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Original Article

Harbouring *Blochmannia* incurs costs: a trade-off between the necessity of the obligate primary endosymbiont for brood development and its costs for adult carpenter ants (Hymenoptera: Formicidae)

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Abstract

All ants of the species-rich genus *Camponotus* ("carpenter ants") possess the obligate intracellular bacterial mutualist *Blochmannia*. We tested the relevance of the endosymbiont *Blochmannia* for offspring rearing using cross-fostering experiments between *Camponotus* sp. colonies, which were either treated with antibiotics to remove *Blochmannia* or untreated. Our antibiotic treatment reduced the level of *Blochmannia* endosymbionts in eggs, larvae, and workers significantly. Corroborating previous results, we found that eggs from treated colonies had a significantly reduced probability to develop into larvae and almost zero probability to become adults. Surprisingly, workers treated with antibiotics (symbiont-free workers) had a significantly higher success in raising their own and foreign eggs both from treated and untreated colonies than untreated workers. This indicates that the *Blochmannia* symbiosis entails substantial costs for the host in terms of brood rearing, that is, antibiotic-treated workers are more successful in brood rearing than symbiont-harbouring workers. If confirmed, this would be a case where the costs of a symbiosis can be empirically measured and quantified. Alternatively, the antibiotic treatment increased, as a side effect, the brood rearing effort of workers leading to the observed increase in brood rearing success of treated workers. But even if that would be the case, it still indicates that workers that have either lost or have a significantly reduced number of endosymbionts can still raise brood from antibiotic-treated and untreated colonies better than untreated workers. Thus, *Blochmannia,* although crucial for brood development in general, may reduce the amount of brood a colony can raise due to negative effects on ant workers.

Key words: Bacterial mutualist, symbiosis, brood-rearing success, antibiotics, cross-fostering, conflict.

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Introduction

Insects are one of the most successful animal taxa regarding species diversity and geographic distribution. Their evolutionary success might be facilitated by mutualistic relationships with intracellular bacterial endosymbionts (Feldhaar & al. 2007, Jackson & al. 2020), whereby mutualism is defined as a reciprocal and beneficial interaction between organisms (Barker & al. 2017). This mutualism ideally results in remarkable new capacities, which an organism would not be able to realise by itself (Buchner 1953).

The mutualistic relationship with intracellular endosymbionts of some insect species may have granted them access to novel ecological niches because of their tolerance to nutritionally deficient diets (Buchner 1953). This strategy might represent a driving force of insect evolution and a key to the success of these taxa (Boursaux-Eude & Gross 2000). Approximately 20% of all insect species are hosts to intracellular endosymbiotic bacteria, which provide them with essential nutrients that are deficient in the insects' diet (Baumann 2005). The endosymbionts

are tightly interconnected with their host's metabolic processes, for example, recycling of nitrogen and provisioning with vitamins and essential amino acids (Nogge 1981, Cochran 1985, Baumann & al. 1997, Douglas 1998, Heddi & al. 1999). Among Hymenoptera, only members of the family Formicidae (ants) are well-known for possessing endosymbionts in bacteriocytes (Schröder & al. 1996, Sauer & al. 2002, Zientz & al. 2005, Klein & al. 2016, Moreau 2020). These bacteriocytes are specialised cells, which are intercalated between midgut and ovaries and are closely linked to the host's development (Buchner 1953, Kolb 1959, Schröder & al. 1996, Sauer & al. 2000, 2002).

One of the best-studied mutualistic relationships between endosymbionts and ants occurs among the ant tribe Camponotini (carpenter ants) and the bacterium *Blochmannia*. These bacteria are obligate intracellular endosymbionts of ants and were first discovered in the genus *Camponotus* in 1887 by Friedrich Blochmann (Blochmann 1892). Preceding phylogenetic studies imply that these endosymbionts were first transferred horizontally from a group of secondary symbionts of mealybugs to the first common ancestor of the Camponotini about 51 million years ago (Gil & al. 2003, Wernegreen & al. 2009, Ward & al. 2016, Rafiqi & al. 2020). Furthermore, these bacteria are most closely related to the endosymbionts of aphids (*Buchnera*) and tsetse flies (*Wigglesworthia*), thereby belonging to the ɣ-subdivision of Proteobacteria (Aksoy & al. 1995, Baumann & al. 1995, Schröder & al. 1996, Chen & al. 1999, Sauer & al. 2000).

