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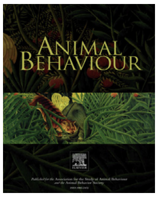

Size variation does not act as insurance in bumble bees; instead, workers add weight in an unpredictable environment

Evan P. Kelemen [a, *], Goggy Davidowitz [b], Anna Dornhaus [a]

[a] *Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, U.S.A.*
[b] *Department of Entomology, University of Arizona, Tucson, AZ, U.S.A.*

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Complex systems (e.g. eusocial insect colonies) exhibit emergent behaviours as a result of the interactions of their components. These components often vary in several traits. Such variation may improve system performance by increasing its efficiency or its robustness to environmental change. These two outcomes, efficiency and robustness, are often thought to be in opposition. Therefore, variation may be beneficial only under certain environmental conditions. Here we aim to understand why variation evolved in a particular system, bumble bee (*Bombus impatiens*) colonies. Workers in these colonies vary in body size, which affects the tasks they perform as well as their starvation resistance, suggesting potential impacts on efficiency and robustness, respectively. We examine how this variation affects colony performance under different environmental conditions and how colonies respond physiologically to these conditions. We maintained colonies of equal biomass but with either variable or less variable worker body sizes using targeted worker removal. We found that colonies with variable body sizes did not produce more brood (i.e. did not show evidence of increased performance) under predictable or unpredictable food environments. However, workers that developed under the unpredictable environment were smaller relative to their weight at eclosion. This effect was due in part to an increase in stored lipids, particularly in smaller workers. These physiological changes may explain why mortality rates did not differ between the predictable and unpredictable environment. Therefore, our finding that size variation did not affect colony performance suggests that size variation may be a neutral trait, present because selection is not acting against it. Our results also suggest that workers respond physiologically to differences in environmental conditions, which is important to consider when testing system robustness.

A central theme throughout biology, from molecular networks to ecosystems, is that the function of complex systems arises from the actions and interactions of their components. These interactions form stable patterns and structures that define system function (Ladyman, Lambert, & Wiesner, 2013). Components can vary along one trait axis such as behaviour (e.g. neurons within the brain exhibiting differences in firing potential) (Padmanabhan & Urban, 2010) or morphology (leaves on a single plant differing in size) (de Casas et al., 2011), or along several trait axes forming distinct types or castes (e.g. soldier and queen phenotypes in the social trematode *Cerithidea californica*; Poulin, Kamiya, & Lagrue, 2019). The variation that components exhibit in a trait, not just the average of the trait, is thought to be important for understanding the behaviour of a system. While evidence of the effects of trait variation on system performance is accumulating (Billick, 2002; Billick & Carter, 2007; Jehn, Northcraft, & Neale, 1999; Parker, 1999; however, see ; Schmid-Hempel, 1992) and it has evolved repeatedly at all levels of biological organization (Carroll, 2001; Smith & Szathmary, 1997), how exactly variation affects system function is often not clear.

One common assumption is that variation among components is adaptive in the context of division of labour (Ferguson-Gow, Sumner, Bourke, & Jones, 2014) because systems are more efficient when components specialize in different tasks (Oster & Wilson, 1978). Here we use the term ‘complementarity’ when different types of components perform particularly well at different co-occurring tasks, thereby improving the system's efficiency when these components are allocated to those tasks. Being particularly adapted for one skill, however, can come at the expense of generalized skills (Carroll, 2001; Oster & Wilson, 1978; Smith, 1776).

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* Correspondence: E. P. Kelemen, Department of Biology, York University, Toronto, ON, M3J 1P3, Canada.

E-mail address: evankelemen@email.arizona.edu (E. P. Kelemen).

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Thus, the implicit cost of specialization is that specialized components may lose the flexibility to respond to rapid shifts in task demands. This rigidity can impose fitness costs on a system in dynamic and unpredictable environments (Jongepier & Foitzik, 2016). Therefore, increased efficiency may come at a cost to robustness to environmental change in complex systems.

Alternatively, the variation that components exhibit may buffer complex systems against environmental change. If different components are better suited for different conditions, then this variation may prevent catastrophic failure of the system in extreme environments (Loreau et al., 2001; Seger & Brockmann, 1987; Venable, 2007). Here we use the term ‘insurance’ when different types of components are particularly adapted to different environments, thereby making the system more robust by protecting it from failure due to environmental fluctuations. In this case, variation improves robustness but is costly during stable conditions as system efficiency is lowered because not all of the components are best suited to the current environment.

As social insects, bumble bees (*Bombus* spp.) provide a unique tool for testing different hypotheses about the effects of variation in a complex system. The social insect colony is a system of integrated components (workers and queens) that has been shaped by ecological and evolutionary processes. In eusocial insects, the colony is the reproductive unit, and theory suggests that the morphology, demography and behaviour of its workers have evolved to maximize its success (Hasegawa, 1997; Oster & Wilson, 1978; Wilson, 1968). Since selection occurs at the level of the colony, variation among worker traits that make this system more efficient and/or robust will be selected.

Size is one dimension along which the components of this complex system, i.e. the workers in the colony, vary. In bumble bees, there is a large range of body size variation present within a colony (Couvillon, Jandt, Duong, & Dornhaus, 2010). Workers may vary up to 10-fold in body weight (del Castillo & Fairbairn, 2012; Goulson, 2010). Worker body size often correlates with division of labour; that is, workers of different size perform different tasks (suggesting possible complementarity) (Goulson, 2003; Goulson et al., 2002; Jandt, Huang, & Dornhaus, 2009; Wilson, 1984). Worker body size may also correlate with worker survival (suggesting possibly an insurance effect) (Couvillon & Dornhaus, 2010; Couvillon, Jandt, Bonds, Helm, & Dornhaus, 2011). Differences in size distributions among colonies could thus influence colony efficiency or robustness under different environmental conditions.

By manipulating the body size distribution of bumble bee colonies under different environmental conditions, we aimed to test (1) whether and when size variation within a colony might influence colony performance and (2) whether there is a physiological response by colonies or workers to different environmental conditions. We manipulated worker body size distributions within colonies through worker removal. We created colonies with high (variable) and low (less variable) worker size variation and measured its impact on fitness under different environments. If size variation improves colony performance through complementarity, then variable colonies in an environment with predictable food access should produce more worker and reproductive biomass. If size variation improves colony performance through an insurance effect, then variable colonies in an environment with unpredictable food access should produce more worker and reproductive biomass, but not under a predictable (and constant) environment. Additionally, we tested whether size variation improved colony robustness specifically through smaller workers being hardier to starvation events (Couvillon & Dornhaus, 2010) by measuring whether smaller bees live longer when the colony has no access to food. This starvation resistance of smaller workers is thought to be due to their relatively higher lipid stores (Couvillon et al., 2011), so we examined the relationships between worker weight and lipid storage with worker size.

