki (Engler 2004) as well as for the fungus growing ants (Jaccoud et al. 1999). In contrast, other termites were found to be repelled by virulent fungal strains, either upon direct contact with conidia (Staples and Milner 2000) or from a distance (Mburu et al. 2009). Thus, the use of olfactory cues in termites to avoid infectious conidia is highly variable and may be species specific. In our case, termites may be motivated to move toward conidia in an attempt to limit its spread. By depositing antifungal exocrine secretions or walling off the fungus (Logan et al. 1990; Milner and Staples 1996; Rosengaus et al. 1998b, 2004), individuals may restrict the potential threat posed by entomopathogens to their nest. In addition, the fungus may alter the olfactory cues emanating from infected nestmates to render them more attractive. This may be similar to the necrophoretic behavior (removal of dead nestmates) exhibited by ants in response to postmortem changes in cuticular chemistry (Choe et al. 2009). Termites might be similarly drawn to dead or dying nestmates to maintain colony antisepsis. Alternatively, the conidia themselves may be inherently attractive. A substantial selective advantage could be gained by such means to enhance the probability of horizontal transmission (Luong and Kaya 2005). Host manipulation appears to be a common phenomenon across many host–parasite and host–pathogen associations (Sheldon 1993, Adamo 1997, Knell 1999, Moore 2002, Luong and Kaya 2005, Biron et al. 2005) and the possibility exists that *M. anisopliae* manipulates termite behavior to increase the amount of time spent with infected conspecifics.

Our results on fitness-related parameters indicate that socially-exposed females are not as severely affected as those females that were directly exposed to fungal conidia. Although the former had survival rates and reproductive development similar to those of controls, the latter were impacted significantly, exhibiting reductions in survival, fresh body mass gain, and vitellogenic oocytes (Fig. 4A–C). One reason for these differences is the relative conidia dosage experienced by socially- versus directly-exposed insects. Although social exposure certainly allows for the transmission of conidia to the female (Rosengaus and Traniello 1997, Rosengaus et al. 2000a), these females were subjected to dosages that were likely orders of magnitude lower than those of directly-exposed females. Thus, it is probable that the severity of infection, and the immune response required to combat it, were insufficient to divert resources away from reproduction.

The reduced fitness parameters of females directly exposed to fungal conidia provides further evidence for in vivo trade-offs between reproduction and immunity in *Z. angusticollis*, as reported previously by Calleri et al. (2006, 2007). Calleri et al. (2006) suggested that such trade-offs could be driven by the conflicting needs to use limited nitrogen resources for the production of vitellogenic eggs on the one hand and the production of immune proteins on the other. However, a preliminary examination of the endocrine response to pathogen exposure (C.S.B. and R.B.R., unpublished data) indicates that relative to controls, infected *Z. angusticollis* females have a reduced circulating titer of juvenile hormone, a change that also would have an adverse affect on vitellogenesis (reviewed in Brent 2009). The reduced fitness parameters of females directly exposed to fungal conidia indicate that either fungal disease itself or the immunological responses of females to cope with fungal infection have a significant impact on the female's reproductive development. Given that the females for which fitness parameters were measured survived at least 20-d postexposure, it is reasonable to assume their survival reflected an induction of successful immune responses that controlled the fungal disease (Vilcinskas and Götz 1999; Rosengaus et al. 1999a, 2007). Therefore, the reduced reproductive parameters likely reflect trade-offs between immunocompetence and reproduction, rather than the pathogenic effects of the fungus (i.e., disease) on the female's reproductive output.

Conclusion. In spite of the potential costs of pathogen exposure, female reproductives of *Z. angusticollis* do not appear to assess male partners based on pathogen load or their disease symptomology. More imminent ecological pressures, including predation, desiccation, or both, may override the need for a careful and time-consuming assessment of the health of a potential mate before colony establishment. Because both mates and nesting sites have scattered and unpredictable distributions (Hamilton 1978, Nalepa and Jones 1991), the cost of extensive searching can be significant (Nalepa and Jones 1991, Rosengaus and Traniello 1991), tipping the balance toward rapid decision-making without significant mate quality assessment. Furthermore, infectious diseases (whether sexually or socially transmitted) may have evolved cryptic strategies, host manipulative strategies, or both to increase the rates of horizontal transmission. Although not tested here, we expect that males, facing the same ecological constraints as the females, also should use similarly rapid mate-choice decisions. This strategy may be particularly effective if both the endocrine and immune systems can respond accordingly to increase survival during the incipient stages of colony foundation, albeit at the expense of short-term reproductive output. Long-term field studies are required to establish whether the proposed mate choice dynamics and fitness consequences described here also are observed in more natural settings.

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