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Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants

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Abstract The paired exocrine metapleural glands present in the large majority of ant species produce compounds with antibiotic properties. In the leaf-cutting ant, *Acromyrmex octospinosus*, the secretion consists of more than 20 different compounds and it has generally been assumed that the glands serve as a general defence against various infectious microbes of fungal and bacterial origin. We present results illuminating the direct costs and benefits of these metapleural gland defences in *A. octospinosus*. We show that major workers of this leaf-cutting ant experience a significant reduction in their respiration rate when the metapleural glands are experimentally closed, indicating that metapleural gland secretion incurs a substantial cost and that the production of compounds from these glands is terminated when the ants are incapable of secreting them. In another set of experiments, we show that the ability to secrete antibiotic compounds from the metapleural glands is of significant importance when ants are exposed to a general but potentially virulent insect pathogen, *Metarhizium anisopliae*. Infection with this fungus is lethal within a few days when ants have their metapleural glands experimentally closed, but relatively harmless when the metapleural glands are functional. These findings support experimentally the view that the metapleural glands play an important hygienic role in leaf-cutting ants.

Keywords Fungus-growing ants · *Acromyrmex* · *Metarhizium anisopliae* · Pathogens · Metapleural glands

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Introduction

Protection is crucial when microbial parasites and pathogens challenge health, which explains why many insects produce compounds with antibiotic properties (e.g. Hajek and St. Leger 1994; Schmid-Hempel 1998; Vilcinskas and Götz 1999). Rapid spread of infectious diseases is especially problematic in social insects, due to the large number of closely related individuals that live together in colonies (Hamilton 1987; Schmid-Hempel 1994, 1998, Baer and Schmid-Hempel 1999). Among the social insects, effective defence mechanisms may be particularly needed in the leaf-cutting ants, since they both have to protect themselves and a mutualistic basidiomycetous fungus (Lepiotaceae, Leucocoprineae) (Chapela et al. 1994) that serves as the main food source for the ant brood (Möller 1893; Quinlan and Cherrett 1979; Hölldobler and Wilson 1990). Almost all ants have paired exocrine metapleural glands located at the posterolateral end of the mesosoma (Hölldobler and Engel-Siegel 1984), which have repeatedly been hypothesised to fulfil general defence functions against common diseases (e.g. Maschwitz 1974; Beattie et al. 1985, 1986; Ortius-Lechner et al. 2000; Bot et al. in print), but direct experimental evidence for this hypothesis has never been obtained (Currie 2001). The present study presents such evidence by experimentally manipulating metapleural gland function and investigating the effects on ant mortality after exposure to a common insect pathogenic fungus.

Entomopathogenic fungi are characterised by their ability to replicate internally within the host after having entered the insect either by penetrating the cuticle or, in some cases, by entering through the insect gut after

ingestion (Boucias and Pendland 1998). *Metarhizium anisopliae* is such a fungal pathogen (Genthner et al. 1998 and references therein; Vilcinskas and Götz 1999) that has previously been isolated from many insects, including a number of species of *Atta* leaf-cutting ants (Humber 1992). *M. anisopliae* hyphae penetrate the cuticle by a combination of physical forces and the secretion of enzyme complexes (Hajek and St. Leger 1994; Boucias and Pendland 1998; Vilcinskas and Götz 1999). After entering the hemocoel, the fungus normally kills the host within a matter of days due to tissue penetration and nutrient depletion (Boucias and Pendland 1998). Furthermore, *M. anisopliae* is known to produce secondary metabolites that debilitate certain cells of the insect innate immune defence, so that an appropriate defence preventing spore germination and early hyphal growth is essential (Hajek and St. Leger 1994; Boucias and Pendland 1998; Vilcinskas and Götz 1999). *M. anisopliae* and other entomopathogenic fungi (e.g. *Beauveria bassiana*) have been extensively investigated for their usefulness as biological agents in insect pest control as an alternative to chemical insecticides (e.g. Diehl-Fleig et al. 1988, 1993; da Silva and Diehl-Fleig 1988; Genthner et al. 1998; Jaccoud et al. 1999; Vilcinskas and Götz 1999).