METHODS

Bumble Bee Colonies and Maintenance

We obtained 24 colonies of *Bombus impatiens*, from Koppert Biological Systems (Howell, MI, U.S.A.; $N = 14$) and Biobest (Romulus, MI, U.S.A.; $N = 10$) in 2016–2018. We housed the colonies in wooden nestboxes (38 × 23 × 8 cm) lined with pine cat litter (Nature's Earth Products, Inc., West Palm Beach, FL, U.S.A.) to reduce moisture and covered them with transparent Plexiglas. Nestboxes were connected to a foraging arena (76.2 × 64.8 × 38.1 cm). Colonies were kept under laboratory conditions (12: 12 h light:dark cycle; 25 ± 1 °C; ~35% humidity) at the University of Arizona, Tucson, U.S.A. Colonies were fed pollen (fresh frozen and ground, obtained from Koppert) directly into the nestbox, and a 1.43 M sucrose solution from gravity feeders elevated off the ground (to induce flying during foraging) in the foraging arena. The amount of food that colonies were fed depended on which environmental conditions they were assigned to (see below).

Before the start of the experiment, we fed colonies ad libitum for 5–7 days and measured each worker's thorax width and weight. Thorax width is a standard measure of body size in bumble bees (Goulson, 2003); we measured it using digital callipers (Neiko Tools, U.S.A.) to the nearest 0.1 mm. We measured a worker's live body weight with a digital scale (Ohaus, U.S.A.) to the nearest 0.00001 g (0.01 mg). Workers were immobilized while being weighed using a 50 ml centrifuge vial partially filled with cotton. Additionally, after weighing, we glued a uniquely numbered plastic tag (‘Opalithplättchen’) to each worker. We then paired colonies roughly by colony size (worker number) and randomly assigned one member of each pair to one of two treatment groups: less variable group ($N = 12$) or variable group ($N = 12$), where ‘variable’ refers to the (manipulated) amount of variation in worker bee body size. For both groups, we recorded the thorax width, weight and tag number of all newly emerged workers weekly, as well as which workers had died. Then for the less variable treatment, we removed all new workers that were more than ± 0.2 mm from the colony's average thorax width at this weekly census. At the same time, for the variable treatment, we randomly removed new workers until the biomass in the colony was within ± 0.05 g (approximately the weight of one bee) of its less variable partner colony. The starting biomass of the colony pairs at each week's start did not significantly differ ($P = 0.27$; Appendix, Fig. A1). We matched the amount of worker biomass in variable and less variable colonies to ensure they contained workers of approximately equal total resource investment. The number of colony pairs per environment to be used in this study was determined by a power analysis on the results of an initial study with two colony pairs per environment. We then pseudorandomly assigned each pair of colonies to an environmental treatment: predictable or unpredictable.

Predictable Environment

Colonies had stable (constant) access to pollen and sucrose solution, with the amount for each colony scaled to the total weight of the colony, roughly similar to amounts that bumble bees can bring back to the nest in the field (Allen, Cameron, McGinley, & Heinrich, 1978): colonies were given 0.45 g of pollen and 6 ml of 1.43 M sucrose/g of worker biomass every day.

Unpredictable Environment

The unpredictable environmental condition was intended to approximate landscapes with unpredictable foraging conditions (i.e. sporadic flower availability or limited foraging opportunities due to bad weather). Bumble bee colonies store food within the nest and can survive several days off their food stores when food is withheld, but since whole-colony mortality can occur after 3 days of starvation with empty food stores (Couvillon & Dornhaus, 2010), we gave each colony pair assigned to the unpredictable environment a 40% chance of no access to food for each day. Both colonies in the pair either had food available or did not have food available on a given day, as determined by a random number generator. The 40% chance was chosen to minimize the chance of an extended starvation interval that colonies could not survive. When colonies had access to food, they were given 0.45 g of pollen and 6 ml of 1.43 M sucrose/g of worker biomass as above. To ensure any differences between the unpredictable environment and the predictable environment were due to the predictability in food access and not to changes in the overall amount of food, colonies in the unpredictable treatment were provided access to the food that they had missed during days with no food access, which we made available on the first day that the starved colonies had access to food again. Providing bees with access to the food that they had missed also gave colonies a chance to replenish their stores of food, and we did not observe (total) colony mortality.

Lipid Extraction

When we removed newly emerged workers from the nests to produce the desired size distributions, we transferred them to separate nestboxes, according to their colony of origin. We provided sugar, ad libitum, but discontinued pollen feeding for these workers for 2 days to allow their crops to be emptied of any pollen, as pollen is rich in fat. Workers were then frozen. This method was chosen as it is quick and effective (Van Huis & Feed, 2019) and it is the preferred way to kill insects to minimize pain compared to alternative methods (Fischer & Larson, 2019). We then gently scraped the number tag off each bee using a razor blade and quartered each bee along the mid-sagittal and transverse planes. We then put the workers on weigh boats and dried them in an oven at 60 °C for at least 7 days. During preliminary measures using nonexperimental bees, we found that bees stopped losing weight after 7 days in the oven (i.e. when they were completely dry). Bees were then stored in Eppendorf tubes until lipid extraction. Prior to lipid extraction, we measured the dry weight of each sample. We extracted lipids using a Soxhlet apparatus for 6 h using petroleum ether as the solvent. Extractions were run for 6 h as samples did not lose any more weight after 6 h. After extracting the lipids from the samples, we measured the weight of each sample. The difference in weight after and before extraction represents the weight of lipids originally contained in the sample.

Animal Welfare Note

Although this experiment included periods of food deprivation of whole colonies followed by euthanization of individuals within colonies, we made every effort to meet and maintain the high standards of animal welfare required by *Animal Behaviour*'s Guidelines for the treatment of animals in behavioral research and teaching. To determine the effect of food predictability on the potential benefit of worker size variation to a colony, we periodically withheld access to food from the colonies in an unpredictable food environment. Bumble bees store food within the nest, and these stores can last several days. Bees survived off these stores when we withheld access to forage from the colony. Precautions were taken to prevent prolonged periods of food being withheld, and no colony succumbed to starvation. After periods of access to food being withheld, we gave colonies access to food equal to the food that they had missed over that period. Giving colonies access to this food allowed them to replenish their food stores, and thus, any observed effect of the environment would be due to the predictability of food and not to the total amount of food that colonies had access to.

