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Chapter 3

The individuality of a colony

Title: Effect of food restriction on survival and reproduction of a termite

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Abstract

Dietary restriction is well-known to affect the trade-off between maintenance and reproduction in a wide range of organisms, but its effects on social insects remain poorly understood. In social insects, the maintenance-reproduction trade-off seems absent in individuals but may appear at the colony level, although this is rarely investigated. In this study, we restricted food availability in a termite species with low social complexity to investigate how it affects survival and reproduction, both at the individual and colony level. Using Bayesian multivariate response models, we found minor negative effects of food restriction on the survival of individual queens and workers as well as on the colonies. In contrast, queen fecundity was significantly reduced while colony-level fecundity (i.e., the number of dispersing alates, future reproductives) increased under food restriction. Workers gave up cooperation within the colony and became alates that dispersed. Thus, there seems to be a re-allocation of resources from maintenance (i.e., workers in the nest) to

reproduction (i.e., workers developing into alates), suggesting a colony-level trade-off between maintenance and reproduction. We argue that non-superorganismal social insects with low degrees of sociality are analogous to organisms with low biological complexity, such as hydra, without a clear separation between germline and soma.

Impact summary

A social life modulates ageing and its associated life history trade-offs such as that between fecundity and longevity. But how and why this happens is less understood. We performed a dietary restriction experiment in a termite of low social complexity to test whether the survival of individuals increased at the cost of fecundity, as has been shown in solitary organisms. We detected a significant decrease in queen fecundity while workers gave up cooperation and developed into winged sexuals and dispersed. The survival of individuals was merely affected. Our study shows how sociality modulates ageing and life-history trade-offs with food restriction not only affecting fecundity and longevity but also social behaviour. It reveals striking analogies between insect societies with low social complexity and *Hydra*, a multicellular animal of low biological complexity.

3.1 Introduction

Resource availability plays an important role in the regulation of ageing (Kirkwood, 1977, 2017; Gavrilov & Gavrilova, 2002). In particular, restricted food intake without malnutrition (i.e., dietary restriction, DR) can extend longevity in many organisms (Partridge *et al.*, 2005; Houthoofd & Vanfleteren, 2006; Kaeberlein *et al.*, 2006; Longo & Mattson, 2014; Redman *et al.*, 2018; Savola *et al.*, 2021) and generally reduce fecundity across taxa (Moatt *et al.*, 2016). Currently, the majority of DR studies have been done in a small number of classical model organisms such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* (Moatt *et al.*, 2016). During the past two decades, social insects have gained considerable interest to study the causes of senescence because of the apparent absence of the longevity-reproduction trade-off in queens (and kings in termites) that are both extremely long-lived and highly fertile. In social insects, such as ants, bees, and termites, the effects of food manipulation on ageing have been less investigated. Only a few DR studies exist in social Hymenoptera (Rueppell *et al.*, 2007; Altaye *et al.*, 2010; Dussutour & Simpson, 2012; Negroni *et al.*, 2021) and only two in termites (Korb & Linsenmair, 2001; Lo Pinto & Agrò, 2019). More DR studies using social insects are needed to understand how a social life modulates ageing.

Social insects are characterised by a reproductive division of labour; only one or a few queens (and kings in termites) reproduce, while the other colony members (workers and sometimes soldiers) help to feed and maintain the colony. The reproductive division of labour resembles the separation of cells into germline and soma within multicellular organisms. Indeed, social insect colonies have been regarded as superorganisms (i.e., high superorganismality) when they have sterile workers (e.g., *Monomorium* ants, higher termites) (Korb & Heinze, 2016; Bernadou *et al.*, 2021). Not being able to reproduce, sterile workers can only gain indirect fitness by increasing the reproductive success of their non-sterile relatives. In that sense, they represent the “soma”, maintaining “homeostasis” within their colony and promoting the reproduction of the “germline” which is represented by the