Leaf-cutting ants have several behavioural and chemical ways to protect themselves and their mutualistic fungus against pathogens. Examples are active cleaning (Wilson 1980; Currie and Stuart 2001), waste management (Hölldobler and Wilson 1990; Bot et al. 2001; Hart and Ratnieks 2001), and the production of antibiotics by mutualistic bacteria (Currie et al. 1999). The secretion from the metapleural glands is known to have bactericidal and fungicidal properties in this group of ants (Maschwitz et al. 1970; Schildknecht and Koob 1970, 1971; Nascimento et al. 1996). Recent work by Ortius-Lechner et al. (2000) has identified about 20 different compounds in the metapleural gland secretion of *Acromyrmex octospinosus*, in addition to the 2 compounds already known from this species (Nascimento et al. 1996). In a recent study by Bot et al. (in print), it has been found that many of the metapleural gland compounds have antibiotic properties against *M. anisopliae*, as well as against a number of other microbes. The presence of a high number of compounds with antibiotic properties in the metapleural gland secretion suggests that these glands serve as a broad defence mechanism against a variety of microbes (Beattie et al. 1985, 1986; Ortius-Lechner et al. 2000; Bot et al. in print). Although the production of compounds with antibiotic properties does suggest a hygienic role for the metapleural glands, as pointed out by Hölldobler and Engel-Siegel (1984) and Currie (2001), experimental evidence *in vivo* is required to eliminate the possibility that these compounds serve a different role and just suppress the growth of some microbes in the artificial conditions of *in vitro* bioassays.

It is expected that the production of defensive compounds in the metapleural glands is energetically costly to workers. Metabolic energy is thus expected to be saved and to become available for other functions when the secretion of these glands is experimentally prevented. When open, however, the compounds secreted from the metapleural glands should inhibit infection after exposure to *M. anisopliae* spores (Bot et al. in print), so that mortality is expected to increase when the ants have their metapleural glands experimentally closed. The experiments reported in this paper investigate this trade-off and quantify some of the essential aspects of costs and benefits of antibiotic production in the metapleural glands of *A. octospinosus* leaf-cutting ants. We estimate metabolic cost by measuring respiration rates of ant workers with and without functioning metapleural glands, and experimentally examine the benefits of the glands by comparing the mortality of ants with and without functioning metapleural glands when exposed to spores of the insect pathogen *M. anisopliae*.

Methods

Respiration rates of ants with open and closed metapleural glands

Three colonies of *A. octospinosus*, collected in Gamboa, Panama, in 1994 (colonies 18 and 19; Bot and Boomsma 1996) and 1998 (colony 86) were used to measure respiration rates of workers. After collection, colonies were kept under standardised conditions in a climate room at 25°C and 70% RH at Aarhus University as described in Bot and Boomsma (1996). From each colony, 30 major workers of approximately the same (young) age, determined by their similar light-brown colour, were taken out. Only workers of the major caste were used in order to gain the most favourable signal-noise ratios possible. Half of the workers from each colony were randomly selected and had their metapleural glands closed with nail polish, using a capillary made of a pipette tip that was attached to a plastic syringe. Gland closure was conducted using a binocular microscope to ensure that blocking was complete and that the spiracles were not blocked by the nail polish. The metapleural glands of the other half of the workers were left untouched, but these workers had a similar volume of nail polish applied between the pronotal spines. For each of the 3 original colonies, 1 sub-colony was set up, consisting of approximately 2 g of fresh fungus and 30 minor workers. Major workers of both treatments were subsequently transferred to these sub-colonies. All workers survived the treatment and behaved normally afterwards (see also below).

Respiration rates were measured 1, 8 and 15 days after the glands had been closed, since pilot experiments had shown that more frequent measuring, e.g. every 2nd day, was too stressful for the ants. To avoid movement during measurements, the ants were anaesthetised for 5 min with a 3% v/v solution of enflurane (efrane) in light paraffin oil. Holm-Jensen et al. (1980) have shown that ants recover from exposure to 4% v/v enflurane for up to 20 h and that enflurane is not metabolised and does not cause any muscular excitations, which makes it ideal for such measurements. Respiration rates were estimated by measuring CO_2-production using a flow-through analyser (model LI-6251) connected to a data acquisition and analysis system (Sable System, Salt Lake, Utah, using Datcan V software) (Nielsen et al. 1999). The air-flow through the system was 150 ml min^{-1} and the equipment was very sensitive, measuring concentrations as low as 50 ppb CO_2. Measurements were conducted on individual workers and lasted for 5.33 min. The respiration chamber (length=60 mm, diameter=13 mm) had rubber plugs at both ends and was placed in a 25°C temperature-regulated water bath. Background control measurements monotonously decreased with time, so that ant measurements were adjusted for background noise by the insertion of two background measurements after every sixth ant measurement in the linear regression equation: background measure=α*time

(minutes)+β. After adjustment, the CO_2 production was converted to ml CO_2 per hour per gram ant wet weight.