We based the sample size of colonies used in this study off a power analysis on the results of an initial study with two colony pairs per environment. This initial power analysis determined that six colony pairs per environment, for a total of 24 colonies, were needed to detect a significant difference in colony performance between size treatments based on the observed effect size. The number of individual bumble bees in this study was a consequence of the number of colonies used in this study.

Statistics Analysis

We performed all analyses using R v.4.0.0 (R Development Core Team, 2013). Linear mixed models were conducted using ‘nlme’ (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2016), and power analyses were conducted using ‘pwr’ (Whitehorn, O'Connor, Wackers, & Goulson, 2012) and ‘nlmeU’ (Galecki & Burzykowski, 2015). To test whether variation increases colony efficiency or robustness, we calculated whether the relative difference in biomass produced between variable and less variable colonies differed from zero using a one-sample Wilcoxon signed-rank test. This measure was calculated as follows: (variable colony biomass produced − less variable colony biomass produced)/((variable colony biomass produced + less variable colony biomass produced)/2) − a value of zero would indicate no difference in biomass; a negative value would indicate that the less variable colony produced more biomass. For each colony pair, we calculated the relative difference in worker, reproductive and total biomass production separately. We quantified biomass produced as the sum of individual weights as measured at eclosion. Following the same method, we tested whether there was a difference in the relative number of workers produced between variable and less variable colonies.

We also tested whether size variation or environmental condition influenced the average size of workers or variation in size of workers. First, we used a linear mixed model with colony as a random factor and environment as a fixed factor. We tested for heterogeneous variance (variation in worker sizes produced) using a log-likelihood test to compare a full model with heterogeneous variances between the environments using the weight function (varIdent) (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) and a reduced model without heterogeneous variances. If heterogeneous variance significantly improved the model, we used this full model to test the effect of the environment on the average worker size. Similarly, we then compared the size of workers produced between the variable and less variable colonies following a similar method but using a linear mixed model with colony as a random factor and variable colony or less variable colony as a fixed factor.

To determine whether smaller workers are hardier against starvation, we tested whether life span differed between the environments and whether size affected this relationship. We analysed the life span data for bees for which we had recorded the exact day they died and which were not alive prior to the start of the experiment. We compared the age at death of workers under a predictable and unpredictable environment relative to their thorax width and compared Akaike's information criterion (AIC) values to determine whether a linear or nonlinear (quadratic) model better fitted the data. The models included environment, thorax width, week eclosed and their interaction as fixed factors, and colony (and thus the specific schedule of starvation days colonies experienced) as a random factor. We also tested whether the body size (thorax width) of workers who died decreased with each consecutive day of starvation, as previously found (Couvillon et al., 2011), using a linear mixed model with consecutive days without food and treatment as fixed factors, and colony and week number as random factors. At the colony level, we tested whether worker mortality (as measured by the average number of workers who died per day) increased with consecutive days of starvation using a linear mixed model with consecutive days without food, treatment and their interaction as fixed factors, and colony as a random factor.

Furthermore, we tested whether the environment influenced resource allocation among the developing workers by size, by comparing the relationship (on a log–log plot) between worker weight and thorax width between environments. We compared this relationship on a logarithmic scale because we expected a nonlinear scaling relationship as thorax width is a linear measure and weight relates to volume. We used a linear mixed model with colony as a random factor and log (weight) and environment as fixed factors.

To see whether any changes in the relationship between weight and thorax width were due to changes in the relationship between worker lipid weight and total weight (on a log–log plot), we tested whether the lipid weight of workers changed using a linear model with dry weight, environment and their interaction as fixed factors. We compared lipid weight and total weight on a logarithmic scale because we were interested in how the proportion of lipids changed (i.e. the slope of their relationship). We did not include colony as a random factor due to the low sample size (<5 individuals) of some colonies. The low sample size was caused by including only individuals that came from weekly worker removal cohorts that included workers from both the variable and less variable colonies in a colony pair. Since the colonies in each colony pair were sized-matched, we did not always remove workers from both colonies. We included only concurrent individuals from both colonies in a colony pair to account for seasonal and annual differences among the colony pairs.

RESULTS

Colony Productivity - Biomass

Worker size distribution did not affect colony productivity in our study (Fig. 1). Under a predictable environment, the relative increase in worker, reproductive and total biomass produced by variable colonies compared to less variable colonies did not differ from zero (Wilcoxon signed-rank test: worker: $T = 13$, $P = 0.68$; Fig. 2a; reproductive: $T = 10$, $P = 0.59$; Fig. 2c; total: $T = 10$, $P = 0.56$; Fig. 2e; all $N = 6$). Because of the large standard deviation in relative difference in biomass produced between colony pair mates (mean ± SD; worker: 10.09 ± 32.46%; reproductive: 33.12 ± 85.39%; total: 12.24 ± 36.28%), with a sample size of six colony pairs, we had low power (approximately a 33%, 8% and 33% power, respectively) to detect a 20% difference in productivity (a difference found in fire ants; Porter & Tschinkel, 1985) between variable and less variable colonies. For the conventional 80% power (Cohen, 1992), we would have needed to run the experiment using 96 colonies to test for differences in worker and total biomass and 504 colonies to test for differences in reproductive biomass. Under an unpredictable environment, the relative increase in worker, reproductive and total biomass produced by variable colonies compared to less variable colonies also did not differ from zero (Wilcoxon signed-rank test: worker: $T = 8$, $P = 0.68$; Fig. 2b; reproductive: $T = 9$, $P = 0.79$; Fig. 2d; total: $T = 9$, $P = 0.31$; Fig. 2f; all $N = 6$). Again, because of the large standard deviation in relative biomass produced between pair mates (mean ± SD; workers: -25.95 ± 96.46%; reproductive: -21.16 ± 79.67%; total: -11.66 ± 30.71%), we had low power (approximately a 12%, 34% and 57% power, respectively) to detect a 20% difference. When combining the data from all the colonies and environments, the total amount of biomass produced relative to the total biomass in the colonies also did not correlate with the size variation within the

