Disruption of the Termite Gut Microbiota and Its Prolonged Consequences for Fitness

Rebeca B. Rosengaus, Courtney N. Zecher, Kelley F. Schultheis, Robert M. Brucker and Seth R. Bordenstein


Published Ahead of Print 13 May 2011.

Updated information and services can be found at:
http://aem.asm.org/content/77/13/4303

**SUPPLEMENTAL MATERIAL**

These include:
http://aem.asm.org/content/suppl/2011/06/17/77.13.4303.DC1.html

**REFERENCES**

This article cites 72 articles, 24 of which can be accessed free at: http://aem.asm.org/content/77/13/4303#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://aem.asm.org/site/misc/reprints.xhtml
To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Disruption of the Termite Gut Microbiota and Its Prolonged Consequences for Fitness†

Rebeca B. Rosengaus,¹* Courtney N. Zecher,² Kelley F. Schultheis,¹ Robert M. Brucker,³ and Seth R. Bordenstein²,³

Department of Biology, Northeastern University, 134 Mugar Life Sciences Building, 360 Huntington Avenue, Boston, Massachusetts 02115-5000; Marine Biological Laboratory, Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, 7 MBL Street, Woods Hole, Massachusetts 02543; and Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235

Received 6 August 2010/Accepted 27 April 2011

The disruption of host-symbiont interactions through the use of antibiotics can help elucidate microbial functions that go beyond short-term nutritional value. Termite gut symbionts have been studied extensively, but little is known about their impact on the termite’s reproductive output. Here we describe the effect that the antibiotic rifampin has not only on the gut microbial diversity but also on the longevity, fecundity, and weight of two termite species, Zootermopsis angusticollis and Reticulitermes flavipes. We report three key findings: (i) the antibiotic rifampin, when fed to primary reproductives during the incipient stages of colony foundation, causes a permanent reduction in the diversity of gut bacteria and a transitory effect on the density of the protozoan community; (ii) rifampin treatment reduces oviposition rates of queens, translating into delayed colony growth and ultimately reduced colony fitness; and (iii) the initial dosages of rifampin had severe long-term fitness effects on Z. angusticollis. Taken together, our findings demonstrate that the antibiotic-induced perturbation of the microbial community is associated with prolonged reductions in longevity and fecundity. A causal relationship between these changes in the gut microbial population structures and fitness is suggested by the acquisition of opportunistic pathogens and incompetence of the termites to restore a pretreatment, native microbiota. Our results indicate that antibiotic treatment significantly alters the termite’s microbiota, reproduction, colony establishment, and ultimately colony growth and development. We discuss the implications for antimicrobials as a new application to the control of termite pest species.

The long-standing associations between termites and prokaryotic and eukaryotic microorganisms have been crucial to the evolutionary and ecological success of this social insect group. The presence of cellulolytic microorganisms in the hindguts of termites is one of the key events that allowed termites to thrive on nitrogenously deficient food resources (49, 63). Fossil records (80) and the similarity in gut flora and other microbial endosymbionts with those of their roach relatives (59) support the hypothesis that these associations existed in the termite ancestor (3, 50, 59). Termite gut symbionts reside in the lumen or are attached to the wall of the hindgut region and can represent more than 40% of the termite’s weight (6). They are horizontally transmitted through coprophagy, a common behavior in termites. Indeed, the need for transfaunation of hindgut symbionts has been proposed as one of the main factors favoring group living (44) and specifically favoring the evolution and maintenance of termite sociability (18). However, little is known about the impact that termite gut symbionts have beyond their role in cellulose degradation and host nutrition.

Here we report on the impact that antibiotic treatment has on the reproductive survival and fecundity of the dampwood termite Zootermopsis angusticollis and the Eastern subterranean termite Reticulitermes flavipes. Although previous experiments demonstrated that antibiotics compromise and/or eradicate the gut microbiota (protozoa and/or bacteria) of termites (11, 26, 53), no studies have yet characterized the short- and long-term fitness costs associated with antibiotics in these social insects or their impact on colony growth and development. Our findings suggest that rifampin disrupts one or more mutualistic interactions essential for normal termite reproduction and longevity.