queens and kings. Workers propagate copies of their genes by promoting the success of the colony, just like the soma propagates copies of its genes by promoting the success of the organism. Colonies of social insects, in which workers can and do reproduce, also show some superorganismal features, but they should not be classified as *bona fide* superorganisms (Korb & Heinze, 2016; Bernadou *et al.*, 2021). Instead, they can be categorised as species with low or intermediate superorganismality (Korb & Heinze, 2016; Bernadou *et al.*, 2021). Species of low superorganismality have workers that can develop into sexuals (e.g., Polistes wasps, wood-dwelling termites). They are characterised by small colonies of a few hundred workers. Species of intermediate superorganismality have workers that have reduced reproductive options but still regularly reproduce (e.g., stingless bees, Reticulitermes termites).

So far, the limited number of DR studies in social insects that we know of have all focussed on species with intermediate or high superorganismality (Korb & Linsenmair, 2001; Rueppell *et al.*, 2007; Altaye *et al.*, 2010; Poissonnier *et al.*, 2018; Negroni *et al.*, 2021). Studies on species with low superorganismality are lacking. Including studies on species with low superorganismality is important as the patterns of ageing vary systematically across social insects with different levels of social complexity/superorganismality (Korb & Thorne, 2017; Kramer *et al.*, 2022). Note, we use social complexity and superorganismality interchangeable as they strongly correlate; the former being more familiar and the latter focussing on the organism analogy (Queller & Strassmann, 2009; Korb & Heinze, 2016). Thus, we might observe different DR responses depending on the degree of superorganismality.

In this study, we restricted food availability in the termite *Cryptotermes secundus* (*Kalotermitidae*) in order to test how it affects the survival and reproduction of individuals and colonies. *C. secundus* feed exclusively on a piece of wood that also serves as the colonies' shelter. They continuously sense wood availability via vibrations generated during gnawing on the wood (Evans *et al.*, 2005) and workers adjust developmental trajectories almost

immediately when wood availability is reduced (Korb & Lenz, 2004; Korb & Schmidinger, 2004). As is typical for such wood-dwelling termites, *C. secundus* has low superorganismality; the workers are totipotent immatures that develop into replacement reproductives that inherit the nest when the natal queen or king dies or into dispersing reproductives (alates). To gain a more comprehensive understanding of how food manipulation affects the longevity-reproduction trade-off, survival and fecundity were measured at both the individual and colony level (for detailed definitions, see **Table 3.1**).

3.2 Methods

Experiment

Sample collection, transportation, colony maintenance, and the experimental setup were performed as previously described (Korb & Lenz, 2004; Korb & Schmidinger, 2004; Weil *et al.*, 2007). Briefly, *C. secundus* colonies were collected from dead *Ceriops tagal* trees in Darwin, Northern Territory, Australia (12°30'S 131°00'E) and kept in *Pinus radiata* wood blocks under lab conditions in Germany (28 °C, 70% relative humidity and a 12 h day/night cycle). This setup allows colonies to develop as in the wild (Korb & Lenz, 2004; Korb & Schmidinger, 2004). Food availability (which *C. secundus* can sense via vibrations; Evans *et al.*, 2005) was manipulated by adjusting the size of wood blocks (Korb & Lenz, 2004). 14 low-food colonies (1 termite: 2.5 cm³ of wood) and 21 control colonies (1 termite: 10.0 cm³ of wood) were set up for this experiment. All experimental colonies developed from standardized groups consisting of 50 workers, which derived from a total of 15 stock colonies (i.e., field colonies) (**Fig. 3.1**). Among these workers, a pair of reproductives developed and an experimental colony with reproductives of known age and similar size was established (Weil *et al.*, 2007; Korb *et al.*, 2009). The age (i.e., time since a pair of reproductives was present) of all these colonies was 3-4 months old. Together with standardized culture conditions, this setup enabled us to control for confounding factors such as social (e.g., large variation in colony size) and environmental factors (i.e.,

temperatures and humidity). The experiment lasted for 18 months. In the end, we obtained fitness data for 34 colonies from 15 stock colonies (**Table 3.1**).