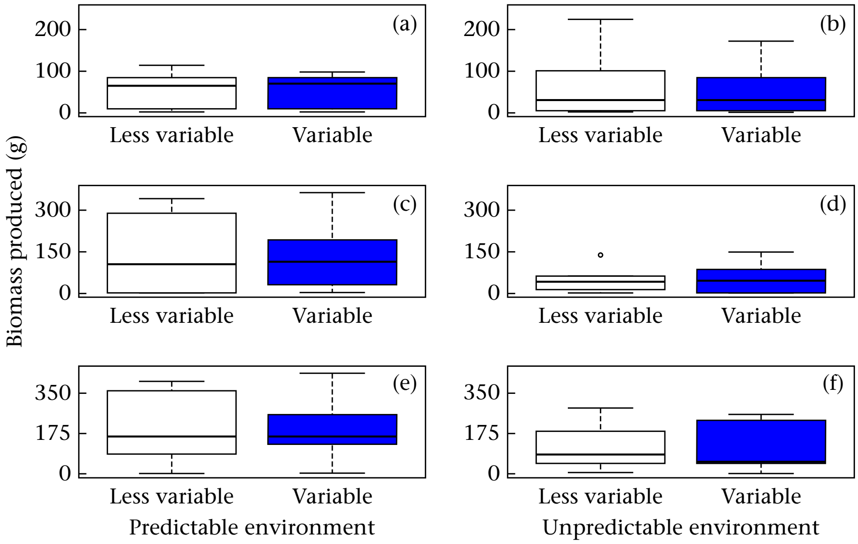

Figure 1. Biomass produced by (a, b) all workers and (c, d) reproductives, and (e, f) total biomass produced by size-matched pair mates in colonies with less variable and variable worker sizes in predictable and unpredictable environments, respectively. Mean biomass of the colonies was 51.0 ± 38.5 g (as measured by all adult workers that were concurrently present within each colony; i.e. those not removed as part of the experimental manipulation), and mean number of workers was 361 ± 284. Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles).

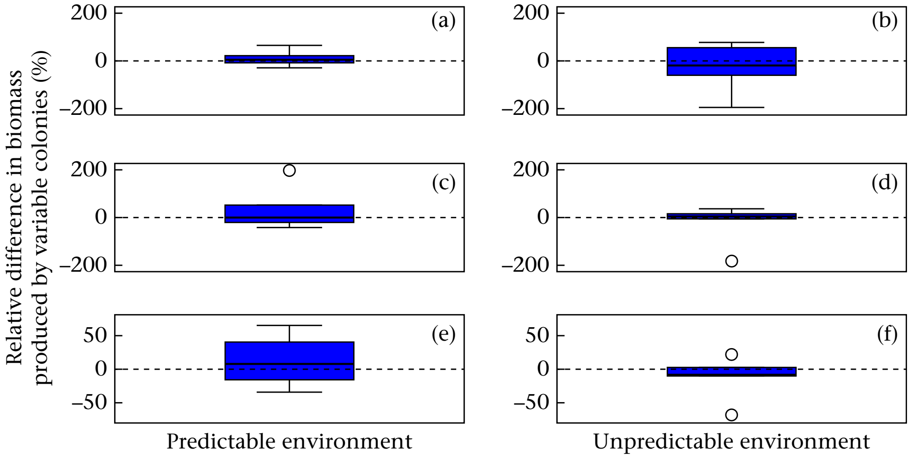

Figure 2. Relative difference in biomass produced by (a, b) workers and (c, d) reproductives in colonies with variable worker sizes, and in (e, f) total biomass produced by a size-matched colony with less variable workers in predictable and unpredictable environments, respectively.

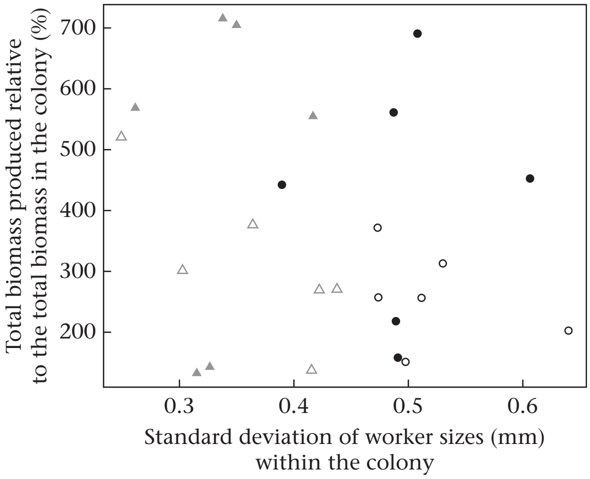

Figure 3. Relation between the standard deviation of the thorax width of all adult workers concurrently present in each colony (i.e. those not removed as part of the experimental manipulation) and the total biomass that a colony produced relative to its total biomass. Unfilled symbols represent colonies in an unpredictable environment. Filled symbols represent colonies in a predictable environment. Triangles represent less variable colonies. Circles represent variable colonies. Model: linear model, fixed; total biomass produced/total biomass in the colony ~ SD.

colony (linear regression: $r^2 = 0.05$, $N = 24$, $P = 0.28$; Fig. 3). Again, because of the large variation in relative biomass produced, our power was low (6%).

Colony Productivity — Number of Individuals

Worker size distribution also did not affect the number of individuals produced. Under a predictable environment, the relative difference in worker, reproductive and total number of individuals produced by variable colonies compared to less variable colonies did not differ from zero (Wilcoxon signed-rank tests: worker: $T = 13$, $P = 0.69$, power of 33% to detect 20% effect; reproductive: $T = 14$, $P = 0.56$, power of 8% to detect 20% effect; total: $T = 14$, $P = 0.56$, power of 74% to detect 20% effect; all $N = 6$), nor did it under an unpredictable environment (worker: $T = 10$, $P = 1$, power of 15% to detect 20% effect; reproductive: $T = 14$, $P = 0.44$, power of 33% to detect 20% effect; total: $T = 12$, $P = 0.84$, power of 69% to detect 20% effect; all $N = 6$).

Effects on the Size Distribution Produced

Under the unpredictable environment, colonies produced more variably sized workers, and this increase in size variation was more pronounced in the variable colonies. There was no change in the average size of workers produced among colonies in either predictable or unpredictable environments (mixed-effect model: effect size = 0.10 ± 0.12 mm decrease in variable colonies, $F_{22} = -0.85$, $P = 0.40$; power of 80% to detect 8% effect). However, workers produced from colonies in the unpredictable environment were more variable in size (log-likelihood test: $\chi^2_1 = 68.71$, $P < 0.001$). Additionally, while there was no difference in the size variation of workers produced by the variable and less variable colonies in the predictable environment ($\chi^2_1 = 1.00$, $P = 0.16$; power of 80% to detect <1% effect), the variable colonies in the unpredictable environments produced more variably sized workers ($\chi^2_1 = 105.35$, $P < 0.001$). We had higher power for these results, suggesting that we would be able to detect an effect if one was present in our nonsignificant results. Therefore, colonies produced more worker size variation in unpredictable environments and produced even more size variation when they already had high size variation.