MATERIALS AND METHODS

Collection and maintenance of termites. Two mature colonies of Z. angusticollis were collected from Huddart Park, San Mateo, CA, and maintained as described by Rosengaus et al. (57). Reproductive of R. flavipes, an important structural pest in the United States, were collected from two stock colonies from West Roxbury, MA.

Establishment of incipient colonies. Incipient colonies were bred in the laboratory from virgin alates (winged adult dispersal forms). These fully pigmented individuals were collected, sexed, and paired only if their wings could be removed easily when folded anteriorly along the humeral suture (57). These selection criteria guaranteed that only ready-to-disperse virgin females and males were used in our studies. To prevent mating prior to colony establishment, the dewinged reproductives were housed in same-sex/same-colony containers (18 by 12 by 8 cm) lined with moist paper towels and some nest material. Within 7 days of removal from the parental nest, reproductives were placed inside petri dishes (60 by 15 mm) lined with filter paper (Whatman qualitative no. 1) and approximately 5.0 g of decayed birch wood. Subsequently, the filter paper was
moistened with either distilled water (controls) or rifampin (Sandoz Inc., Princeton, NJ; 300-mg capsules) (see below for details). Rifampin is bacteriostatic or bactericidal depending on dosage and acts by specifically inhibiting RNA-dependent RNA polymerase activity in eubacterial cells (27). It is a broad-spectrum compound active against a variety of Gram-positive and Gram-negative organisms (8, 16, 55, 76). The dishes, stacked in covered plastic boxes (30 by 23 by 10 cm), were maintained at 22°C.

**Effects of antibiotic ingestion on Z. angusticollis gut microbiota.** To determine if rifampin affected the composition of the termite’s gut microbial community, Z. angusticollis reproductive pairs were established in incipient colonies as described above. The diet of four incipient colonies was supplemented with 300 μl of a 0.5% suspension of rifampin on the day of pairing and 14 and 34 days after the initial dose. Three corresponding control colonies were similarly established but received distilled water instead. Subsequently, these colonies were left undisturbed until day 85 postpairing, when control and rifampin-fed females were surface sterilized with 2% NaClO and then their guts dissected in sterile phosphate-buffered saline (PBS) and preserved in 70% molecular-grade ethanol. This time frame was chosen because it was approximately at this time that the initial differences in oviposition rates became evident. Each sample was then centrifuged at 12,000 × g, and the ethanol was decanted. The DNA of the guts was extracted using the Qiagen DNAeasy blood and tissue kit per the manufacturer’s instructions for “quick and easy isolation of total DNA from eukaryotic tissues.” All samples were homogenized and treated with proteinase K for 3 h at 55°C before the column extraction procedure. Aliquots of the resulting DNA samples were then pooled and stored at 4°C until PCR, cloning, and sequencing (see the supplemental material for a detailed protocol). Extraction controls of sterile water were treated identically to samples and carried through all subsequent procedures. Negative water controls, as expected, showed no PCR amplification and did not yield clones containing an insert.

**Survival of Z. angusticollis and R. flavipes reproductives and colony fitness.** Incipient colonies were established as described above to examine the effect of rifampin on termite survival and fitness. These colonies ensured the monitoring of complete families throughout colony ontogeny by performance of periodic censuses. The filter paper was initially moistened with either 300 μl of water or rifampin and then again on the third day for the first 50 days postpairing. During these frequent initial censuses, colonies were sprayed with distilled water as needed. PD indicates that incipient colonies were housed in petri dishes. Q and K denote queen and king, respectively. See text for details.