The *Blochmannia* genome contains genes for biosynthesis pathways for essential amino acids (except arginine) necessary for the host's development, especially for sclerotization, recycling of ammonia, and sulphate reduction (Hopkins & Kramer 1992, Zientz & al. 2004, Jackson & al. 2022). In contrast, biosynthesis pathways for non-essential amino acids have been lost (Gil & al. 2003, Zientz & al. 2004, Degnan & al. 2005, Jackson & al. 2022). This supports the hypothesis that the mutualism between host and *Blochmannia* has a nutritional basis (Feldhaar & al. 2007, Jackson & al. 2022). In turn, ants provide their endosymbionts with a protected environment within their bacteriocytes allowing a maternal transmission route of *Blochmannia* through the germline (Sauer & al. 2002, Stoll & al. 2009, 2010, Kupper & al. 2016, Ramalho & al. 2018).

General importance of *Blochmannia* for its host has been measured in preceding experiments when the colonies' success in raising brood has been used as a fitness measure. In contrast to earlier assumptions, treatment with antibiotics did not affect adult workers (Zientz & al. 2006). However, it dramatically impacts larval and pupal development: Ant groups treated with antibiotics had significantly reduced success in raising their own and foreign brood. The brood is fed via trophallaxis (Meurville & al. 2021), and it is assumed that food provided by adult workers is of lower quality due to the absence of endosymbiotic bacteria (Zientz & al. 2006).

Zientz and colleagues (Zientz & al. 2006) studied the relevance of *Blochmannia* for workers' ability to raise brood and found this symbiosis between ants and bacteria important for the development of the entire colony. Here, we extend their work by asking what effects the antibiotic treatment had on the brood itself, for example, fewer endosymbionts? As previously shown, bacterial transcriptional activity and bacterial genome copy number increases during development, peaks during pupation and young workers, and declines with age in adult ants (Wolschin & al. 2004, Zientz & al. 2006), with the greatest increase during embryogenesis and larval development (Sauer & al. 2002, Wolschin & al. 2004, Zientz & al. 2006, Bézier & al. 2009, Rafiqi & al. 2020). *Blochmannia* has co-speciated with its hosts, and the endosymbionts are transferred vertically (Buchner 1953, Kolb 1959, Schröder & al. 1996, Sauer & al. 2000). Hence, it could be that brood treated with antibiotics has a lower chance to develop into larval or pupal stage due to the reduced number of endosymbionts, additionally to the effect of lower food quality provided by *Blochmannia*-free adult workers. To answer this question, we conducted cross-fostering experiments and additionally tested if untreated colonies are able to raise brood from treated colonies (Zientz & al. 2006). We found that both workers from antibiotic-treated and untreated colonies (in the following referred to as "treated" and "untreated" workers, colonies or subcolonies, respectively) had lower success in raising brood from antibiotic-treated colonies than brood from untreated colonies (in the following referred to as "treated" and "untreated" brood or eggs, respectively). Surprisingly, and in contrast to observations by Zientz & al. (Zientz & al. 2006), treated workers were substantially better in raising brood from either treated or untreated colonies than untreated workers, indicating potential costs of the endosymbionts for the host in terms of brood rearing.

Materials and methods

Ant culture

Fourteen mated queens of *Camponotus* sp. were collected in the field from April to June 2018 in Comoé National Parc, Ivory Coast (8° 46' 11" N, 3° 47' 21" W). Within one year, each queen produced a nest with 150 - 200 workers. Colonies of *Camponotus* sp. were kept in artificial plaster nests in a climate chamber at the University of Münster, Germany, at 25 °C (20 – 8 h) and 29 °C (8 – 20 h), 60% humidity, and a 12 h day-night rhythm. They were fed ad libitum with honey water (50% honey, 50% water), half a cockroach (*Blaptica dubia*), and water twice a week. Voucher specimens of workers from the laboratory colonies have been submitted to the Museum für Naturkunde in Berlin, Germany.