Table 3.1. Fitness variables.

Variable	Definition	Type
Queen survival	Survival status of the queen at the end of the experiment	Response, Binary
Colony survival	Survival status of the colony at the end of the experiment	Response, Binary
Worker survival	Number of workers that survived until the end of the experiment	Response, Binomial
Queen fecundity	Eggs+larvae+workers produced during the experiment	Response, Poisson
Colony fecundity	Number of alates	Response, Poisson
Treatment	Control or food-restricted	Predictor, fixed effect
Colony ID	Individual colony identifier	Predictor, random effect
Incipient colony size	Number of workers at the beginning of the experiment	Predictor, fixed effect

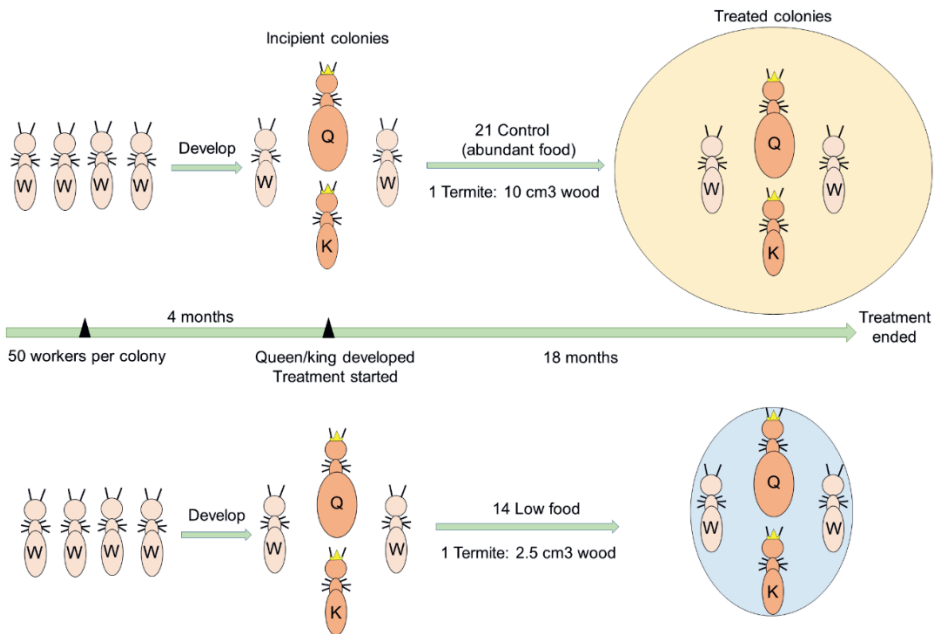


Figure 3.1. Experimental setup of food manipulation. During the first four months before the start of the experiment, incipient colonies developed from worker-only groups. These incipient colonies were randomly allocated into wood blocks representing different food conditions (control: 10.0 cm³ wood per termite; low food: 2.5 cm³ wood per termite). After 18 months of treatment, we split the wood blocks and recorded colony compositions. Incipient colony size was also measured before treatment to account for potential confounding effects. Q: queen; K: king; W: worker.

Statistical analysis

All data analyses were conducted using R version 4.0.4 (R Core Team 2021) in Rstudio version 1.4.1106 (Rstudio Team 2021). Since we measured multiple and possibly correlated fitness components per colony, we fitted Bayesian multivariate response models using the brms package (Bürkner, 2017, 2018), which is an interface to the MCMC sampler Rstan (version 2.21.2; Stan Development Team 2020). All response variables, fixed predictors, and random-effect variables are listed in **Table 3.1**. See **Supplementary methods** for more details about model settings and model comparisons.