Worker Mortality

Larger workers were slightly more robust to the unpredictable environment (Table 1, Fig. 4; ΔAICc to next model >3 in all comparisons; Appendix, Table A1). Under the predictable environment, life span had a quadratic relationship with body size, such that medium-sized workers lived the longest. Surprisingly, in the unpredictable environment, workers lived longer than in the predictable one, with both smaller and larger workers experiencing an increase in life span and larger workers living the longest. The increase in life span by the extreme-sized workers resulted in no relationship of body size with life span when we analysed data from only the unpredictable environment (Appendix, Table A2).

Smaller workers did not act as insurance against food shortages as they did not live longer through periods without access to food. The thorax width of workers that died was also unaffected by the size variation in the colony (mixed-effect model: effect size = 0.16 ± 0.23 mm decrease in variable colonies, $F_{1,10} = -0.69$, $P = 0.51$; power of 80% to detect 18% effect), the number of consecutive days without food (effect size < 0.001 ± 0.01 mm increase per day, $F_{1400} = 0.001$, $P = 0.99$; power of 80% to detect < 1% effect), or the interaction between the two (effect

Table 1
The best fit linear mixed model predicting the life span of bumble bee workers

Parameter	B^a	SE	*df*	*t*	*P*
Intercept	-95.94	34.96	2420	2.74	0.006
Environment (unpredictable)	123.94	39.91	22	3.11	0.005
Thorax width (linear term)	55.26	16.09	2420	3.43	0.006
Week eclosed	-2.04	0.16	2420	-3.30	<0.001
Thorax width (quadratic term)	-6.08	1.84	2420	-12.53	0.001
Thorax width (linear term)*environment (unpredictable)	-55.19	18.36	2420	-3.006	0.03
Thorax width (quadratic term)*environment (unpredictable)	6.18	2.11	2420	2.94	0.003
	Marginal $r^2 = 0.08$				
	Conditional $r^2 = 0.33$				

The model included colony as a random factor. Positive effect sizes indicate an increase in life span predicted by the respective factor; for example, the unpredictable environment and higher thorax width were associated with a longer life span.

[a] Effect size.

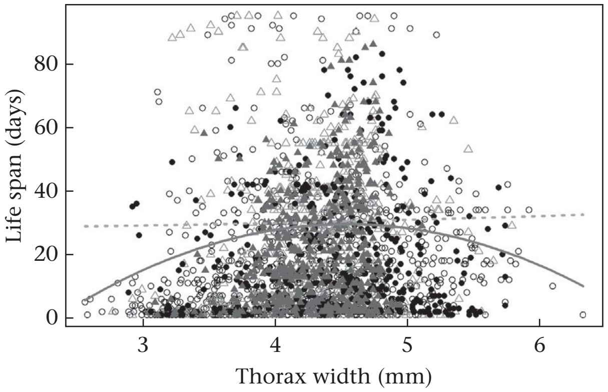

Figure 4. Relation between worker thorax width and age at death in unpredictable (dashed line) and predictable (solid line) environments. Filled symbols represent workers from colonies in a predictable environment (variable: $N = 503$; less variable: $N = 476$). Unfilled symbols represent workers from colonies in an unpredictable environment (variable: $N = 600$; less variable: $N = 870$). Triangles represent less variable colonies. Circles represent variable colonies. Model: linear mixed model, fixed: life span ~ environment × thorax width^2 + environment × thorax width + week eclosed, random: colony.

size = 0.03 ± 0.02 mm increase in variable colonies per day, $F_{1,1400} = 1.60$, $P = 0.11$; power of 80% to detect 1% effect; Appendix, Fig. A2).

At the colony level, size variation did not buffer colonies from worker losses during starvation events. Worker mortality increased with each consecutive day without access to food (mixed-effect model: effect size = 3.14 ± 1.41 deaths per day, $F_{1,44} = 2.23$, $P = 0.03$), and mortality did not differ between less variable and variable colonies (effect size = 3.68 ± 7.17 additional deaths in variable colonies, $t_{10} = 0.51$, $P = 0.62$; power of 80% to detect 182% effect) or with the interaction between the two factors (effect size = 1.36 ± 1.99 fewer deaths per day in variable colonies, $t_{44} = -0.68$, $P = 0.49$; power of 80% to detect 115% effect). Overall, there was no difference in worker mortality between the unpredictable or the predictable environment (mixed-effect model: effect size = 0.19 ± 0.59 fewer deaths in the unpredictable environment, $F_{1,20} = 0.32$, $P = 0.75$; power of 80% to detect 133% effect) or its interaction with size variation within the colony (effect size = 0.41 ± 0.84 more deaths in the unpredictable environment in variable colonies, $F_{1,20} = 0.49$, $P = 0.63$; power of 80% to detect 319% effect).

Worker Physiology

The environment that workers developed under influenced their size (as measured by thorax width) relative to their weight at eclosion (Fig. 5). The thorax widths of workers that did not develop entirely under either environmental condition (emerged during the first 3 weeks of the experiment) increased with weight (mixed-effect model: effect size = 0.34 ± 0.003 increase in log (thorax width) with log (weight), $F_{1,3601} = 112.47$, $P < 0.001$; Fig. 5a), but were unaffected by the environment (effect size < 0.001 ± 0.01 decrease in log (thorax width) in unpredictable environments, $F_{1,22} = -0.34$, $P = 0.74$; power of 80% to detect <1% effect), or its interaction with weight (effect size < 0.001 ± 0.004 decrease in log (thorax width) with log (weight) in unpredictable environments, $F_{1,3601} = -0.02$, $P = 0.98$; power of 80% to detect <1% effect). However, the thorax widths of workers that developed completely within the two experimental environments (emerged after the third week) not only increased with weight (mixed-effect model: effect size = 0.30 ± 0.004 increase in log (thorax width) with log (weight), $F_{1,3155} = 68.78$, $P < 0.001$), but was affected by the environment (effect size = 0.06 ± 0.02 increase in log (thorax width) in unpredictable environments, $F_{1,17} = 3.88$, $P = 0.001$) and its interaction with weight (effect size = 0.04 ± 0.005 increase in log (thorax width) with log (weight) in unpredictable environments, $F_{1,3155} = 8.09$, $P < 0.001$; Fig. 5b). This difference between the environments was driven by a change in the relationship between weight and thorax width among workers in the predictable environment (Appendix, Table A3). Thorax width changed faster than weight under the unpredictable environment but slower than weight under the predictable environment. So, changes in the weight obtained during development had a greater effect on the size of workers in an unpredictable environment. Therefore, bees under the predictable environment with fewer resources (lower weight at eclosure) developed into larger adults, leading to a smaller effect of resources on body size than in an unpredictable condition, where resources strongly affected body size.