Antibiotic treatment

To remove endosymbionts, half of the colonies (i.e., seven out of 14 colonies) were fed twice a week for 12 weeks with the antibiotic rifampicin (Serva Elektrophoresis

GmbH, Heidelberg, Germany), dissolved in honey water (50 / 50) at a final concentration of 1% (10 mg / ml).

DNA extraction and quantitative PCR (qPCR)

qPCR of bacterial DNA was used to quantify the *Blochmannia* levels in brood and workers (for PCR conditions, see Tab. S1, digital supplementary material to this article, at the journal's web pages). For each of the seven nests treated with antibiotics, 10 eggs (pooled sample) and two workers were collected before and after the treatment, for a total of 28 worker samples and 14 samples of 10 pooled eggs. DNA was extracted using the QIAGEN QUIAmp DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions, quantifying the amount of *Blochmannia* DNA using 16S ribosomal DNA (rDNA) primers (F 5'-AAACCCTGATGCAGCTATACCGTGTGTG-3', R 5'-CCATTGTAGCACGTTTGTAGCCCTACTCA-3'), and sequencing all samples at GATC (Eurofins Genomics, Cologne, Germany) to confirm that *Blochmannia* DNA was indeed extracted. As a control, 18S rDNA (F 5'-AGGCAGTTAARGAAATTCAA-3', R 5'-TATTGTCCAGWCAYTACGGGARKC-3') and 28S rDNA (F 5'-AAGCTAAVCAGAAAGCGGGGA-3', R 5'-AAAACCATTCGTCTTGACCRC-3') primers to the host's rDNA were used. qPCRs were performed using a KAPA SYBR FAST kit (Merck, Darmstadt, Germany), according to the manufacturer's instructions. In each qPCR, a 0.5 µl DNA template was used. Quantification was performed based on independent DNA preparations and was measured in duplicate. For these measurements, samples were taken from the colonies before and after antibiotic treatment, respectively. For this reason, the bacterial DNA did not originate from the same individual or egg before and after antibiotic treatment.

Cross-fostering approaches

Antibiotic treatment affected the raising of brood (Zientz & al. 2006). This might be due to (i) lower ability of nurses to raise brood, and / or (ii) less viable brood. To test hypotheses (i) and (ii), a full-factorial cross-fostering experiment was set up.

From each of the 14 colonies (seven treated with antibiotics, seven untreated), four queenless subcolonies containing 20 workers were separated, resulting in a total number of 28 subcolonies of 20 workers from untreated ("Untreated") and 28 subcolonies from antibiotic-treated mother colonies ("Treated"). Within four weeks, each subcolony received two times 20 eggs: once eggs from an antibiotic-treated colony ("Treated"), once from an untreated colony ("Untreated") (Tab. 1). To test whether workers would discriminate or prefer eggs of foreign colonies, also the acceptance of eggs from the own mother colony ("Same") were compared with eggs from different mothers ("Different").

Every day, the number of eggs, larvae, and pupae were counted for each subcolony, and pupae were removed from colonies. After 28 days, the first cross-fostering experiment (experiment 1) was terminated, and all brood was removed from the subcolonies. The next day, the experiment (experiment 2) was repeated, and 20 eggs were introduced from another colony and treatment. These two experiments will be referred to as replicates in the statistical analysis. Thereby, survival of the brood (also referred to as viability) was defined by the number of brood items (i.e., eggs, larvae, and pupae) that were still present in the nests after 28 days of experiment. The overall rearing success was measured by counting the number of pupae that developed from eggs to pupae within the 28 days of experiment.

Statistical analysis

All statistical analyses were performed using R version 4.3.0 (R Core Team 2020). Mann-Whitney-U-tests were used to analyse statistical differences in 16S rDNA before and after antibiotic treatment detected by qPCR. For analyses of differences in brood rearing success between no treatments and antibiotic treatments, generalized linear mixed models (GLMMs) were used with a logit link function (lme4 package, Bates & al. 2015), including random factors for the origin of ants and eggs used for cross-fostering experiments and a fixed factor for the

Tab. 1: Overview of the cross-fostering approach. *Same: eggs came from mother queen of the isolated workers; Different: eggs were from another queen, not related to the workers.