To test hypotheses for model parameters, we computed the probabilities of direction (p_d -values; (Makowski *et al.*, 2019a). These are posterior probabilities that a parameter is positive (if the posterior median is positive) or negative (posterior median is negative). P_d -values can be regarded as the Bayesian equivalent of frequentist p -values. We used the BayestestR package version 0.10.0 (Makowski *et al.*, 2019b) to compute p_d -values and equally tailed credible intervals (Cis). We adopted the rules listed in **Table S3.1** to interpret p_d -values.

3.3 Results

Survival of colonies, queens, and workers under food restriction

The survival of queens, colonies, and workers did not differ significantly between food-restricted and control colonies (**Fig. 3.2a, 3.2b, and 3.2c, Table 3.2**). Incipient colony size (i.e., colony size at the start of the experiment) was positively related to the survival of queens (not significantly), colonies (not significantly), and workers (significantly). Moreover, there were no significant interactions between treatment and incipient colony size (**Fig. 3.2d, 3.2e, and 3.2f, Table 3.2**).

Table 3.2. Results of Bayesian analyses. FR: food restriction; CO: control; ns: not significant. Estimates of mean and CI are on logit scale for survival variables and log scale for fecundity variables.

Variable	Posteriors	Mean	p_d (%)	95% CI	Significance
Queen survival	Queen survival in CO	2.19	94.5	[-0.56, 7.56]	ns
	Queen survival in FR vs CO	-0.02	51.2	[-1.8, 1.77]	ns
	Effect of incipient colony size on queen survival in CO	0.64	79.2	[-0.95, 2.2]	ns
	Difference in incipient colony size effect between FR and CO	0.76	79.9	[-1.08, 2.55]	ns
Colony survival	Colony survival in CO	4.54	100.0	[1.41, 11.22]	***
	Colony survival in FR vs CO	-0.25	60.5	[-2.05, 1.57]	ns
	Effect of incipient colony size on colony survival in CO	1.21	92.0	[-0.50, 2.89]	ns
	Difference in incipient colony size effect between FR and CO	0.76	79.9	[-1.04, 2.56]	ns
Worker survival	Worker survival in CO	-1.24	99.9	[-2.17, 0.39]	***
	Worker survival in FR vs CO	-0.03	52.1	[-1.1, 1.07]	ns
	Effect of incipient colony size on worker survival in CO	0.64	95.7	[-0.09, 1.38]	*
	Difference in incipient colony size effect between FR and CO	0.37	76.9	[-0.66, 1.38]	ns
Queen fecundity	Queen fecundity in CO	0.82	96.4	[-0.09, 1.51]	*
	Queen fecundity in FR vs CO	-1.72	99.9	[-2.80, 0.62]	***
	Effect of incipient colony size on queen fecundity in CO	0.58	97	[-0.03, 1.21]	**
	Difference in incipient colony size effect between FR and CO	0.36	77.4	[-0.56, 1.32]	ns
Colony fecundity	Colony fecundity in CO	-1.55	100.0	[-2.90, 0.56]	***
	Colony fecundity in FR vs CO	1.06	96.5	[-0.10, 2.18]	*
	Effect of incipient colony size on colony fecundity in CO	0.11	59.4	[-0.81, 1.06]	ns
	Difference in incipient colony size effect between FR and CO	0.56	84.7	[-0.5, 1.66]	ns