This changing relationship between body size and weight was associated with, and possibly caused by, a difference in the relationship in worker lipid weight and dry worker mass under the predictable and unpredictable environments (linear regression: $P = 0.005$, $r^2 = 0.35$) (Table 2, Fig. 6). The slope of this log–log relationship among workers in an unpredictable environment (slope = 0.78) did not differ from one ($F_{1,103} = 0.99$, $P = 0.243$), indicating that bees of different dry weight store similar proportions of lipids. This slope in a predictable environment (slope = 1.39) was greater than one ($F_{1,92} = -2.35$, $P = 0.027$), indicating that heavier workers store relatively more lipids. In an unpredictable environment, the percentage of dry weight that was lipids was 4.12 ± 2.04% for the smallest quartile of workers (<0.036 g) and 3.16 ± 1.34%. for the largest quartile of workers (>0.062 g). In the predictable environment, the percentage of dry weight that was lipids was 2.93 ± 1.37% and 4.16 ± 2.27% for the smallest and the largest quartiles of workers, respectively. This

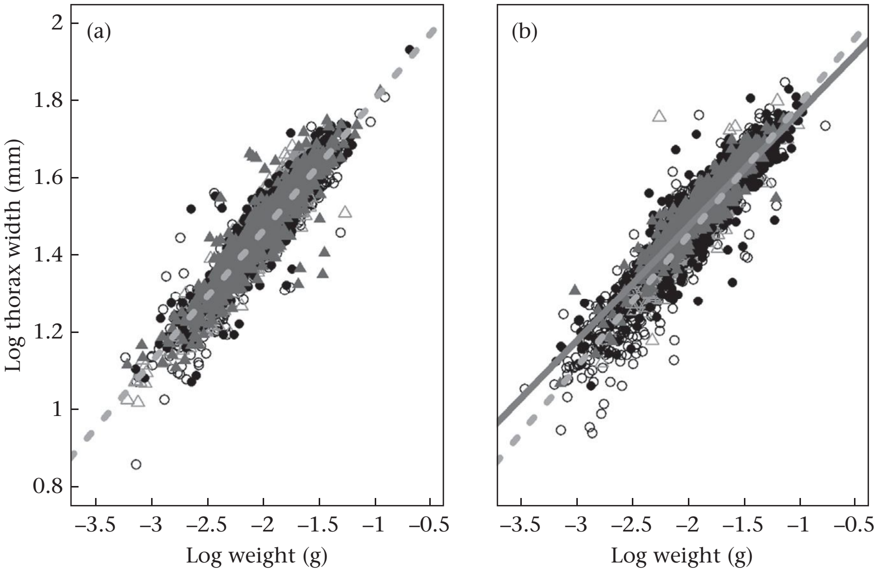

Figure 5. Relation between worker weight and thorax width (dashed line) of (a) workers in the different environmental conditions at the start of the experiment and (b) workers that developed under predictable (solid line) and unpredictable (dash line) food environments: in particular, workers who weighed little were morphologically larger in the predictable environment and smaller in the unpredictable environment. Model: linear mixed model, fixed: log (thorax width) ~ log (weight) × environment, random: colony. In (a), marginal $r^2 = 0.86$ and conditional $r^2 = 0.89$. In (b), marginal $r^2 = 0.85$ and conditional $r^2 = 0.88$. Filled symbols represent workers that developed in a predictable environment (a: variable: $N = 902$; less variable: $N = 1156$; b: variable: $N = 993$; less variable: $N = 550$). Unfilled symbols represent workers that developed in an unpredictable environment (a: variable: $N = 725$; less variable: $N = 850$; b: variable: $N = 583$; less variable: $N = 1053$). Triangles represent less variable colonies. Circles represent variable colonies.

suggests that, in an unpredictable environment, workers stored the same relative amount of lipids regardless of their body size, while in a predictable environment, smaller workers stored relatively fewer lipids than larger workers. This might indicate that colonies prioritized producing larger workers over workers with greater lipid stores in the predictable environment.

DISCUSSION

We found that bumble bee colonies with more body size variation performed no better than colonies with less body size variation under predictable and unpredictable food environments. We cannot exclude the possibility that the effect of variation was below our ability to detect as there was large variation in colony performance (biomass production), resulting in low power. However, our results suggest that there may not be selection on size variation as it may be an effectively neutral trait. We did find that environmental conditions caused physiological differences among the workers. We found that extreme-sized workers lived longer under an unpredictable than a predictable environment. This gives some support to the insurance hypothesis as it means that workers of different sizes are well suited to different environmental conditions, as suggested by Couvillon and Dornhaus (2010). Interestingly, we found that the increase in worker life span in the unpredictable environment may be due to workers in this environment developing, for their weight, into relatively smaller (in thorax width) workers with increased lipid stores (i.e. with higher specific weight). These results, therefore, suggest that physiological responses of workers can increase system robustness.

The size that workers achieved with a given amount of resources was sensitive to the environment in which they were raised. Under an unpredictable environment, larvae with more mass became smaller adults instead of trying to maximize their size with those resources. In other insects, more resources during development increases adult size (Quezada-Euán et al., 2011) and weight (Davidowitz, D'Amico, & Nijhout, 2004; Teder, Tammaru, & Esperk, 2008). Less is known about how resource quantity affects the relationship between weight and linear size. Changes in resource quantity can, but do not always (Angelo & Slansky, 1984; Wissinger, Steinmetz, Alexander, & Brown, 2004), influence the relationship among different body parts, affecting wing length (Nijhout & Emlen, 1998) and sexual ornament size (McCullough, Ledger, O'Brien, & Emlen, 2015; Nijhout & Emlen, 1998). Poorer-quality environments typically lead to smaller adult size (Quezada-Euán et al., 2011). Smaller adult sizes are achieved to maintain overall body compositions (Teder et al., 2008), although some developing insects continue to grow despite changes in relative body composition (Dmitriew, Cooray, & Rowe, 2007; Strobbe & Stoks, 2004). While lower-quality diets lead to relatively more resources being stored as fat (Dmitriew, Carroll, & Rowe, 2009), little is known about how resource predictability affects resource storage. Our study indicates that developing workers allocate resources differently according to the predictability of their food. Such environmentally induced changes in body composition may explain the poor relationship between linear measures of body size and body weight (Kendall et al., 2019). Our findings suggest that it is important to consider resource predictability, not just quality, when investigating compensatory physiology.