Group	Number colonies	Treatment subcolony	Experiment 1		Experiment 2	
			Treatment eggs	Origin eggs*	Treatment eggs	Origin eggs*
1	7	Untreated	Untreated	Same	Treated	Different
2	7	Untreated	Untreated	Different	Treated	Different
3	7	Untreated	Treated	Different	Untreated	Same
4	7	Untreated	Treated	Different	Untreated	Different
5	7	Treated	Treated	Same	Untreated	Different
6	7	Treated	Treated	Different	Untreated	Different
7	7	Treated	Untreated	Different	Treated	Same
8	7	Treated	Untreated	Different	Treated	Different

replicate. Additionally, a penalized maximum likelihood estimation (blme package, Chung & al. 2013) was used to account for potential problems of data separation (eggs to larvae survival) or overfitting (larvae to pupae survival), details are provided in the comments of the R-script. Output summaries of the best-fitting statistical models are provided in Table S2. To assess the quality of the model estimation, the DHARMA package (Hartig 2022) was used. Confidence intervals of the survival probabilities were computed via bootstrap. Statistical significance was determined to the significance level $\alpha = 0.05$. The R-scripts to run the analyses are attached as Appendix S1 and are additionally available in the following github repository: https://github.com/pczuppon/BlochmanniaStats.

Results

All adults and pooled egg samples were positive for *Blochmannia*. Treatment with antibiotics significantly reduced the amount of *Blochmannia* by about 55% in workers (Mann-Whitney-U-test, $p = 0.02622$, $U = 7$), and 99.98% in eggs (Mann-Whitney-U-test, $p < 0.001$, $U = 0$) (Fig. 1).

In general, eggs from colonies treated with antibiotics were less viable: Out of 20 eggs from treated colonies, on average only 6.4 (standard deviation (SD) = 5.01, n = 56) brood items (larvae, pupae) were recovered after the end of the experiments (four weeks). In contrast, 19.8 (SD = 0.93, n = 56) brood items were recovered from eggs that came from untreated colonies.

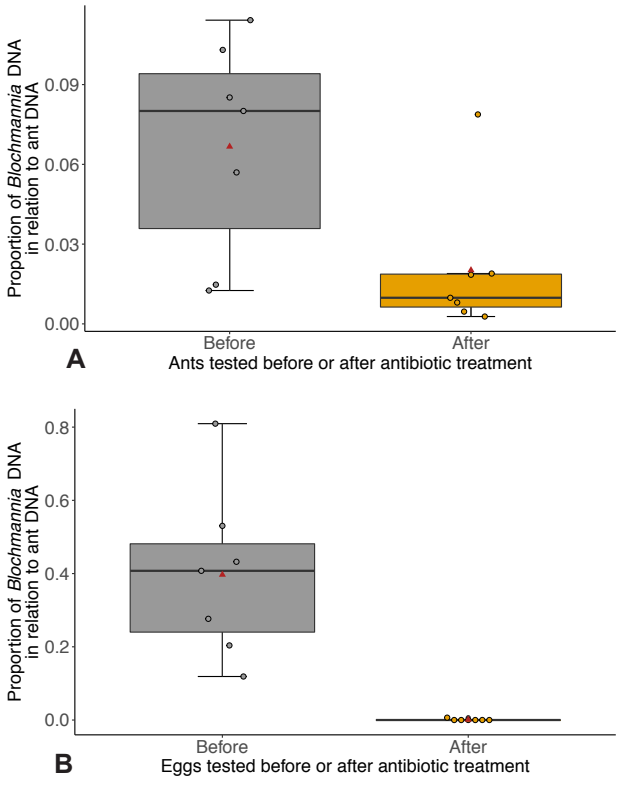

Fig. 1: Reduction of bacterial DNA content in ants (A) and eggs (B) after three months of antibiotic treatment. Boxplots show the median (thick horizontal line), mean (red triangle), interquartiles (box boundaries), and 1.5 times the interquartile range (whiskers). Standardization was based on DNA concentration of the geometric mean of 18S and 28S DNA in relation to the 16S DNA concentration (determined by qPCR).