**RESULTS**

**Effects of antibiotic ingestion on Z. angusticollis gut microbiota.** Diets of Z. angusticollis reproductives were supplemented with a low dose of rifampin antibiotic suspension (0.005 g of rifampin in 1 ml of sterile deionized water) on days 0, 14, and 34 after pairing. Sampling of the gut bacterial diversity by cloning and sequencing of 16S rRNA gene amplicons at day 85 indicated there was a significant difference in the bacterial population structures between the control and rifampin-treated Z. angusticollis termites (P = 0.01, UniFrac) (Table 1). As expected, rifampin treatment reduced the 16S rRNA gene bacterial diversity (Table 1). Of the 87 clones sequenced from the control termite 16S rRNA gene library, 17 operational taxonomic units (OTUs) were represented based on a 97% identity cutoff (mean Chao1 = 23 ± 6 OTUs; mean ACE = 21). However, among the 85 clone sequences in the rifampin-treated termites, only six OTUs were represented (mean Chao1 = 6 ± 1 OTUs; mean ACE = 6), amounting to a 64% reduction in bacterial diversity. The rarefaction analyses of the two libraries also showed that despite similar sequencing ef-
Ingestion of rifampin also had a significant short-term negative impact on the number of gut protozoa per gram of termite. In a separate experiment, nymphs fed rifampin for 3 days had a significantly lower median number of protozoa (± interquartile range) in their guts than the controls ($9.7 \times 10^6 \pm 3.4 \times 10^6$ for rifampin versus $2 \times 10^7 \pm 9.4 \times 10^6$ for control; $P = 0.01$) (medians are reported given that the frequency with which gut protozoa were recorded was not normally distributed). However, in subsequent dissections on days 8 and 14 postfeeding, the median number of protozoa per gram of termite did not differ significantly between the two treatments ($2 \times 10^7 \pm 7.6 \times 10^6$ for rifampin versus $2 \times 10^7 \pm 8.8 \times 10^6$ for control; $P = 0.5$) on day 8; $9.8 \times 10^6 \pm 1 \times 10^7$ for control versus $7 \times 10^6 \pm 9.3 \times 10^6$ for rifampin [$P = 0.5$] on day 14). Thus, although rifampin temporarily affected the number of protozoa in termite guts, it did not destroy them completely. Collectively, our results indicate that rifampin has only a moderate and transitory effect on the density of the culturable protozoan gut community and has a prolonged effect on the diversity of bacteria in termite guts.

**Survival of *Z. angusticollis* reproductives and colony fitness.**

The effects of the antibiotic on survival were evaluated throughout the first 2 years of colony life. A Cox proportional regression model with the variables “colony of origin” (either BDTK17 or BDTK19), “gender,” “sibship” (nestmate or non-nestmate pairs), and “treatment” (control or antibiotic fed) revealed that colony of origin (Wald statistic [WS] = 7.3, df = 1, $P = 0.007$) and treatment (WS = 25.1, df = 1, $P < 0.0001$) had significant effects. First, reproductives from colony 

---

**TABLE 1. Numbers of 16S rRNA OTUs in *Zootermopsis* control and treated guts**

<table>
<thead>
<tr>
<th>Class</th>
<th>Bacterial genus</th>
<th>No. of 16S rRNA OTUs in:</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control gut</td>
<td>Rifampin-treated gut</td>
</tr>
<tr>
<td>Endomicrobia</td>
<td>Termite group 1</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Bacteroides</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Propionibacter</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Deferrribacteres</td>
<td>Lincoln Park 3’</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacteria</td>
<td>Treponema</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>Verrucomicrobia genus</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Clostridia</td>
<td>Clostridales genus</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Clostridiales genus</td>
<td></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Uncultured rumen bacterium (&lt;95%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Uncultured sludge (&lt;95%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>Opitutaceae</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Epsilonproteobacteria</td>
<td>Sulfurispirillum</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Uncultured betaproteobacterium</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>Uncultured gammaproteobacterium (&lt;95%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Deltaproteobacteria</td>
<td>Uncultured Desulfovibrionales genus (&lt;95%)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>Pseudomonas</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Bacilli</td>
<td>Enterococcus</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>Providencia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Oxalobacteraceae genus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>Methylobacterium</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>Alphipia</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Acinetobacteria</td>
<td>Arthrobacter</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Flavobacteriales</td>
<td>Cytophaga</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>Ralstonia</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Uncultured bacterium (&lt;95%)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Bacilli</td>
<td>Streptococcus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>Sphingomonas</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Total no. of clones 91 86