In our study, workers in the unpredictable environment may have stored more resources as fat. Workers of equal weight in both environments were smaller with a higher composition of lipids in the unpredictable environment. This might indicate that, in unpredictable environments, colonies prioritize producing workers with greater lipid stores over larger workers. However, the change in the percentage of lipids was very small, so it may not be a major cause of the biomass difference. The biomass differences may instead be due to the storage of protein (Otis, Wheeler, Buck, & Mattila, 2004) or carbohydrates (Surholt, Greive, Hommel, & Bertsch, 1988), with stores of the latter know to fuel flight in bees (Blatt & Roces, 2001; Suarez et al., 2005; Surholt et al., 1988). The lipids that differed in this study may be membrane lipids, and not meaningful energy storage. The percentage of total worker biomass that was lipids in our study was similar to foraging bumble bees (Adhikari, Burkle, O'Neill, Weaver, & Menalled, 2019; Smith,

Table 2
The linear model predicting worker lipid content

Parameter	B^a	SE	*df*	*t*	*P*
Intercept	-2.439	0.507	193	-4.82	<0.001
Environment (variable)	-1.650	0.658	16	-2.51	0.013
Dry mass	1.394	0.168	193	8.30	<0.001
Environment (variable)*dry mass	-0.607	0.215	193	-2.82	0.005
	$r^2 = 0.35$				

[a] Effect size.

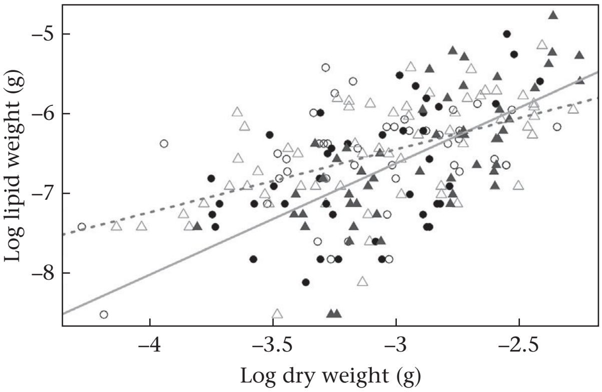

Figure 6. Relation between worker lipid weight and total weight in predictable (solid line) and unpredictable (dashed line) environments (also see Table 2). Filled symbols represent workers from colonies in a predictable environment ($N = 93$). Unfilled symbols represent workers from colonies in an unpredictable environment ($N = 104$). Triangles represent less variable colonies. Circles represent variable colonies. Model: linear model, life span ~ environment × dry mass.

Graystock, & Hughes, 2016) but less than laboratory-fed bumble bees (Couvillon et al., 2011). We fed bees at quantities similar to what they would be able to forage in nature (Allen et al., 1978), and therefore, our experimental set-up appears to represent a similar quality environment.

There was variation in relative colony performance in this study, despite the fairly controlled laboratory environment. This variation may be due to intrinsic differences among colonies in performance either due to genotype or queen quality (Rangel, Keller, & Tarpy, 2013) or worker interactions (Free & Williams, 1972). Variation in bumble bee colony performance has been observed previously in the laboratory (Ribeiro, Duchateau, & Velthuis, 1996; Whitehorn et al., 2012), and these differences can be compounded over the lifetime of a colony. In addition, we may not have found an effect of worker size variation because of the difficulty of measuring colony performance in the laboratory (Keller, 1993), given the differences between the laboratory and the field in bee foraging behaviour and sources of mortality. A major benefit of larger workers is their performance while foraging (Spaethe & Weidenmüller, 2002), where works may have foraging ranges miles in diameter and have to negotiate complex floral landscapes (Greenleaf, Williams, Winfree, & Kremen, 2007; Osborne et al., 1999, 2008). Natural foraging conditions also come with an increased risk of mortality, particularly for larger bumble bees (Kerr, Crone, & Williams, 2018; Müller, Blackburn, & Schmid-Hempel, 1996). Therefore, our experiment may not have captured all of the costs and benefits of size variation within a colony.

Our study suggests that worker size variation may not benefit a colony as predicted by the complementarity hypothesis. The explicit assumption of the complementarity hypothesis is that components perform well at different co-occurring tasks; however, this may not be the case in bumble bees. Despite smaller workers being more likely to perform in-nest tasks, such as brood care (Jandt & Dornhaus, 2009), larger workers appear to be more efficient at every task (foraging: Goulson et al., 2002; Spaethe & Weidenmüller, 2002; brood care: Cnaani & Hefetz, 1994; cognitive tasks: Riveros & Gronenberg, 2010; Spaethe, Brockmann, Halbig, & Tautz, 2007). So, while complementarity may not explain the evolution of worker size variation in bumble bees, this hypothesis is supported in other social insect species (Hölldobler & Wilson, 1990; Porter & Tschinkel, 1985; Weber, 1972). However, a large body of literature has found no clear complementarity effect (reviewed in Schimd-Hempel, 1992), suggesting that it is not the only reason for size variation to evolve in social insects or in complex systems in general.

The insurance hypothesis, while commonly considered in the ecological literature, has not been considered in the social insect literature. Several studies (Beshers & Traniello, 1994; Davidson, 1978; Fowler, 1984; Herbers, 1980; Kelemen & Dornhaus, 2018) have examined whether worker variation within a colony is plastic with regard to environmental condition, but few studies have measured colony performance as measured here (Billick & Carter, 2007; Porter & Tschinkel, 1985). With regard to plasticity, we found similar results as previous studies: under more stressful conditions, the size variation in the workers produced increased. Interestingly, we found that colonies with more variable worker sizes produced even greater size variation when food resources were limited than did colonies with less variation. The reason for this is unclear. If size variation indicates colony stress, then these colonies may have been more stressed. Colony performance was not improved by a variable workforce, as predicted by the insurance hypothesis (Couvillon & Dornhaus, 2010). Similar to Couvillon and Dornhaus (2010), we found size-dependent differences in worker responses to environmental conditions, suggesting that the environment may well affect optimal body size. However, in contrast to Couvillon and Dornhaus (2010), we found that larger workers, not smaller workers, lived marginally longer when colonies were exposed to starvation events. Our results may differ from Couvillon and Dornhaus (2010) because of differences in the strength and the duration of the starvation events that colonies experienced. In our study, colonies experienced repeated frequent short starvation events, while colonies in their study experienced an extreme starvation event that lasted until complete colony mortality. Therefore, colonies in our experiment may have had time to acclimate to such variable environmental conditions. The variable access to food may also explain why larger and smaller workers lived longer under the unpredictable environment; for example, fruit flies, *Drosophila melanogaster*, that develop with fewer resources also live longer (May, Doroszuk, & Zwaan, 2015). While colonies in predictable and unpredictable environments had access to the same total amount of food, individual workers may have received different totals while developing due to the specific feeding schedule that their colony experienced. At the colony level, size variation did not improve worker mortality rates. We also may not have detected an effect of size variation due to the large variation in mortality rates among the colonies, as has been seen in other studies (Kelemen, Cao, Cao, Davidowitz, & Dornhaus, 2019). Whether or not differences in worker life span can lead to benefits of variation, as predicted by the insurance hypothesis under some conditions, remains an open question; here, however, we did not find empirical support by directly measuring colony productivity for bumble bees, but it is an interesting hypothesis to consider for social insects more broadly.