There was a strong effect on survival depending on the ants attending the brood. At the end of both experiments, eggs (n = 20) from treated colonies, attended by treated workers, produced 9.4 (SD = 5.25, n = 28) brood items (= 47%), whereas only 3.3 (SD = 2.04, n = 28) brood items (= 16.5%) survived when tended by untreated workers (Fig. 2, Tab. 1).

For brood from untreated colonies, we did not find any difference in survival depending on the ants attending the brood: Ants treated with antibiotics kept all brood from untreated colonies alive (n = 28); whereas untreated ants kept on average 19.6 items alive (five out of 28 groups did not manage to keep all brood alive).

When raised by treated workers, 20.0 out of 20 (100%) (SD = 0.0) eggs from untreated colonies developed into larval stage, while on average 13.4 of 20 (67%) (SD = 4.68) eggs from treated colonies could be reared by those workers. In contrast, when raised by untreated workers, 19.6 of 20 (98%) (SD = 1.14) eggs from untreated colonies and 7.0 of 20 (35%) (SD = 4.45) eggs from treated queens became larvae.

During the experiments, eggs from treated queens had a lower chance to develop from eggs to pupae in comparison with eggs from untreated queens: In total, 1.3 (6.5%) (SD = 1.76) eggs from treated colonies and 18.3 out of 20 (91.5%) (SD = 1.46) eggs from untreated colonies reached pupal stage when tended by treated workers, whereas only 0.7 out of 20 (3.5%) (SD = 0.98) eggs from treated colonies and 8.3 (41.5%) (SD = 2.91) eggs from untreated colonies reached pupal stage when reared by untreated ants. Thus, treated ants were at least twice as successful as untreated ants in raising eggs from antibiotic-treated queens to pupae.

Workers from treated colonies were better at raising eggs to pupae than workers from untreated colonies. Taking the number of hatched larvae as a baseline, instead of the number of eggs at the beginning of the experiment, we observed that 11.1% of larvae deriving from eggs from treated colonies and 91.4% larvae from eggs from untreated colonies could be reared to pupal stage by treated workers. In contrast, only 6.6% larvae from treated and 42.2% of larvae from eggs from untreated colonies reached pupal stage when raised by untreated workers.

Overall, we detected statistically significant effects of the treatment of eggs and ants on the likelihood to develop from an egg to larval stage ($p < 0.001$, slope value = 5.45, standard error (SE) = 0.55, and $p = 0.002$, slope value = -1.66, SE = 0.52, respectively). Untreated ants reared eggs less successfully than treated ants, and eggs from untreated colonies had a higher probability to survive to the larval stage than eggs from treated colonies (Tab. 2).

For the probability to develop from larval to pupal stage, we found that the GLMMs with the treatment of eggs and ants as fixed effects did not match the observation

