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Evolutionary biology

Reduced cellular immune response in social insect lineages

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Social living poses challenges for individual fitness because of the increased risk of disease transmission among conspecifics. Despite this challenge, sociality is an evolutionarily successful lifestyle, occurring in the most abundant and diverse group of organisms on earth—the social insects. Two contrasting hypotheses predict the evolutionary consequences of sociality on immune systems. The social group hypothesis posits that sociality leads to stronger individual immune systems because of the higher risk of disease transmission in social species. By contrast, the relaxed selection hypothesis proposes that social species have evolved behavioural immune defences that lower disease risk within the group, resulting in lower immunity at the individual level. We tested these hypotheses by measuring the encapsulation response in 11 eusocial and non-eusocial insect lineages. We built phylogenetic mixed linear models to investigate the effect of behaviour, colony size and body size on cellular immune response. We found a significantly negative effect of colony size on encapsulation response (Markov chain Monte Carlo generalized linear mixed model (mcmcGLMM) $p < 0.05$; phylogenetic generalized least squares (PGLS) $p < 0.05$). Our findings suggest that insects living in large societies may rely more on behavioural mechanisms, such as hygienic behaviours, than on immune function to reduce the risk of disease transmission among nest-mates.

1. Introduction

A key challenge of living in large societies is dealing with pathogens and parasites, which is as true for primates, including humans, as it is for naked mole rats or social insects. Because of crowded living conditions and frequent physical interactions, social living makes individuals potentially more vulnerable to socially transmitted pathogens and the diseases they cause [1]. For example, since the origin of agriculture and large settlements, diseases—from the bubonic plague to HIV—have threatened human populations [2]. However, humans have controlled the risk and spread of some diseases with the development of public health, antibiotics and vaccination [3]. Insect societies also confront and respond to strong selective pressures from diseases [4], worsened by the high genetic relatedness among nest-mates that enables pathogens and parasites to easily sweep through a host insect colony [5]. Even though social insects have been remarkably successful ecologically and evolutionarily [6], the mechanisms behind their ability to resist disease remain enigmatic.

Heightened individual immunity could combat disease risk in social insects, as purported in hominid evolution [7]. However, increased immune function can be costly whether because of metabolic costs or, as is the case in humans, because of the risk of immune overreaction to host tissue [8,9]. These costs lead to potential evolutionary trade-offs between immune function and other functionally important traits—such as metabolism or reproduction—that could reduce individual fitness and colony productivity [10–12]. In the light of these trade-offs, selection

might favour less metabolically expensive 'behavioural immunity' in social insects who construct nests with antimicrobial properties, exhibit auto- and allogrooming, and many other behaviours that reduce disease transmission and the likelihood of infection [13]. Such behaviours of 'social immunity' may be a key adaptive response to compensate for the increasing risk of disease associated with more frequent interactions among nest-mates as colony size increases [14].

Two hypotheses predict how individual immune systems should respond to the evolution of sociality and larger colony sizes. The social group hypothesis (SGH) argues that social insects might evolve more heightened immune responses than solitary organisms, despite their behavioural immunity [14]. Alternatively, the relaxed selection hypothesis (RSH) predicts that behavioural immunity reduces disease risk such that costly internal immune functions are maintained at lower levels [14–16]. A direct test of these hypotheses would measure the investment in physiological and behavioural immunity as a function of sociality or colony size.

Here, we tested the SGH and RSH by contrasting cellular immune function across multiple insect lineages. We quantified encapsulation response, an important part of the innate immune system in insects, in which haemocytes bind to large parasites after they have entered the host body to immobilize and kill them. Finding higher encapsulation in social insect lineages would support the SGH, whereas higher encapsulation response in solitary species would support the RSH. We used phylogenetic generalized linear mixed models to evaluate the effects of behaviour (eusocial or not), maximum colony size, incubation temperature and body size on the cellular immune response of diverse species of insects.