*a* See Fig. S1 in the supplemental material for the corresponding rarefaction curve.
BDTK17 had 1.3 times the hazard ratio of death as reproductives from colony BDTK19 after controlling for the effect of treatment (Table 2). Second, rifampin-fed reproducitives after 2 years postpairing were 1.7 times as likely to suffer premature mortality than untreated individuals, even after controlling for the effect of colony of origin (Fig. 2; Table 2).

The time course of survival for the reproducitives did not differ significantly between the rifampin and control treatments ($\chi^2 = 2.8$, df = 1, and $P = 0.09$ for BDTK17; $\chi^2 = 0.1$, df = 1, and $P = 0.7$ for BDTK19) (Fig. 2), until after day 150 (Fig. 2). By 465 days, the survival distributions and percent survival were significantly different between the control and antibiotic treatments for each of the stock colonies (Fig. 3). These differences were pronounced by 730 days. At this time, 50% of the original control reproducitives had died. In contrast, the rifampin-fed reproducitives reached 50% mortality by the 465-day census (Fig. 2; Table 2). Thus, on average, the control termites lived approximately 265 additional days before reaching the 50% mortality mark (median lethal time [LT$_{50}$] estimate [Table 2]). These findings indicate that the effects of rifampin treatment significantly affect survivorship of reproducitives from both stock colonies, with mortality differences being most prominent between 465 and 730 days (Fig. 2; Table 2).

Rifampin-fed reproducitives originating from both colonies had consistently fewer offspring than their corresponding untreated controls. Because none of the reproductive output met statistical significance by 465 days postpairing (Mann-Whitney U tests [MW]), statistical analyses were carried out by combining all colonies within a treatment. Given the longitudinal nature of this study, we present a detailed description of the effects of antibiotic treatment on colony fitness at each of the census dates.

(i) Census at 150 days postpairing. The addition of low dosages of rifampin during the initial stages of colony foundation in Z. angusticollis resulted in a significant reduction in fecundity. Figure 3 shows a significant disparity between the frequency distributions of offspring number between surviving control and rifampin-treated colonies. The percentage of surviving control colonies with eggs, larvae, and soldiers on day 150 postestablishment was higher than that of rifampin-treated colonies; furthermore, a higher percentage of control colonies produced the highest number of eggs, larvae, and soldiers (Fig. 3). At 150 days postpairing, surviving control colonies also had a significantly higher median number of eggs, larvae, and soldiers than their rifampin-treated counterparts (Fig. 4). Furthermore, the effect of the antibiotic on Z. angusticollis reproductive output appeared to be immediate, since it significantly delayed first oviposition, by approximately 47 days (MW = 1,220, $z = -1.8, P = 0.086$) (Fig. 5a), and had a tendency to delay first hatching by roughly 33 days (MW = 1,220, $z = -1.8, P = 0.086$) (Fig. 5b) relative to controls.

(ii) Census at 465 days postpairing. The antibiotic continued to have a long-term negative effect on colony reproduction. All fitness parameters of surviving rifampin-fed reproducitives were significantly reduced relative to those of controls (Fig. 5).

(iii) Census at 730 days postpairing. At 2 years postpairing, the negative effect of rifampin on colony fitness persisted despite the antibiotic treatment being provided only during the first 2 years postestablishment.