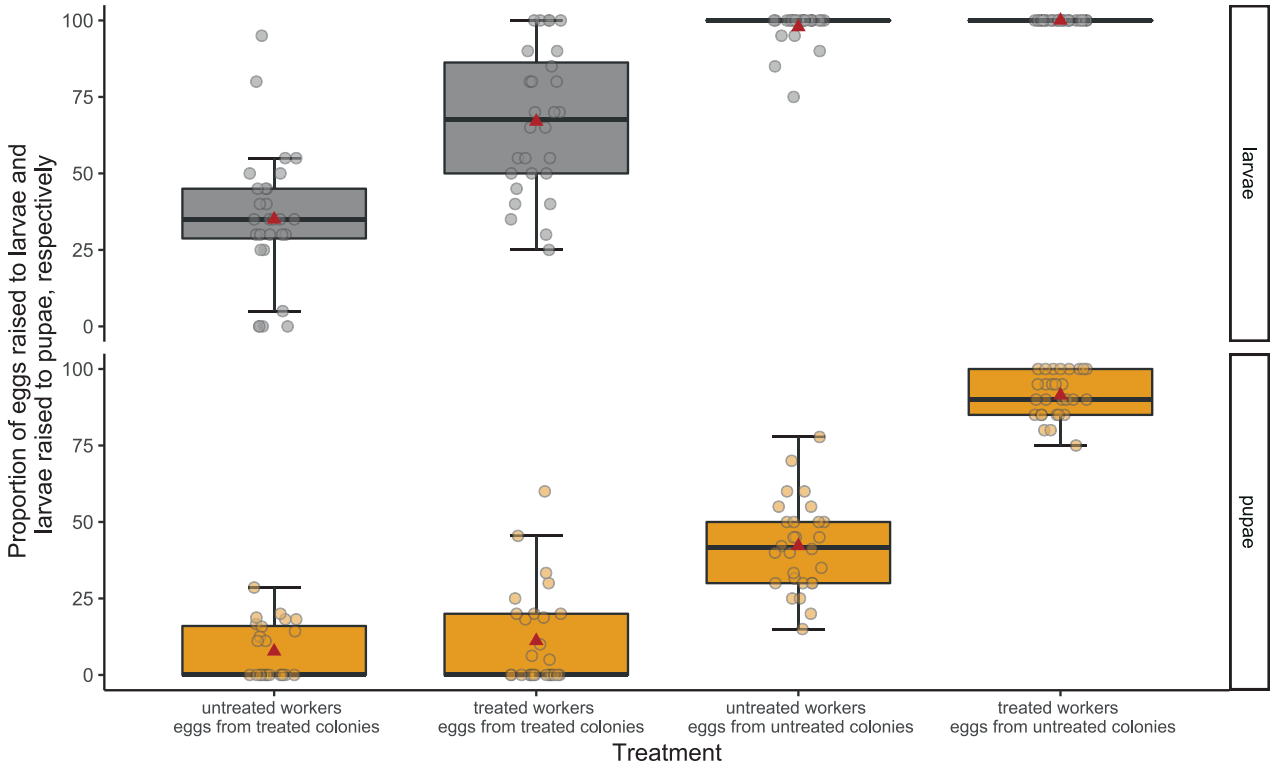

Fig. 2: Success rate of rearing eggs to larvae and pupae. Eggs from treated colonies develop less often into larvae and even less often into pupae. Treated worker ants are better in rearing eggs (from treated and untreated colonies) than untreated worker ants. Twenty eggs from treated and untreated colonies were taken care by 20 (un)treated workers (full factorial design). Each group of ants raised eggs twice, once from untreated and once from treated colonies. Treatment consisted of antibiotic supplement (rifampicin) in honey water for 90 days. Boxplots show median (thick horizontal lines), mean (red triangles), interquartiles (box boundaries), and 1.5 times the interquartile range (whiskers).

Tab. 2: Estimated development probabilities per treatment. Confidence intervals (95%) are stated in brackets and were obtained by bootstrapping the data 1000 times. Values are averaged over the two replicate estimates per bootstrap.

Colonies (egg source)	Ant workers	Development probability	
		Eggs to larvae	Larvae to pupae
Treated	Untreated	0.33 (0.17, 0.51)	0.10 (0.06, 0.15)
	Treated	0.71 (0.51, 0.85)	0.10 (0.07, 0.13)
Untreated	Untreated	0.99 (0.97, 1.00)	0.42 (0.37, 0.48)
	Treated	1.00 (1.00, 1.00)	0.92 (0.89, 0.94)

(diagnostic Figs. S1, S2). We therefore included an interaction between the treatment of ants and treatment of eggs, which explained the data better (Akaike information criterion (AIC) without interaction = 441, AIC with interaction = 391). This statistical model showed that the treatment of eggs significantly affects the likelihood to develop from larval to pupal stage ($p < 0.001$, slope value = 4.68, SE = 0.25). The simple effect of treatment of ants was not statistically significant ($p = 0.836$, slope value = 0.03, SE = 0.32), but the interaction between the treatment of eggs and ants was ($p < 0.001$, slope value = -2.78, SE = 0.35). Larvae that emerged from eggs from untreated colonies had a much higher chance to reach pupal stage compared with eggs from treated colonies (Tab. 2). However, eggs from untreated colonies that were reared by untreated ants had a strongly decreased chance to develop from larva to pupae compared with eggs from untreated colonies that were reared by treated ants (Tab. 2).