2. Material and methods

We collected specimens from 11 insect lineages, including bees, wasps, ants, termites and cockroaches, for the immune assays (figure 1). Live individuals were immunologically challenged using 3 mm nylon microfilaments coated with lipopolysaccharide, to induce an immune response, and inserted in the specimen abdomen using sterile technique [17,18]. Individuals were anaesthetized on ice to immobilize them and minimize stress during the procedure. After the probes were introduced in the insect bodies, individuals were incubated for a 4 h period in two *temperature treatments* (25°C and 35°C). Then, we removed the probes and photographed them under a scope Nikon SMZ 800 using consistent light and camera settings. Encapsulation density was quantified from the melanin deposition on the probe as the mean greyness of the nylon microfilament through image analyses in the software ImageJ [19] (electronic supplementary material, appendix S1).

Lineages were coded as 'eusocial' if they exhibited a reproductive division of labour and overlapping generations (electronic supplementary material, appendix S2). However, this binary classification of *eusociality* is a simplification of the variability in social complexity between the focal species. Therefore, we included *colony size* as a secondary predictor variable of immune response in our analysis with the assumption that species with larger colonies tend to be at increased risk from socially transmitted pathogens. To control for differences in *body size* across the different lineages, we measured body length of each taxa and included this continuous variable in our model. Colony size was log transformed to normalize the data and improve their fit to our model.

To quantify the phylogenetic signal in our dataset, we calculated lambda for the two continuous variables in our dataset: *colony size* and *body size*. To account for the non-independent evolutionary history among the 11 lineages in our dataset, we

calculated the inverse phylogenetic covariance matrix with the function *inverseA* in 'ape' [20], after building a maximum likelihood phylogeny using seven genes (electronic supplementary material, appendix S3). This pairwise phylogenetic matrix was incorporated as a random effect in a multivariate generalized linear mixed model using the R package 'MCMCglmm' [21] (electronic supplementary material, appendix S3). For this analysis, we estimated the effect of sociality, colony size, temperature and body size on encapsulation response. The Heidelberg and Welch diagnostic was used to corroborate convergence of the MCMC runs. We used Deviance Information Criterion (DIC) to determine the models that best explain the variability in immune function across the focal lineages. Interaction terms between factors were also assessed. We used an ordinary generalized least-square (GLS) model and a phylogenetic GLS with Pagel's 'lambda' correlation structure using the R package 'ape' [20] to quantify the effect of the predictor variables on encapsulation response (electronic supplementary material, appendix S3).

3. Results

We detected that encapsulation response was lower in insect species with larger colony size using the MCMCglmm (posterior mean = -4.93 ; 95% CI = $[-10.20, -0.05]$) and PGLS approaches (-2.55 , $p = 0.037$). This corresponds to a 2% change in encapsulation response (mean greyness) $[-4.93$; CI = $-10.20, -0.049]$ with every 10-fold increase in colony size. The high lambda values detected for the continuous variables (colony size $\lambda = 0.78$; body size $\lambda = 0.77$) and the lower Akaike Information Criterion (AIC) values for phylogenetically corrected models suggest that the best models included phylogeny fitted as a random effect (table 1). Encapsulation response was not significantly lower in eusocial than in non-eusocial lineages (posterior mean = 17.84 ; 95% CI = $[-5.03, 40.04]$; figure 1). However, DIC values indicated that colony size, eusociality and body size similarly contributed to the variability in encapsulation response. We found a significant effect of body size in the PGLS model (2.89 ; $p = 0.043$) but no significant interaction was detected between body size and other variables (table 1). No significant effect of temperature (posterior mean = -3.52 ; 95% CI = $[-10.21, 3.40]$) was found on encapsulation response (table 1).

4. Discussion

Our results suggest that encapsulation response of social insects decreases with increasing colony size, in support of RSH. Even though the effect size we found was small $[-4.93$; CI = $-10.20, -0.049]$, we detected reduced encapsulation in species with larger colonies after correcting for phylogeny. The significantly negative association between colony size and encapsulation response suggests that species with larger colonies rely more heavily on behavioural immune mechanisms to decrease disease transmission among group members [22]. Other aspects of 'social behaviour' that do not relate to colony size may be more subtle than we could capture in this categorical metric [23].