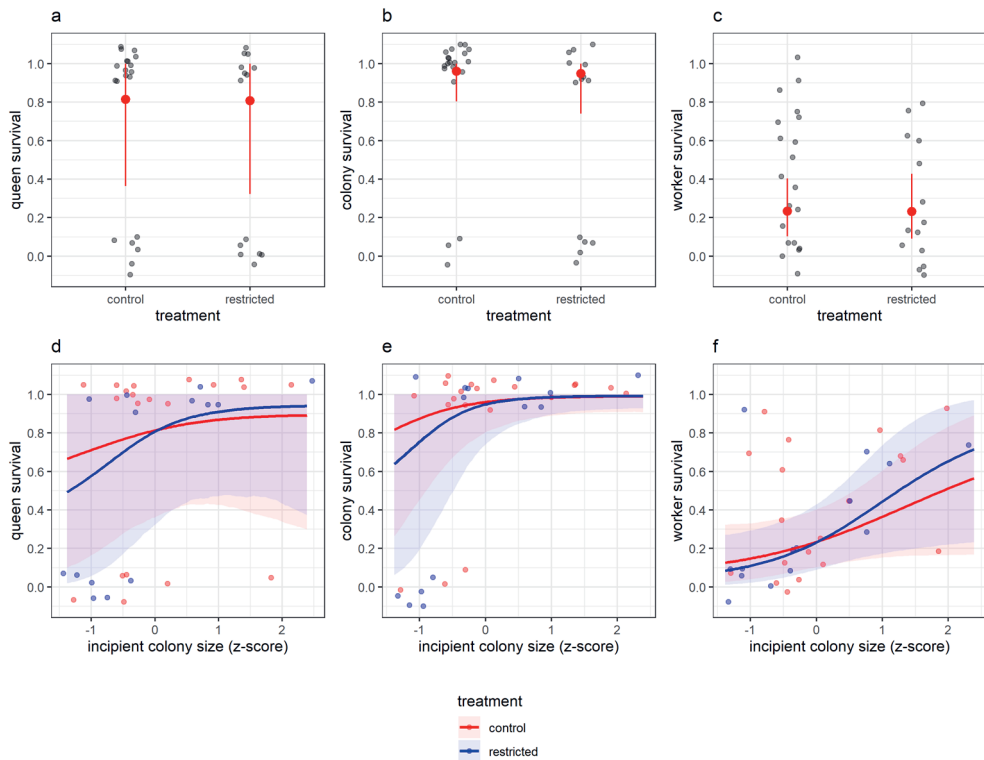


Figure 3.2. Plots of raw data (jittered) and model predictions for survival. Treatment effect on survival of (a) queens, (b) colonies, and (c) workers. Treatment effect for different incipient colony sizes for (d) queens, (e) colonies, and (f) workers. All dots represent the raw data points except for the red dots in panel a, b and c which represent the posterior means of the predicted survivability. The red error bars and light blue and red shaded areas represent 95% credible intervals. The curves indicate the posterior means of predicted response values.