Mortality of ants with open and closed metapleural glands after exposure to *Metarhizium anisopliae*

To examine the role of the metapleural glands in protecting workers from microbial pathogens, we conducted a two-by-two factorial experimental design, crossing the presence/absence of the pathogen *M. anisopliae* with workers with and without functioning metapleural glands. The experiment was too labour-intensive to test more than one colony at the time, and was thus first carried out with colony 19 and later repeated for colony 18 to assure reproducibility. From each colony, 80 minor, 80 medium and 80 major workers were taken out. Half of these workers had their metapleural glands closed and the other half had the glands left untouched, following the same procedure as described above. The workers (all castes combined) with open and closed glands, respectively, were kept in separate sub-colonies each consisting of approximately 5 g of fresh fungus from the original colony, and were fed with fresh leaves of bramble (*Rubus fruticosus*). The next day, half of the ants of each caste and metapleural gland treatment were sprayed with *M. anisopliae* using a garden pump sprayer containing approximately 50,000–100,000 *M. anisopliae* spores per millilitre demineralised water and 1 µl Tween 20 per millilitre (Calbiochem, Novabiochem, La Jolla, Calif.) to avoid spore clumping. The *M. anisopliae* spores were obtained from cultures purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM 1490). Control ants were given a similar treatment, except that they were sprayed with demineralised water containing only Tween 20.

After the spraying treatment of the ants, new miniature gardens for 40 smaller sub-colonies were set up, each containing 80 mg fresh fungus from the original colony. These were placed in small vials (height 3.5 cm; diameter 2 cm) with moist tissue in the bottom and a small bramble leaf for food. One major worker pupa was given to each sub-colony to minimise the possibility of abnormal worker behaviour resulting from the absence of brood. Each sub-colony was subsequently given six workers (two minors, two media, two majors), which all had either open or closed metapleural glands and which all had either been exposed to *M. anisopliae* or not. All combinations of treatments were replicated ten times. Two days after the start of the experiment, the sub-colony fungus garden was weighed, ant mortality was recorded and the sub-colonies were given a new vial with clean tissue and a fresh bramble leaf. After another 2 days, fungus weight and ant mortality were recorded again and the experiment was terminated.

Testing the assumption that metapleural gland closure only affects gland secretion

Our treatment of closing the metapleural glands with nail polish makes the implicit assumption that there is a direct negative feedback between the pressure building up in the metapleural gland reservoir and secretion. We thus assume that secretion is terminated soon after gland closure (saving the metabolic costs of producing the secretion), but that the individual ant concerned does not suffer any secondary health effects, at least for the duration of the experiments. We tested this assumption with a series of separate experiments. The results of these tests are reported here (instead of in the results section of this paper), because they are essentially only a technical component of our study, and were only done to justify the experimental approach outlined in the methods sections.

Firstly, we extended the observations on treated ants. We followed major *A. octospinosus* workers with closed metapleural glands for much longer than 4 days. This experiment was part of another study, where we could demonstrate that treated workers survived essentially without mortality for 8 weeks. The cumulative mortality in the treatment group (60 workers from 3 colonies) was only 0.08%, whereas an equally large group of control ants with open glands had a mortality rate of 0.15% (M. Poulsen, A.N.M. Bot, J.J. Boomsma, unpublished data). In addition, we performed additional respiration-rate measurements (M. Poulsen, unpublished data) and were able to show that closing only 1 of the 2 metapleural glands of individual major workers (n=12) gave respiration rates (after 8 days) that were approximately intermediate between those of ants with both glands open or closed. Both results indicate that gland closure does not damage the health of treated ants. Intermediate respiration rates with one gland closed are predicted by the assumption that gland functioning stops after gland closure, but such additive effect would be unlikely if the treatment had damaged the ants.