It is surprising that we found no difference in colony performance after we manipulated worker size variation, especially since worker variation is so often argued to be a major adaptation of social insect colony organization (Hasegawa, 1997; Oster & Wilson, 1978; Wilson, 1968). However, many previous studies have also failed to find such effects (Calabi & Traniello, 1989; Jandt &

Dornhaus, 2014; Rissing & Polloek, 1984; Wood & Tschinkel, 1981; Wilson, 1983; see also Schmid-Hempel, 1992). Therefore, the most parsimonious explanation for why size variation did not seem to matter, at least in this study and for bumble bees, is that variation is not adaptive. This is congruent with other studies in bumble bees that have also found a lack of a relationship between size variation and colony performance (Herrmann, Haddad, & Levey, 2018; Jandt & Dornhaus, 2014). Size variation in bumble bees is due to higher feeding rates of centrally located larvae compared to those in the periphery of the nest (Couvillon & Dornhaus, 2009; Kelemen & Dornhaus, 2018). If size variation is neutral, selection may not act against it. So, while it is assumed that size variation is adaptive, it may not be. Instead, there may be no real cost to variation. Size variation may be an effectively neutral trait in other social insects as well, and it may explain why other studies have failed to find evidence that size variation increases colony performance (Calabi & Traniello, 1989; Colin, Doums, Péronnet, & Molet, 2017; Rissing & Polloek, 1984; Wilson, 1983).

Overall, we have shown that worker size variation does not increase brood production of *B. impatiens* under predictable or unpredictable environments in the laboratory. We found that environmental condition affects worker physiology. Smaller and larger workers experienced an increase in life span in an unpredictable environment. This size-dependent response by workers supports the insurance hypothesis by suggesting that workers may be suited for different environments and thereby increase colony robustness. The changes in worker life span we found were likely mediated by changes in resource storages as the relationship between size at eclosure and weight differed based on the environment. However, size variation ultimately did not increase colony robustness to environmental perturbations. Therefore, our results suggest that at least within bumble bees, phenotypic variation may not have evolved to increases the efficiency or robustness of the system; instead, a physiological response of workers to environmental conditions may increase system robustness.

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Appendix

Table A1
The AICc comparisons for the linear and quadratic mixed models predicting the life span of bumble bee workers under predictable and unpredictable food environments

Model	ΔAICc	*df*	Weight
Thorax width (quadratic) × environment + week enclosed	0	9	0.991
Thorax width (quadratic) + environment + week enclosed	11.6	7	0.006
Thorax width (linear) × environment + week enclosed	11.8	7	0.001
Thorax width (linear) + environment + week enclosed	13.3	6	0.001
Thorax width (quadratic) + week enclosed	14.8	6	<0.001
Environment + week enclosed	15.8	5	<0.001
Thorax width (linear) + week enclosed	16.6	5	<0.001
Week enclosed	167.6	4	<0.001
No fixed factors	176.7	3	<0.001

All models included colony as a random factor.

Table A2
Worker size did not affect the life span of workers that developed under an unpredictable environment

Parameter	B[1]	SE	*df*	*P*
Intercept	-25.44	19.94	1455	0.20
Thorax width (linear term)	0.80	9.10	1455	0.93
Week enclosed	0.04	1.05	1455	0.97
Thorax width (quadratic term)	-1.84	0.20	1455	<0.001
	Marginal r^2 = 0.08			
	Conditional r^2 = 0.37			

[1] Effect size.

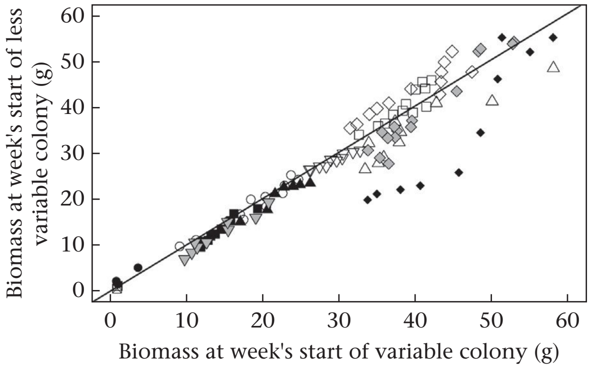

Figure A1. Biomass of size-matched pairs in the variable colony and in the less variable colony at the start of each trial week throughout the duration of the experiment. The slope did not differ from 1 as the colony pairs had the same biomass starting each week (slope = 1.02, *df* = 105, *P* = 0.27). Unique symbols represent different size-matched pairs. Open points are pairs reared in the variable environment and closed points are pairs reared in the constant environment. Model: linear mixed model, fixed: less variable ~ variable, random = size-matched pair.

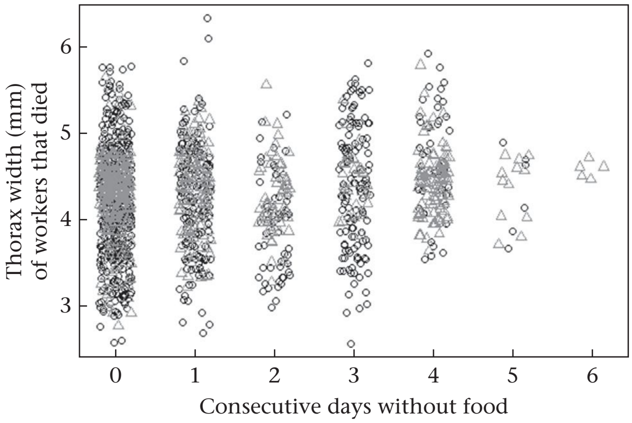

Figure A2. Relation between thorax width of workers that died and the number of days a colony was without food. Lighter grey triangles represent less variable colonies ($N = 870$). Black circles represent variable colonies ($N = 600$). Model: linear mixed model, fixed: thorax width ~ consecutive days × variable_or_less variable, random: colony/week number.