Table 2. Survival parameters and mass estimates for Z. angusticollis primary reproducitives originating from two parental colonies. 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Significance</th>
<th>Value for:</th>
<th>Significance</th>
<th>Value for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT$_{50}$ on day 730</td>
<td>$P &lt; 0.0001, BS = 8.6$</td>
<td>730</td>
<td>$P &lt; 0.0001, WS = 100$, df = 1</td>
<td>730</td>
</tr>
<tr>
<td>% Survival on day 730</td>
<td>$P &lt; 0.0001$</td>
<td>465</td>
<td>$P &lt; 0.0001$</td>
<td>465</td>
</tr>
<tr>
<td>Hazard ratio of death</td>
<td>$t = 3.8, df = 111.7, P = 0.001$</td>
<td>Reference</td>
<td>$t = 3.8, df = 100$, $P = 0.001$</td>
<td>Reference</td>
</tr>
<tr>
<td>Mass (g) on day postpairing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>$0.0518$</td>
<td>0.0604</td>
<td>$0.0518$</td>
<td>0.0604</td>
</tr>
<tr>
<td>100</td>
<td>$0.0549$</td>
<td>$0.0584$</td>
<td>$0.0549$</td>
<td>$0.0584$</td>
</tr>
<tr>
<td>150</td>
<td>$0.0461$</td>
<td>$0.0488$</td>
<td>$0.0461$</td>
<td>$0.0488$</td>
</tr>
</tbody>
</table>

For LT$_{50}$ and percent survival, values indicate differences in the survival distributions between control and rifampin-treated reproducitives. These distributions are depicted in Fig. 2a and b. BS, Breslow statistic; WS, Wald statistic (Cox proportional regression). For mass, values indicate differences in the average mass between control and rifampin-treated reproducitives within each parental stock colony (test, SPSS).
initial stages of colony foundation (Fig. 4). After controlling for the effects of mass (see below), sibship, and colony of origin, treatment significantly influenced colony fitness ($t = -2.9$ and $P = 0.004$ for eggs, $t = -3.8$ and $P < 0.0001$ for larvae, and $t = -4.1$ and $P < 0.0001$ for soldiers as determined by multivariate linear regression with SPSS [65]). By the last census, 69.8% of the 128 originally established control colonies had oviposited at least one egg, while only 38.6% of the 120 original rifampin-treated colonies had done so (Pearson’s $\chi^2 = 24.0$, df = 1, $P < 0.0001$). Moreover, 63.5% of the original control colonies produced at least one larva, whereas only 28.6% of the original rifampin-treated colonies did (Pearson’s $\chi^2 = 30.0$, df = 1, $P < 0.0001$).

Survival of Reticulitermes flavipes primary reproductives and colony fitness. R. flavipes reproductives treated with antibiotic had a survival rate comparable to that of the controls for the first 5 months of colony life. A Cox proportional regression indicated that neither colony of origin, sibship, gender, nor treatment was a significant and independent predictor of ter-

Survival distributions of control (solid lines) and rifampin-treated (dashed lines) male and female Z. angusticollis reproductives originating from colonies BDTK17 (a) and BDTK19 (b) during the first 2 years of colony life. Filled and open circles represent the percent survival of control and rifampin-treated individuals at each of the major census dates (indicated by the arrows), respectively. These percentages differed significantly on days 465 and 730 postestablishment (Pearson’s $\chi^2$, $P < 0.004$). * and NS, significant and insignificant differences in the median survival time at each of the census dates, respectively (MW test; see text). Additional survival parameters are shown in Table 2.
mite survival (Wald statistic [WS] = 0.2, 1.5, 0.002, and 0.07, respectively; df = 1, P > 0.2). In regard to fecundity, after 5 months of colony formation, 69.4% of the original control established colonies oviposited at least one egg, relative to 53.3% of the original rifampin-treated colonies (Pearson’s $\chi^2 = 2.5$, df = 1, $P = 0.05$). Approximately 45% and 44% of the original control and rifampin-treated colonies, respectively, hatched at least one larva (Pearson’s $\chi^2 = 0.002$, df = 1, $P > 0.05$). After 150 days postestablishment, no soldiers had differentiated. Although these proportions were not statistically significant, several additional reproductive parameters of the rifampin-treated reproductives were negatively impacted relative to controls. Rifampin-fed *R. flavipes* reproductives had a lower maximum number of eggs (MW = 242.5, $z = -2.7$, $P = 0.007$), lower maximum number of larvae (MW = 159.0, $z = -2.8$, $P = 0.005$), and lower number of larvae on day 150 postpairing (MW = 112.0, $z = -3.5$, $P < 0.0001$) (Fig. 6). Although some additional reproductive parameters were reduced for rifampin-fed reproductives, they were not statistically different. Larger sample sizes and longer surveys past the first 150 days postestablishment are needed to elucidate if rifampin has long-term effects on *R. flavipes* reproduction similar to those it had in *Z. angusticollis*.