Reproduction of queens and colonies

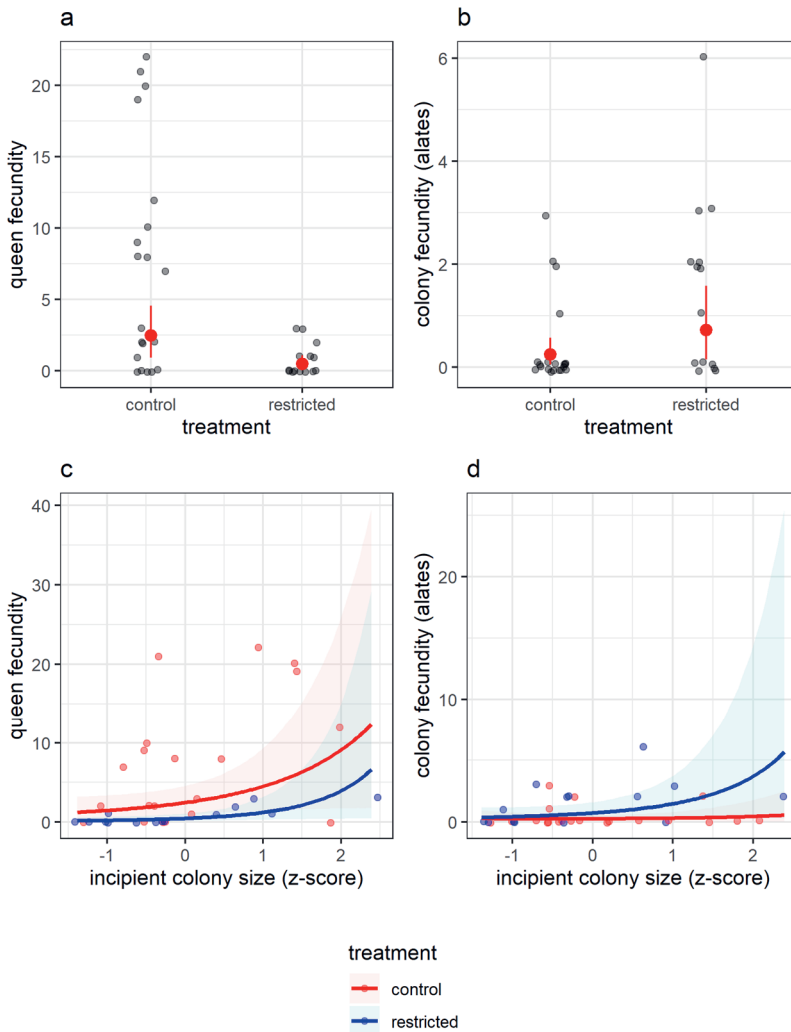


Figure 3.3. Plots of raw data (jittered) and model predictions for fecundity. Treatment effect on fecundity of (a) queens (i.e., eggs + larvae + new workers) and (b) colonies (i.e., number of dispersing alates). Treatment effect at different incipient colony sizes (standardized, z-scores) on the fecundity of (c) queens and (d) colonies.

The fecundity of queens declined significantly under food restriction (**Fig. 3.3a** and **Table 3.2**) and was significantly positively correlated with incipient colony size (**Fig. 3.3c** and **Table 3.2**). This positive correlation seemed to be negatively affected by food restriction, but interactions between treatment and incipient colony size were not significant (**Fig. 3.3c**, **Table 3.2**). In contrast, the fecundity of colonies significantly increased under food restriction and was not correlated with incipient colony size (**Fig. 3.3b** and **3.3d**, **Table 3.2**). Restricted food availability seemed to positively affect the correlation between colony fecundity and incipient colony size, but interactions between treatment and incipient colony size were not significant (**Fig. 3.3d**, **Table 3.2**).

3.4 Discussion

Effect of restricted food availability on survival

We did not detect a life-extending effect on either individual queens, workers, or colonies, which is generally observed under DR in solitary model organisms like *D. melanogaster* (Moatt *et al.*, 2016). We propose at least three potential explanations. First, sample sizes were too small. We think this is unlikely because there is little hint in the data that DR leads to higher survival (**Fig. 3.2**, **Table 3.2**), although larger sample sizes could have provided more precise results. Second, the experimental period was too short to detect a significant effect. Our experimental period should have been long enough to reveal an effect as *C. secundus* detects and responds rapidly to such changes in food availability (Korb & Lenz, 2004; Korb & Schmidinger, 2004; Evans *et al.*, 2005). Also, our experimental period was the same as in a similar study in which temperature manipulation resulted in significant effects on queen and worker survival (Rau & Korb, 2021). Lastly and most likely, the life-extending effect of DR is absent in *C. secundus*. This lack of an effect has also been reported for workers of the termite *Reticulitermes lucifugus* (Lo Pinto & Agrò, 2019) and queens of the ant *Temnothorax rugatulus* (Negroni *et al.*, 2021).

Effect of food restriction on behaviours of queens and workers

Unlike in solitary insects, food manipulation in our study was performed at the colony level to resemble the natural conditions in *C. secundus*, which does not forage outside the nest. Cooperative brood care widely occurs in social insects (Wilson, 1971; Schultner *et al.*, 2017), resulting in increased queen fecundity with more workers. However, brood care is low in lower termites that have a wood-dwelling life type (Korb *et al.*, 2012). *C. secundus* workers do not provide brood care for eggs and young larvae (Korb, 2007b). Thus, the increased fecundity with more workers (**Fig. 3.3c**) was probably caused by workers feeding the queen, although this needs to be confirmed. The positive correlation between queen fecundity and colony size seemed weaker when food was limited (**Fig. 3.3c**), possibly indicating a reduction of food intake by the queen. A previous study with *C. secundus* showed that workers behave more selfishly when food is limited. They feed nestmates less via proctodeal trophallaxis (i.e., anal feeding) and spend more time feeding on the wood (Korb & Schmidinger, 2004). This suggests that food restrictions on the colonies triggered more selfish worker behaviour that might have resulted in reduced food intake for queens.