We included random effects for the origin of the eggs and ants used for the experiment, to account for potential variation between the colonies (origin of eggs and ants). Interestingly, we found that the origin of the eggs and ants had a much stronger effect on the likelihood to develop from eggs to larvae than from larvae to pupae, as visible by comparing the respective cumulative variances, that is, the sum of the estimated variances for egg and ant origin:

egg to larvae = 1.33, larvae to pupae = 0.05 (for more details, we refer to Tab. S2). The replicate was included as a fixed effect because of the low number of levels of this factor (two experiments). We did this to account for potential temporal variation during the experiment. Indeed, the replicates showed non-negligible variation in the survival rates. The probability to reach the larval stage was decreased in replicate 2 (slope value = -0.73, SE = 0.14), yet the probability to reach the pupal stage once the larvae hatched was increased in replicate 2 (slope value = 0.61, SE = 0.14).

Discussion

The aim of this study was to investigate the role of the endosymbiotic bacteria *Blochmannia* sp. for brood development in *Camponotus* sp. carpenter ants using a cross-fostering experiment with ant nurses and eggs of untreated and treated colonies. Antibiotic treatment reduced the endosymbiont levels in workers and eggs substantially, which was confirmed by qPCR.

The cross-fostering experiments confirmed previous results that *Blochmannia* is relevant for brood development, since brood with reduced *Blochmannia* titres had a higher egg mortality than brood containing endosymbionts (Sauer & al. 2002, Wolschin & al. 2004, Feldhaar & al. 2007). In particular, these endosymbionts selectively regulate germline genes in early development stages of their hosts to successfully integrate biosynthesis pathways and facilitate its vertical transfer (Wernegreen & al. 2009, Rafiqi & al. 2020, Jackson & al. 2022). By contrast, the frequency of *Blochmannia* decreases in older workers and the endosymbionts seem to play a minor role for adult ants (Wolschin & al. 2004). This conclusion was also reached by other studies, which also observed degeneration of endosymbionts in adult workers in several Camponotini and linked this to the importance of these endosymbionts in the early stages of host development and decreased importance in the adult stages (Kolb 1959, Sauer & al. 2002).

Surprisingly, we found that treated workers are more successful in raising larvae to pupae than untreated workers, regardless of the origin of the brood. In contrast, there was no effect of the ants on the hatching success of the eggs. During the egg stage, workers provide merely hygienic services and maintain favourable climate conditions but do not provide resources for the egg. However, our statistical model shows a significant interaction between ant origin and the pupation rate. This seems reasonable, given that workers exchange food and information with larvae via trophallaxis.

Overall, our results suggest that the symbiosis between *Blochmannia* and Camponotini is more complex than previously thought, revealing a potential trade-off between (i) benefits and (ii) costs for both endosymbionts and hosts at different developmental stages in this symbiotic relationship:

(i) *Blochmannia* provides access to nutrients necessary for host development (Hopkins & Kramer 1992, Zientz & al. 2004, Jackson & al. 2022), while the ants' bacteriocytes provide their endosymbionts with a protected environment (Sauer & al. 2002, Kupper & al. 2016, Ramalho & al. 2018).