Taken together, these results indicate that small amounts of rifampin provided during the incipient stages of colony foundation alter the reproductive outputs of both termite species for the long term.

**Termite mass.** The mass of each surviving *Z. angusticollis* reproductive was recorded on days 50 and 465 postestablishment (Table 2). Our results show that by day 50, control reproductives were no more than 0.005 g heavier than their
DISCUSSION

This investigation demonstrates that the addition of the antibiotic rifampin to the diet of *Z. angusticollis* and *R. flavipes* during colony establishment reduces bacterial diversity in the reproductive’s guts, as well as colony fitness. Relative to controls, rifampin-treated *Z. angusticollis* reproductives had reduced survival and lower reproductive success. They exhibited a delayed first oviposition and significantly lower production of eggs, larvae, and soldiers throughout the 730 days of colony life (Fig. 4 and 5). Similarly, rifampin treatment in *R. flavipes* led to a reduction in the total number of eggs and larvae during the first 150 days of colony foundation (Fig. 6). How does rifampin treatment mediate the fitness costs on reproduction in these termite species? We propose two possible explanations.

First, the antibiotic could influence the reproductive success of reproductives indirectly by compromising the nutritional health of the royal pair, causing reduced weight gain and reproductive output. Rifampin could have caused defaunation of the eukaryotic hindgut microbes, resulting in malnutrition and/or starvation. The elimination of wood-digesting protozoan symbionts through the use of antibiotics has previously been demonstrated (11, 26, 53). However, in this study, rifampin-treated reproductives had numerous protozoa (median number = $7 \times 10^6 \pm 9.3 \times 10^6$ protozoa per gram of termite) at 14 days posttreatment, and it is the gut protozoa that are primarily responsible for cellulase activity in the digestive tracts of primitive “lower” termites (13, 36). Rifampin does not have a prolonged negative effect on the cellulolytic gut protozoa of *Z. angusticollis*, most likely because this antibiotic specifically inhibits the bacterial RNA polymerase (32). Moreover, the most abundant bacteria in the termite’s hindgut, the spirochetes, play an important role in the digestion process and are highly resistant to rifampin (12). Hence, the facts that (i) rifampin did not eradicate protozoan symbionts of *Z. angusticollis*, (ii) the body mass of *Z. angusticollis* reproductives was transiently affected (Table 2) and that of *R. flavipes* was unaffected, and (iii) the experimental replicates survived up to the 465- and 730-day censuses (for *Zootermopsis*) and the 150-day census (for *Reticulitermes*) while continuing to show a reproductive output biased against rifampin treatment do not support antibiotic toxicity, malnutrition, and/or starvation as a factor reducing fitness. Furthermore, endogenous production of cellulases has been reported in this insect order, and hence nutrition in termites may not be completely dependent on their protozoa communities (7, 25, 71, 72, 77).