Effect of restricted food availability on reproduction

Consistent with DR studies in solitary organisms, the queens' fecundity declined (**Table 3.2 and Fig. 3.3a, 3.3b**). This cannot be explained by redirecting resources from reproduction to maintenance since the survival of queens was not enhanced. Yet, nutrient limitation can directly result in decreased reproduction regardless of the soma maintenance mechanism (Adler & Bonduriansky, 2014).

In our experiment, colony fecundity increased as more dispersing reproductives (alates) developed under food restriction than in the control group. The increased alate production is in line with previous studies in which workers have also been more likely to develop into winged sexuals under food-deprivation (Korb & Lenz, 2004; Korb & Schmidinger, 2004; Hoffmann *et al.*, 2012). Food restriction thus led to a collapse of cooperation within the

colony as workers became more “selfish” (i.e., more workers developed into alates, fed nestmates less and fed themselves more). At the colony level, this means that resources were reallocated from maintenance (i.e., workers helping in the nest) to reproduction (i.e., workers developing into alates), suggesting a colony-level trade-off between maintenance and reproduction.

Analogies between *C. secundus* colonies and hydra

Like wood-dwelling termites in general, *C. secundus* is not classified as a *bona fide* superorganism (Korb & Heinze, 2016). Its colonies have some organismal features that are similar to those of hydra. A hydra does not have a separated germline; its body wall is a flux of stem cells that are capable of developing into all kinds of cells, from somatic to germ cells (gametes) (Galliot *et al.*, 2006; Schaible *et al.*, 2014). *C. secundus* workers are analogous to stem cells of hydra. They are totipotent (immatures) that develop/differentiate into all terminal castes (soldiers and reproductives) via moulting. Our results suggest that worker-to-alate differentiation is regulated by food availability. In hydra, nutrient reduction also leads to changes in differentiation of stem cells and thus reallocation of cell types (Flechtner & Lesh-Laurie, 1984). Strikingly, hydra are very long-lived and there has been debates about their immortality (e.g., Bosch, 2009; Martínez & Bridge, 2012, references therein). They show few signs of senescence if they do not reproduce sexually (e.g., Schaible *et al.*, 2014; Sebestyén *et al.*, 2020 and references therein). Similarly, the totipotent workers of *C. secundus* live very long lives (at least 4-5 years; Korb & Thorne, 2017) for an insect, and especially for a social insect worker, which are typically short-lived (Jemielity *et al.*, 2005; Heinze & Schrempf, 2008). A *C. secundus* colony, the equivalent of a hydra, could also live forever as dead reproductives are continuously replaced by workers that develop into replacement reproductives. The longevity of a *C. secundus* colony is only limited by the tree size the colony nests within; when a tree is consumed the colony will finally die as they do not forage outside (Korb, 2009). These analogies make it tempting to compare *C. secundus* and other wood-dwelling termites with hydra in the future.

3.5 Author Contributions

J.K. designed the study, collected and identified the termite samples and did the fitness experiment. S.L and I.P did the analyses and all authors wrote the paper.

3.6 Acknowledgements

We thank Charles Darwin University (Australia), and especially S. Garnett and the Horticulture and Aquaculture team, for logistical support during termite collection and The Parks and Wildlife Commission, Northern Territory, and the Department of the Environment, Water, Heritage and the Arts, Australia, for granting the collection (Permit number 59044) and export permit (PWS2016-001559), respectively. We also like to thank Daniela Schnaiter for assistance in performing the experiments and in maintenance of the termite colonies and Daniel Elsner for English reviewing. The study was conducted following the Nagoya protocol and was supported by the Deutsche Forschungsgemeinschaft (DFG; KO1895/23-1; KO1895/25-1; KO1895/20-2) within the Research Unit FOR2281.