Secondly, we examined the effect of closing metapleural glands on the amount of secretion accumulating in the gland reservoir, by making semi-quantitative estimates of the relative amounts of secretion present in the reservoir, after a number of pre-determined time periods following gland closure. Eighty major workers of *A. octospinosus* colony 86 were used for the experiment. Ten of these had their reservoir cut open immediately after they were taken out of the original colony and the observed quantities of secretion thus represented the gland contents 0 h after closure. The remaining 70 major workers had their metapleural glands closed using nail polish as described above and were left in a sub-colony containing approximately 2 g of fungus garden material and 40 minor workers. Subsequently, ten ants were randomly chosen and taken out of the sub-colony 1, 2, 4, 8, 16, 24 and 32 h after gland closure. Shortly after each of these each samplings, the reservoir was cut open with a razor blade under a binocular microscope and the secretion present in the reservoir was recorded using the following scale: 0 reservoir empty and dry; 1 secretion present and reservoir moist; 2 reservoir approximately one-third full; 3 reservoir approximately two-thirds full; 4 entire reservoir full; 5 reservoir full and secretion bursting out when opened.

The results of this experiment were tested using a non-parametric contingency table and a correlation analysis between content and time after closure. The results showed that gland secretion did not accumulate within the first 32 h after gland closure (χ^2=40.76; P=0.2318; n=80; df=35). The average score obtained in all dissections was 1.95 (SE=0.135). There was no significant correlation between the content of the reservoir and time (r=0.125; P=0.268; n=80; df=79), and scores of 5 and 0 were rare (3.8% and 5.0%, respectively). We interpret these results as further evidence that the central assumption of this study is basically correct. No secretion is accumulating after gland closure. The amounts of secretion found after the different time spans are similar to the amounts that were present when the glands were closed, which implies that secretion must have stopped shortly after treatment.

The alternative hypothesis that the glands continued to function after closure and that the secretion somehow diffused back into the surrounding tissues while damaging the ants and affecting their respiration rates, could be rejected. This hypothesis is at variance both with the fact that virtually all treated ants survive for 8 weeks and with the fact that we only rarely observe a closed gland with secretion under pressure behind the bulla (category 5). In addition, microscopical cross sections (light microscopy magnifications ×700 and ×1100, electron microscopy ×3000 and ×7000) were unable to reveal any effects of gland closure on the structure of cells and tissues around the gland. These cross sections were made of eight major workers from colony 86 after they had one metapleural gland closed for 4 days, so that the other gland of each ant could serve as an internal control. Transmission electron microscope and light microscope cross sections were made and evaluated by an expert in this field (Professor Johan Billen, University of Leuven, Belgium). No deviations from normal cell structure and tissue morphology could be observed when comparing the cross sections from open and closed glands (J. Billen, personal communication).

Statistical analysis

A Shapiro-Wilk W-test was conducted to test for normality of the respiration data. Two data sets were not normally distributed, but the removal of a single outlier from each data set removed this

problem. The outliers were both very high values and were probably caused by ineffective anaesthesia. The respiration data were subsequently tested using a repeated measurements ANOVA (SYSTAT) with treatment and colony as factors. Both a full model test and a reduced test including only main factors were conducted. The effect of *M. anisopliae* on ant mortality was tested using a non-parametric Cox's proportional hazard model (Volf 1989) (JMP) with *M. anisopliae* exposure or non-exposure, metapleural glands open or closed, and colony-of-origin as main factors. Ants that survived until the termination of the experiment were included as right-censored data. Four models were tested: (1) a full model including all interaction terms; (2) a model including only the main factors and the first-order interaction terms; (3) a model excluding colony-level variation after the previous tests had shown that both colony effects and interaction terms including colony as a factor were non-significant; (4) a model containing only the main factors. The overall change in sub-colony fungus weight from day 1 to 5 was analysed to check the assumption that *M. anisopliae* only affects ant survival and does not have a negative impact on the fungus garden. Differences in fungus weights appeared to be normally distributed according to a Shapiro Wilk *W*-test. The effects of the factors *M. anisopliae* exposure, metapleural gland closure and colony of origin were therefore analysed with a three-way ANOVA (SYSTAT). Three models were tested, one containing only the main factors, another including the first-order interactions and a third testing the full model.