Similar studies using antibiotics in the phylogenetically related roach *Periplaneta americana* resulted in poor growth and reduced reproductive output (54). These effects were attributed to the elimination of *Blattabacterium*, which mobilizes nitrogen from urine waste deposits within the fat tissue. It also provides vitamins, proteins, and essential amino acids to the roach (3, 4, 54, 59). Although *Z. angusticollis* lacks an association with *Blattabacterium* (59), other bacteria, including the rifampin-eliminated *Bacteroidetes* and *Treponema*, are similarly involved in nitrogen fixation (11, 13, 35, 43, 46) and/or the production of NH$_3$ from uric acid (52, 59, 66). The absence of these taxonomic groups may have irreversibly restricted nitrogen availability in female reproductives. Given that dietary nitrogen supplementation is known to significantly increase...
ovariole number and fecundity in Z. angusticollis neotenics and other insects (5, 10), the loss of the Bacteroidetes and Mollicutes may have compromised nitrogen reserves and/or the essential amino acids required for oogenesis. However, some Epsilon- and Gammaproteobacteria, two classes that were overly represented in the treated guts, may perform ammonification, denitrification, and nitrogen fixation (38, 46). Thus, further work is required to associate the fitness cost in treated termites with a shift in the ability to use nitrogen.

A second possible explanation for the long-term fitness costs associated with antibiotic treatment is that rifampin disrupted one or more mutualistic bacterial partnerships within the termite hosts, specifically, a partnership(s) that goes beyond the breakdown of cellulose. Given the long coevolutionary history between the gut symbionts and termites, it is likely that these social insects accrue additional benefits from their microbiota that are unrelated to cellulolytic activity. Microbes can play other important roles within their termite hosts, including detoxification (17), mediation of disease resistance and immune function (15, 23, 31, 51, 58, 60; K. F. Schultheis et al., unpublished data), production of volatile compounds that are coopted to function as aggregation or kin recognition pheromones and defensive secretions (2, 22, 24, 28, 39, 45, 47), and performance of atmospheric nitrogen fixation (5, 11, 35). Results from this work suggest that the microbial communities of Z. angusticollis and R. flavipes may also contribute to the fecundity of reproductives and ultimately to the successful establishment of colonies. One such candidate for affecting reproduction is Wolbachia piipientis, a widespread intracellular bacterium known to infect Z. angusticollis (9). However, based on PCR surveys of the Wolbachia wsp genes from antibiotic-treated and untreated reproductives, Wolbachia was not involved in influencing colony fitness, since all reproductives, nymphs, and eggs from both the experimental and control colonies harbored Wolbachia regardless of treatment and colony of origin.

The bacteria identified in our control animals have previously been associated with termite guts, either as normal symbionts (33, 34, 40, 48, 70) or as opportunistic pathogens (75; Table 1). The long-term fitness costs likely resulted from perturbations in the termite gut symbionts in treated termites. Rifampin is a bactericidal antibiotic that preferentially targets Gram-positive bacteria (78, 81). The consequence of employing this antibiotic is that it shifted the gut microbial community largely toward Gram-negative microorganisms, including known termite symbionts, i.e., termite group 1, Desulfovibrio spp., and Treponema spp. (Table 1).