3.7 Data Accessibility

Raw data and R scripts for the analysis is available at: <https://github.com/silulinlulu/Effects-of-restricted-food-availability-on-termite>.

3.8 Supplementary Materials

Supplementary Methods

Details of statistical analyses

We used Bayesian multivariate response models in which each univariate sub-model was a generalized linear mixed model. For an introduction to Bayesian analysis, we refer to McElreath (2020). Briefly, priors are the prior plausibilities of the parameters we aim to estimate. This plausibility is updated continuously during Markov Chain Monte Carlo (MCMC) sampling until it reaches equilibrium. The plausibility of the parameters after the equilibrium is called posterior. A range of possible posteriors is called a credible interval.

For binomial and Poisson response variables, we used the default logit and log link functions, respectively. As fixed predictors, we used the Z-score of incipient colony size (standardized to have a mean of zero and a SD of one) and experimental treatment (control vs. food restriction), as well as their interaction. A Z-score can be calculated as

$$Z = \frac{x - \mu}{\sigma} \quad (1)$$

where x is the observed value, μ is the mean, σ is the standard deviation of the sample.

Relative model performance was assessed by computing Watanabe Akaike Information Criterion (WAIC scores; (Watanabe, 2010) and associated standard errors for each model. To assess the goodness of fit for each response variable separately, we visually inspected posterior predictive plots using the brms' `pp_check` function, and we computed Bayesian R-squared values (Gelman *et al.*, 2019).

As the count variables were relatively rich in zeroes, we also fitted zero-inflated Poisson models, but this did not improve the performance of the

models, as indicated by WAIC scores (**Table S3.2**), which were computed using the `waic` function implemented in the R package version 2.4.1 (Vehtari *et al.*, 2017). We also allowed for nonlinear relations between incipient colony size and response variables using smoothing splines (using the default thin plate splines with the `brms` `s` function), but this also did not improve the performance of the models (**Table S3.2**).

To account for overdispersion and to allow estimation of correlations between residuals of response variables, we used experimental colony ID as an observation-level random intercept for all response variables (Harrison, 2014). Since some experimental colonies were derived from the same stock colony, we also used the stock colony ID as a random intercept. Also, this did not improve the performance of the models significantly, although its WAIC score was slightly lower (**Table S3.2**). We selected our initial model as the final model because it was the simplest model and other models did not significantly improve the performance of the model.

We used weakly informative Gaussian priors with a mean zero for all fixed effect parameters at the link scale ($SD = 10$ for intercepts and $SD = 1$ for all other regression coefficients). For random effects, we used the default priors of `brms` (t-density with $df = 3$ for standard deviations; LKJ density for correlations).

For each model, we run four chains with 1000 warm-up iterations followed by 3500 sampling iterations, thus yielding 10,000 posterior samples per model. Proper mixing of chains was monitored with trace plots and convergence of chains was assessed with \hat{r} statistics (Vehtari *et al.*, 2020).

Table S3.1. Rules to interpret Bayesian statistics (Makowski *et al.*, 2019a, 2019b).

Bayesian statistics	Value	Interpretation	Significance
Probability of direction (pd)	$\leq 95\%$	uncertain	not significant
	95% ~ 97%	possibly existing	*
	97% ~ 99%	likely existing	**
	99% ~ 99.9%	probably existing	***

Table S3.2. WAIC scores for all fitted models.

Fitted model	WAIC score
Count variables with Poisson distribution	353.8
Count variables with zero-inflated Poisson distribution	366.9
Queen fecundity modelled with spline	354.3
Source colony as random effect	352.6