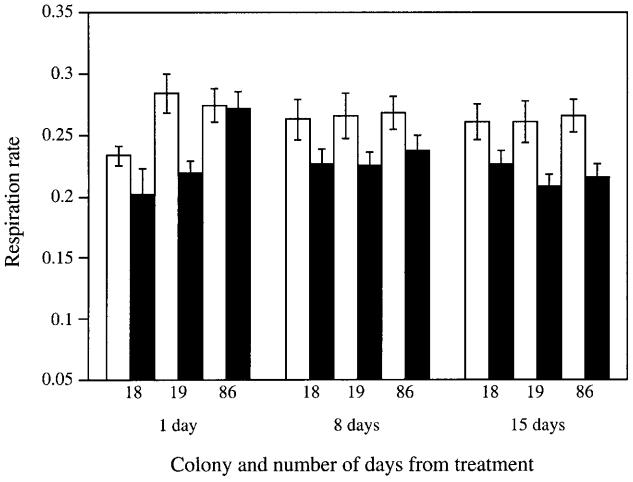

Fig. 1 Respiration rates (ml CO_2 produced per hour per gram wet weight) of major workers of 3 colonies of *Acromyrmex octospinosus* (18, 19, 86) 1, 8 and 15 days after their metapleural glands were closed. *Black bars* represent treatments, whereas *white bars* are respiration rates of control ants from the same colonies. All *bars* are means±SE of 15 replicate workers

Results

Closing the metapleural glands resulted in a significant reduction in ant respiration rate. This effect was already present after only 1 day in two out of the three colonies (Fig. 1). Eight days after the treatment, ants with closed metapleural glands had reduced respiration rates compared to control ants in all three colonies. This reduction was even more pronounced 15 days after gland closure, with reductions of 13.0%, 20.3% and 18.8% for colonies 18, 19 and 86, respectively (Fig. 1). The overall effect of metapleural gland closure on respiration rate was significant ($F_{1,76}$=19.62; P<0.0001), but colony-of-origin did not have a significant effect and neither were any of the interaction terms significant. A reduced model containing only the main factors confirmed this result. Both tests thus indicate that the effect of metapleural gland closure on respiration rate is consistent across colonies.

Infection with *M. anisopliae* leads to an increase in worker mortality, with a disproportionately large effect when the metapleural glands are closed (Fig. 2). In the full model, this effect is quantified in particular by the significant *M. anisopliae**Gland interaction term (χ^2=11.10; df=1; P=0.0009). The overall effect of *M. anisopliae* is also significant (χ^2=41.20; df=1; P<0.0001), but this is to be expected, because the pathogen can only influence mortality in a positive way and a significant interaction could not occur without an effect of *M. anisopliae*. The overall effect of closing the metapleural glands is likewise significant (χ^2=13.49; df=1; P=0.0002), but this result is mostly due to the highly increased mortality in ants with closed glands when *M. anisopliae* is present (Fig. 2). There was no significant difference between colonies (χ^2=13.36; df=1; P=0.821), implying that the effect is universal and consistent across colonies (Fig. 2). None of the other interaction terms were significant; nor did the reduced models show any major changes in the statistical outcome.

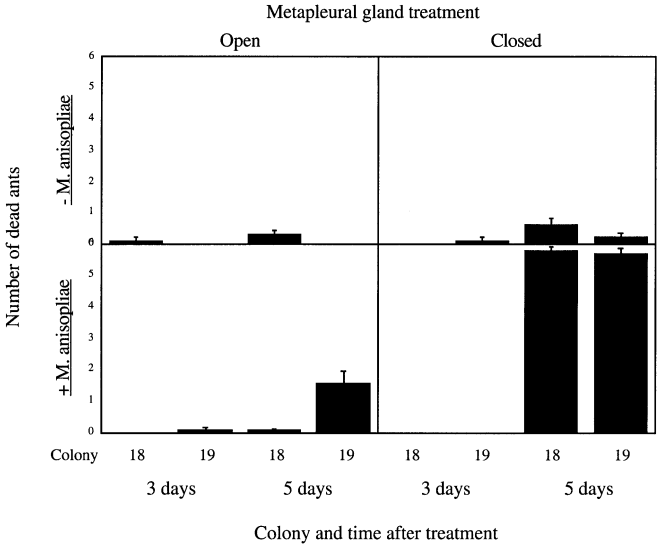

Fig. 2 The effect of *Metarhizium anisopliae* infection on ant mortality 3 and 5 days after treatments were conducted. Treatments were: (1) open and closed metapleural glands, (2) exposure and non-exposure to *M. anisopliae* and (3) colony of origin (18 and 19, respectively). All *bars* are means±SE of six replicate workers