The lower number of immune-related genes in the genome of *Apis mellifera*, compared with dipterans and other hymenopterans, support predictions from RSH [15]. However, new evidence suggests that the reduced repertoire of immune genes in *A. mellifera* precedes the evolution of sociality in bees [24], a complexity not yet well accounted for. Nonetheless, the accelerated rates of evolution in immune

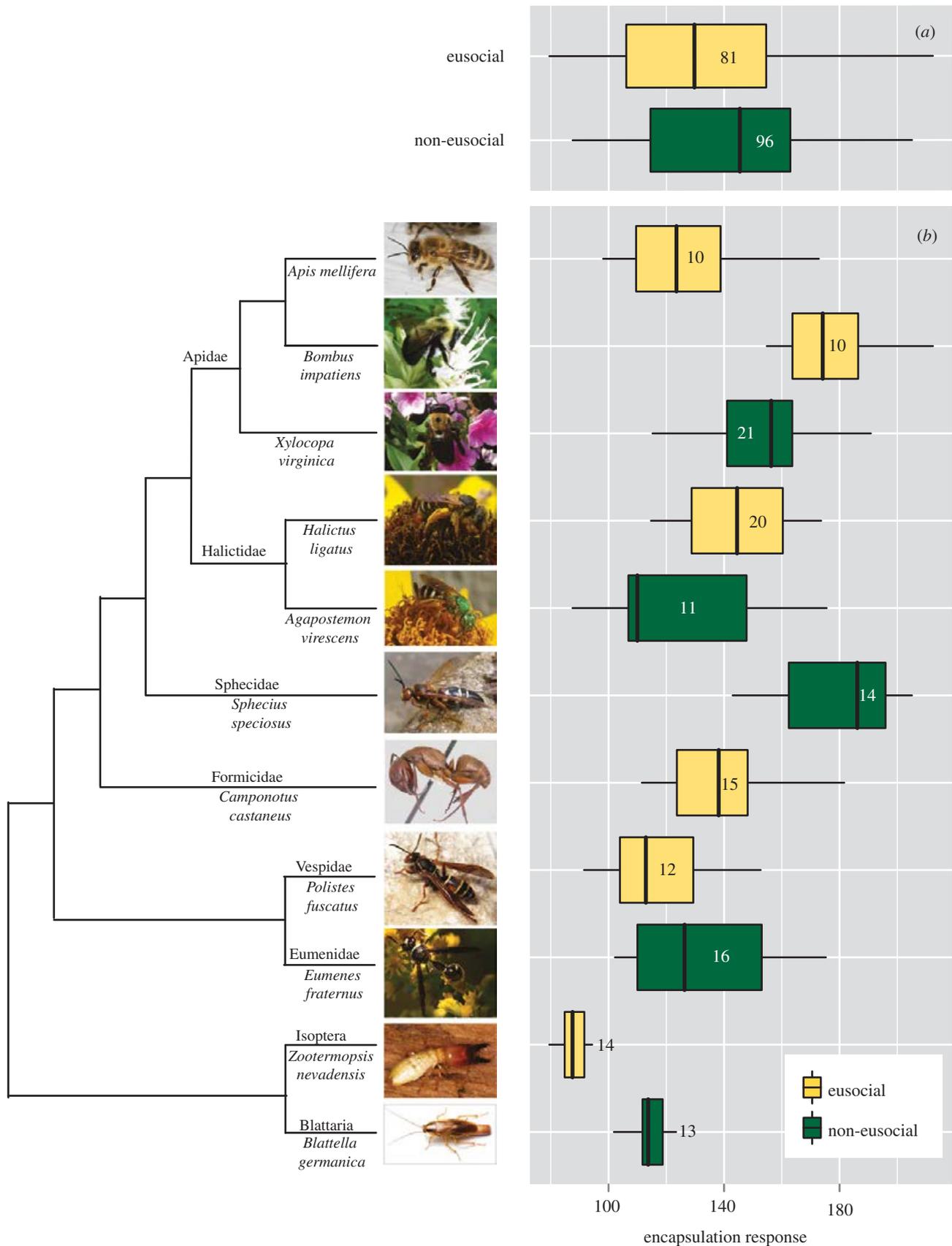


Figure 1. Levels of encapsulation response quantified in eusocial and non-eusocial insect lineages. (a) Boxplot of the encapsulation response in eusocial (yellow) and non-eusocial (green) lineages. (b) Boxplot of the encapsulation response across the sampled insect lineages. Numbers in boxplots show sample size. Colony size, not eusociality, had a significantly negative effect on encapsulation response. See main text for details. Photo credits: *Apis mellifera* (Wikipedia), *Bombus impatiens* (Elsa Youngsteadt), *Xylocopa virginica* (Margarita M. López-Uribe), *Halictus ligatus* (Elsa Youngsteadt), *Agapostemon virescens* (Wikipedia), *Sphecius speciosus* (Elsa Youngsteadt), *Camponotus castaneus* (Wikipedia), *Polistes fuscatus* (Wikipedia), *Eumenes fraternus* (Wikipedia), *Zootermopsis nevadensis* (Will Chatfield-Taylor) and *Blattella germanica* (Matt Bertone).

genes of honeybees and ants also suggest that immune systems are under relaxed selection in social insect lineages, as supported by Harpur & Zayed [15]. By contrast, evidence

from external immunity—immune defence mediated by external non-immunological antimicrobial compounds—shows the opposite trend. A study comparing the strength

Table 1. Statistical models showing coefficients and significance (in parenthesis) of main effects and interactions from the MCMCglmm and PGLS approaches. Significant terms are shown in bold. DIC and AICc values are reported for MCMCglmm and PGLS models, respectively.

	sociality	colony size	body size	temperature
MCMC generalized linear mixed model				
main effect	17.84 (−5.03, 40.04)	−4.93 (−10.20, −0.049)	14.16 (3.27, 25.58)	−3.52 (−10.21, 3.40)
interaction with body size	−6.57 (−29.21, 15.17)	0.81 (−4.99, 6.83)	—	—
interaction with colony size	−92.34 (−471.46, 233.13)	—	—	—
phylogenetic generalized least squares				
main effect	15.27 (0.273)	−2.55 (0.037)	2.89 (0.043)	—
interaction with body size	1.19 (0.675)	0.053 (0.934)	—	—
interaction with colony size	−3.51980 (0.6850)	—	—	—
models of trait evolution				
DIC (independent)	1409.102	1409.286	1409.608	1410.107
DIC (Pagel)	1409.102	1409.005	1409.608	1410.354