The most striking change was the abundance of an epsilon-proteobacterium that was not represented in the control termite library. This bacterium’s 16S rRNA gene sequence is 98% similar to that of a rare symbiont of the termite luminal lining (34) and appears to have increased its proportional representation in the gut microbiota. At least two potential reasons for this shift in the dominant bacteria exist. First, the decline in Gram-positive bacteria may have allowed rare members of the community such as the Gram-negative epsilon proteobacterium to exploit the new, unoccupied niche space of the gut. Members of the rare biosphere potentially offer an unlimited source of microbial diversity that flourishes upon ecological perturbations (64). By altering the normal microbiota of the gut with antibiotics, rare but relatively fast-growing microaerophilic species (i.e., some proteobacteria and Serratia) not susceptible to the antibiotic may now exploit the host niche as well as the levels of available oxygen, ultimately overgrowing and becoming dominant in the gut (see references 14, 27, 41, 58, and 79 and references therein). Second, the appearance of rare or nonnative bacterial members in the rifampin-treated guts may be affected by interactions with other bacteria. For example, the Serratia marcescens 16S RNA gene sequence identified in our study is 99.9% identical to that of an opportunistic pathogen of termites that has been hypothesized to induce replication of normal termite gut bacteria by suppressing the host immunity, changing available oxygen, and producing bacterial growth-promoting enzymes such as carboxymethyl cellulase (1, 19, 68, 75). A Serratia-induced proliferation of the symbiotic community could cause septicemia, which can result in early termite mortality (75). S. marcescens is present in the rifampin-treated termite gut library but not in the control termite gut library. Thus, its appearance in the treated termites may have directly or indirectly led to the proliferation of the rare epsilon proteobacterium symbiont of the luminal lining (48). However, it is important to keep in mind that not all associations with Serratia are necessarily pathogenic. Serratia grimesii, for example, has been implicated as a source of folate compounds important to the maintenance of a functional hindgut microbiota of Z. angusticollis (29). The shift in gut bacterial population structure is strikingly prolonged, since termites were not fed antibiotics for ~50 days prior to dissections. The inability to return to a pretreatment microbial homeostasis (70), coupled with the acquisition of putative opportunistic pathogens and the low growth rates of many of these termite gut microorganisms (27, 30, 41, 42, 62), may help explain the prolonged effects that the antibiotic had on longevity and fecundity.

This study provides the first report of the long-term fitness consequences of disrupting the normal gut microbiota of termites. The long coevolutionary history of termites and their associated microbiota, coupled with the environmentally stable conditions inside their nests, lends itself to study of the nature and dynamics of symbiotic interactions (37). The mutualistic gut partnerships of social insects may not only affect the fitness of individuals but also have significant repercussions at the colony level. Symbionts, whether parasitic, commensal, or mutualistic, pose important selective pressures on their hosts. These host-microbe interactions likely influence the evolution of multiple host life history traits, including longevity, behavior, reproductive biology, immunity, and evolution and maintenance of sociality (see references 20, 37, 56, 61, 69, and 74 and references therein). Furthermore, the use of rifampin and/or other antibiotics has potential applicability for biological control of social insect pests. By disrupting the mutualistic interaction between termite hosts and their symbionts, better management practices for these social insect pests may be achieved without the environmental and ecological drawbacks typically associated with the use of other toxic chemicals.

**ACKNOWLEDGMENTS**

We thank the administrators of Huddart Park for allowing collection of termite colonies as well as Jessica Dumas, Larissa Gokool, Zea Schultz, Patrick Henrick, Brian Lejeune, and Troy Kieran for help with
performing censuses and establishing colonies. We also appreciate the helpful comments and suggestions of two anonymous reviewers.

This research was funded by the Louis Stokes Minority Program, which supported Jessica Dumas, by NSF career award DEB 0447316 to R. B. Rosengaus, and by NSF grant IOS-0852344 and NAI grant NNA04CC04A to S. R. Bordenstein.

REFERENCES


dence of the higher termite, Nasutitermes exitiosus and the lower termite, Coptotermes lacteus on their gut flora. J. Insect Physiol. 24:363–368.


27. Hughes, D. M., N. E. Pierce, and J. L. Boomsma. 2008. Social insect symbi-


29. Kudo, T. 2009. Termite-microbe symbiotic system and its efficient degrada-


31. Leadbetter, J. R., and J. A. Breznak. 1996. Physiological ecology of Metha-


33. Lombardo, M. 2008. Access to mutualistic endosymbiotic microbes: an un-


60. Scarborough, C. L., J. Ferrari, and H. C. J. Godfray. 2006. Microbial diversity in the deep sea and the under- }
66. Reference deleted.
71. Tokuda, G., H. Watanabe, and N. Lo. 2007. Does correlation of cellulase gene expression and cellulolytic activity in the gut of termite suggest synerg-}
78. Wierz, J. T., and J. A. Breznak. 2007. Stenoxbacter acetivorans gen. nov., sp. nov., an acetate-oxidizing obligate microaerophile among diverse \( \text{O}_2 \)-con-}