(ii) Workers still harbouring *Blochmannia* are less successful in raising brood, possibly due to negative effects on body conditions (resource drainage) in adult life stages or by increasing the susceptibility to pathogen infections and affecting the host's immune response (Sinotte & al. 2018). In fact, untreated workers still harbouring *Blochmannia* have raised only half as many brood items as (treated) workers without *Blochmannia* – an observation that we made in all 56 worker-only subcolonies in our study which were provided with 20 brood items each. This effect was already described for other endosymbiont-host-relationships: In sap-feeding insects, Zytynska & al. (2019) showed that hosting facultative endosymbionts, on the one hand, can incur costs due to reduced longevity and fecundity and increased development time, but on the other hand provides also benefits like increased resistance to parasitic wasps. In particular, many aphids harbour facultative endosymbionts (Buchner 1953, Fukatsu & Ishikawa 1993, Fukatsu & al. 1998), which are known for providing heat tolerance (Burke & al. 2009, Brumin & al. 2011), host plant adaptations (Tsuchida & al. 2004, Wagner & al. 2015), and resistance to parasitoid attacks by causing high mortality rates of parasitoid larvae (Oliver & al. 2003, Hendry & al. 2014). Nevertheless, in the absence of natural enemies, the frequency of the inherited endosymbionts decreased, indicating a potential cost of hosting these endosymbionts (Oliver & al. 2008). Gwynn & al. (2005) attributed these findings to a fecundity cost of hosting these facultative endosymbionts resulting in fewer offspring indicating a trade-off between survival and immunity of the host and fecundity due to the presence of the endosymbionts. Furthermore, the facultative endosymbiont *Hamiltonella defensa* of the black bean aphid, *Aphis fabae*, even shortens the longevity of its host causing lower lifetime reproduction rates (Vorburger & Gouskov 2011).

These fitness costs can be associated with the endosymbionts' consumption of the host's nutrients resulting in a nutritional sink, except when the endosymbionts are capable of recycling host by-products (Ankrah & al. 2018). This assumption of a high energetic demand of endosymbionts has already been shown several times for *Drosophila* and aphids, where the presence of a facultative endosymbiont reduced host fitness (Fry & Rand 2004, Weldon & al. 2013, Cayetano & al. 2015, Leclair & al. 2017, Parker 2021). We suspect the same effect in *Blochmannia* (see Sinotte & al. 2018), but in contrast to the studies on facultative endosymbionts, in the Camponotini ants, the obligate endosymbionts are essential during brood development (Hopkins & Kramer 1992, Zientz & al. 2004, Jackson & al. 2022). Therefore, the fitness costs of hosting endosymbionts changes in the Camponotini ants over time. We assume that the ant's immune system aims to actively downregulate the number

of *Blochmannia* after moulting, additionally requiring energy for a proper immune response as shown also for facultative endosymbionts (Ardia & al. 2012, Sinotte & al. 2018, Dolezal & al. 2019, Blow & al. 2020), revealing a coevolutionary process (arms race or trade-offs) between host and endosymbiont (Gwynn & al. 2005, Ratzka & al. 2011, Sinotte & al. 2018, Mathé-Hubert & al. 2019). By downregulating *Blochmannia* in their bacteriocytes, adult workers are more successful in raising brood. This benefits both, the host and the endosymbiont, since *Blochmannia* is transmitted maternally (and workers do not reproduce), and thus ultimately can only spread if its host reproduces which depends on colony size (Schröder & al. 1996, Sauer & al. 2000). *Camponotus* sp. ants reproduce via the production of winged queens and males that leave a colony to mate and found a new colony independently (Hansen & Akre 1991). *Blochmannia* endosymbionts are only transmitted via the queens, and the larger the workforce of a colony, the more queens it can produce (Schröder & al. 1996, Sauer & al. 2000). Therefore, it would also be in the endosymbiont's interest, not just the host's, to reduce its prevalence in adult worker ants, which are in any case a dead end in terms of propagation for the endosymbiont. That *Blochmannia* are not reduced completely in adult workers may be because the timing and / or mechanism to reduce *Blochmannia* in adult workers is hard to evolve because the life span of adult ant workers is usually quite short and individual workers are only involved in brood care for part of their live (Hölldobler & Wilson 1990). Another possibility is that adult ants still benefit from low levels of *Blochmannia* due to their ability to recycle nitrogen from urea (Zientz & al. 2004, Feldhaar & al. 2007).

We therefore consider the *Camponotus-Blochmannia* system as a dynamic symbiosis between endosymbionts and hosts that is more complex than previously appreciated (Rafiqi & al. 2022). Further analyses of brood care, immune responses at different developmental stages, and selection pressures are necessary to improve our understanding of the evolutionary processes and different selection pressures that govern the interactions between the endosymbiont *Blochmannia* and its hosts *Camponotus* spp.

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Competing interests

We declare we have no competing interests. URE was partially funded by the German Research Foundation (DFG) as part of the SFB TRR 212 (NC³) project no. 316099922.

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