The expectation that *M. anisopliae* should not affect fungus weight in a direct and negative way was confirmed, as the fungus gardens increased in weight (Fig. 3) in the presence of *M. anisopliae* ($F_{1,72}$=12.47; P=0.0007). This increase is unlikely to be a direct result of the exposure to *M. anisopliae*, but rather an indirect effect of reduced feeding on the fungus in gardens where ants had died, because they were incapable of coping with *M. anisopliae* infection and/or a result of reduced

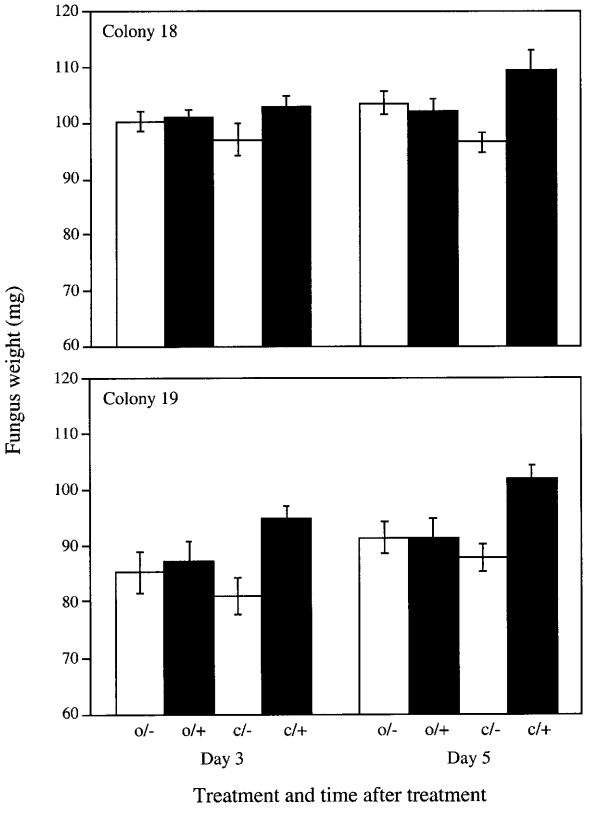

Fig. 3 The effect of *Metarhizium anisopliae* exposure and metapleural gland closure on sub-colony fungus garden weight. *Bars* represent sub-colonies containing workers with open metapleural glands (*o*) or with closed metapleural glands (*c*); + and – signs indicate whether the ants had been exposed to *M. anisopliae*. Each *bar* represents the mean±SE of ten replicate sub-colonies

removal of old fungus material when ant care is absent. This effect is documented by the combined effect of metapleural gland closure and the presence of *M. anisopliae*, the *M. anisopliae**Gland interaction term, which is significant in this analysis ($F_{1,72}$=14.81; P=0.0003). This time, the response was also different across colonies ($F_{1,72}$=30.96; P<0.0001), as fungus weight in colony 18 increased more than fungus weight in colony 19 (Fig. 3; both started at 80 mg).

Discussion

The respiration-rate measurements strongly suggest that metapleural gland secretions incur a significant cost for the ants because gland closure leads to a considerable reduction in the overall metabolic rate. Despite the substantial cost of maintaining the production of metapleural gland compounds, metabolic energy is apparently continuously allocated to this function under natural conditions. The advantage justifying this continuous expense is evident from the disproportionately high mortality of ants with experimentally closed metapleural glands after exposure to *M. anisopliae*. This could indicate that the metapleural glands are either the only or the major defence in *A. octospinosus* that can overcome the negative impacts of exposure to this general and potentially virulent pathogen. This implies that relatively short periods of reduced availability of resources for metapleural gland function may have a profound effect on ant longevity. Under natural conditions, such interruptions could occur during periods of food stress.