AIC (independent)	107.379	107.434	111.164	—
AIC (Pagel)	108.481	103.829	105.377	—
Pagel's lambda	—	0.787	0.771	—

of external antimicrobial secretions between solitary, semi-social and social bees found evidence of progressively stronger antimicrobial activity in species with higher levels of sociality [25]. Stronger antimicrobial activity is a form of heightened immune response [26], as expected under SGH, and suggests that selection is strongly acting on external immune defences in social insects. Internal and external immune responses appear to be under different selective pressures.

In our analyses, variation within species in encapsulation response accounted for a large proportion of the variation present in our study (figure 1). Large heterogeneity in immune response within taxa may result from background differences in immune levels of wild individuals, as a result of age or previous immune challenges. However, we still found significant differences among taxa suggesting that species-specific selective pressures play key roles in immune systems. We did not detect a significant effect of temperature on encapsulation. Suboptimal environmental temperature is often predicted to decrease immune function in insects [27] but temperature can interact with other environmental factors, such as nutrition or population density, resulting in nonlinear responses [28].

Overall, our results suggest that encapsulation response is reduced in social lineages with larger colonies, supporting

the RSH hypothesis that social insects rely on behavioural immunity to reduce disease transmission. These findings help elucidate how sociality and colony-size drive selection on different aspects of immune systems in insects.

Ethics. During field collections and encapsulation experiments, we minimized handling time and stress of individuals as much as possible. Procedures used in this study were based on standard protocols for this type of immune assays.

Data accessibility. Files for raw encapsulation data, seven-gene alignment for phylogenetic reconstruction and maximum-likelihood estimated phylogeny are available from Dryad: <http://dx.doi.org/10.5061/dryad.sj066>.

Authors' contributions. M.M.L.-U., S.D.F., R.R.D and D.R.T. designed the experiment; M.M.L.-U. and W.B.S. conducted the experiment; M.M.L.-U. performed the data analysis and wrote the manuscript; all authors revised, approved the manuscript and are accountable for the work herein.

Competing interests. We declare no conflict of interest.

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