Some of the *M. anisopliae* effects documented in our experiments could potentially have been caused by fungal toxins (Samuels et al. 1988; Hajek and St. Leger 1994; Vilcinskas and Götz 1999) rather than by growth of *M. anisopliae* hyphae. However, in that case, the presence of toxins should have caused immediate ant death after first exposure to *M. anisopliae*. This was not observed in our experiment, where ant mortality mostly occurred after 5 days (Fig. 2). Additionally, extensive *M. anisopliae* growth was observed on the cuticle of all dead ants with closed metapleural glands, which implies that *M. anisopliae* hyphal growth and subsequent tissue penetration had killed the ants. At this stage, however, increasing toxin concentration due to infection spread may be of importance for the rate of ant mortality.

The measurements of respiration rate show that the abortion of function, and the concomitant saving of energy, happen extremely quickly after the ants are experimentally prevented from secreting metapleural gland compounds. The exact physiological mechanism controlling this interruption of function is unknown. However, based on the explicit tests of the assumption that gland closure only affects gland secretion (see Methods), we conclude that the hypothesis that enforced inability to secrete the glandular compounds aborts further production of antibiotic compounds from these glands by some negative feedback mechanism is correct. The microscopical analysis (see Methods) further implies that antibiotic-producing glands do not become atrophied after secretion is aborted, but that they can persist in an inactive state for considerable periods of time. The relatively low amounts of secretion in the gland reservoirs of many ants when sampled directly from their laboratory colonies, and the rapid decrease in respiration rate after metapleural gland closure, both suggest that active regulation of the production of antibiotic compounds takes place, e.g. in response to the instantaneous level of challenge by pathogens, which may allow the ants to periodically save considerable amounts of metabolic energy that can be allocated to other purposes. Such flexible production of defensive compounds could be of considerable importance in natural habitats where both the amount of available resources and the level of exposure to pathogens may vary in space and time.

The expenses associated with the production of metapleural gland compounds documented for major workers will almost certainly be even more pronounced in minor workers. This is due to: (1) the relatively larger size of the metapleural glands in minor workers of *A. octospinosus* compared to major workers (Bot and Boomsma 1996), and (2) independently collected evidence that minor workers are of greater importance for general defence

against various pathogens than major workers (Poulsen et al. 2002).

The antibiotic properties of compounds originating from the metapleural glands have recently been shown to significantly inhibit spore germination and/or growth of a variety of pathogenic bacteria and fungi *in vitro*, including *M. anisopliae* (Bot et al. in print). The results of the present study document for the first time *in vivo* that the compounds originating from the metapleural glands are produced in sufficient quantities to suppress a general pathogen like *M. anisopliae* (cf. Currie 2001). We suspect that the results of our study may well be representative of the way in which ants control a whole series of common microbial pathogens. This would underline the tremendous significance of the metapleural glands and justify the metabolic expenses quantified in this study. The fact that metapleural gland closure and *M. anisopliae* infection had similar effects on worker mortality across the two to three colonies examined indicates that these responses are universal.

The application of *M. anisopliae* and other entomopathogenic fungi in biological pest control of leaf-cutting ants may seem of little use when taking the apparent efficiency of the metapleural gland defence into consideration. However, the virulence of entomopathogenic fungi differs both between fungal strains and between leaf-cutting ant colonies (Diehl-Fleig et al. 1988). Furthermore, the efficiency of the metapleural glands in inhibiting entomopathogens from becoming established in the colony may depend on the concentration of the pathogen infection (see, for example, a study of the effect of *Beauveria bassiana* on *Acromyrmex* spp.; Diehl-Fleig et al. 1993).

As far as we know, few studies have quantified the absolute energetic costs associated with single chemical defence mechanisms in insects (cf. Schmid-Hempel 1998). However, several studies have revealed that there are energetic trade-offs associated with physiological and chemical defence functions (see, for example, Doums and Schmid-Hempel 2000; Moret and Schmid-Hempel 2000). In addition, increased efforts spent on one type of defence may lead to a concomitant reduction in another. In an earlier study (Poulsen et al. 2002), we documented that such a trade-off exists for the general metapleural gland defence function and the specific defence against *Escovopsis* by a *Streptomyces* bacterium growing on the cuticle of *Acromyrmex* workers. Other studies have shown trade-offs of defence functions with other fitness parameters, such as growth rate, weight or fecundity (e.g. Kerfoot 1977; Walls and Ketola 1989; Harvell 1992; König and Schmid-Hempel 1995). Despite their high expenses, it seems nevertheless to be a universal feature that costly defence mechanisms against parasites may be selected for, because their considerable costs are more than compensated for by increased survival and reproduction.

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