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Effect of temperature and social environment on worker size in the ant *Temnothorax nylanderi*

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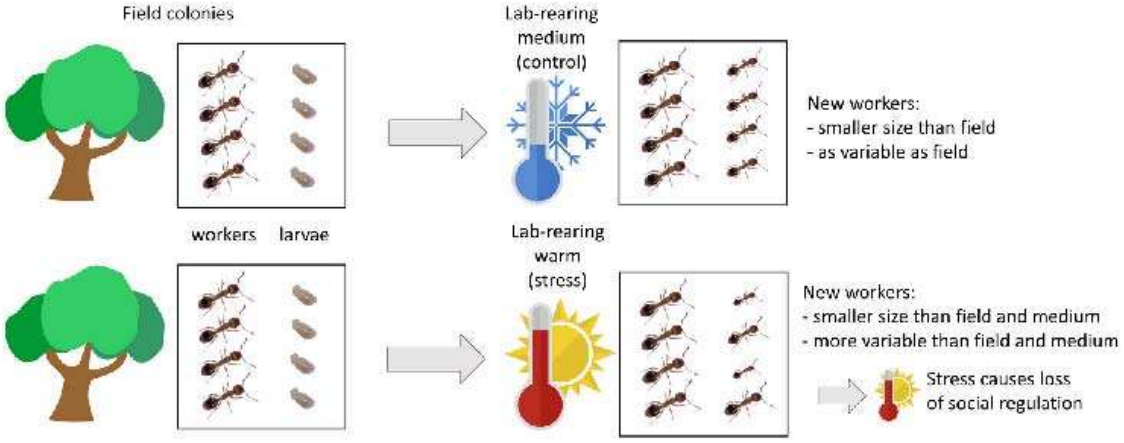
Abstract

Warm temperatures decrease insect developmental time and body size. Social life could buffer external environmental variations, especially in large social groups, either through behavioral regulation and compensation or through specific nest architecture. Mean worker size and distribution of worker sizes within colonies are important parameters affecting colony productivity as worker size is linked to division of labor in insect societies. In this paper, we investigate the effect of stressful warm temperatures and the role of social environment (colony size and size of nestmate workers) on the mean size and size variation of laboratory-born workers in the small European ant *Temnothorax nylanderi*. To do so, we reared field-collected colonies under medium or warm temperature treatments after having marked the field-born workers and removed the brood except for 30 first instar larvae. Warm temperature resulted in the production of fewer workers and a higher adult mortality, confirming that this regime was stressful for the ants. *T. nylanderi* ants followed the temperature size rule observed in insects, with a decreased developmental time and mean size under warm condition. Social environment appeared to play an important role as we observed that (i) larger colonies buffered the effect of temperature better than smaller ones (ii) colonies with larger workers produced larger workers whatever the rearing temperature and (iii) the coefficient of variation of worker size was similar in the field and under medium laboratory temperature. This suggests that worker size variation is not primarily due to seasonal environmental fluctuations in the field. Finally, we observed a higher coefficient of variation of worker size under warm temperature. We propose that this results from a disruption of social regulation, i.e. the control of nestmate workers over developing larvae and adult worker size, under stressful conditions.

Graphical abstract



Keywords: body size, temperature size rule, social environment, phenotypic plasticity, social insects

1. Introduction

Temperature is one of the most important environmental factors for ectotherms such as insects, affecting life history traits and inducing physiological and behavioral responses (Hochachka and Somero, 2002; Hoffmann et al., 2003; Sørensen et al., 2003; Dahlhoff and Rank, 2007). Social life imposes a higher level of complexity for understanding the response of organisms to thermal stress. Emergent properties of complex social systems can be sensitive to disturbance but in the same way can provide a better resilience (Middleton and Latty, 2016). For instance, by constructing complex nest structures, insect societies might be more prone to any disturbance that would lead to nest destruction. On the other hand, constructing complex nest structure can help facing thermal stress by better controlling the brood rearing conditions (reviews in Jones and Oldroyd, 2006; Kadochová and Frouz, 2013). The size of the social groups may then be of major importance with larger groups being potentially more efficient in maintaining group functioning and better in tolerating environmental stress (Linksvayer and Janssen, 2009). For instance, Modlmeier et al. (2013) showed that survival to cold shocks followed by starvation was higher in groups than for isolated individuals.

Temperature is known to have a strong effect on insect development time and size with many insects following the temperature size rule (TSR) for which adult body size is negatively correlated to developmental temperature (Atkinson, 1994; Angilletta and Dunham, 2003; Chown and Gaston, 2010; Klok and Harrisson, 2013; Lee et al., 2015). The mechanisms underlying this relationship are far from clear but body size exhibits a fair amount of plasticity, in spite of its strong link to fitness components (Chown and Gaston, 2010, Kalinkat et al., 2015). In social insects, worker size is a major component of worker phenotypic variation within a colony. According to their size, workers can be more or less efficient for various colony tasks (Hölldobler and Wilson, 1990) such as foraging (Peat et al., 2005; Westling et al., 2014) or colony defence (Nowbahari et al., 1999; Parmentier et al., 2015). Even though recent studies revealed varying degrees of genetic influence on worker size variation (e.g. Hughes et al., 2003; Huang et al., 2013), most size variation within social insect colonies relies on a plastic response of the developing brood to environmental variation (Wheeler, 1986; Smith et al., 2008). Environmental variation can be directly related to external microclimates (Wheeler, 1986; Purcell et al., 2016) but mostly rely on the social environment that affects nutritional intake of developing larvae (Smith et al., 2008, Linksvayer, 2006, Linksvayer et al., 2011, Quezada-Euan et al., 2011; Shpigler et al., 2013). Nurse workers, by providing food and care to larvae and by moving them within the nest, have considerable control over the nutritional intake and the nest microenvironment provided to the larvae.

What would happen to the size distribution of workers produced from brood reared under thermal stress is far from clear. If the social environment is able to play a regulating role, no effect is expected at least for large colony size. Such regulation could involve moving the brood into other chambers in species that nest underground, emigrating to a new nest in species that nest in cavities, or simply providing more intensive care to the brood. On the other hand, if thermal stress disrupts the colony functioning, brood rearing conditions might be disturbed and lead to a change in worker size distribution.Despite the importance of worker size, very few studies have experimentally examined the effect of warming on the mean and distribution of worker size within colonies. Interestingly, even though a decrease in developmental time with increasing temperatures has been reported in some ant species (Elmes and Wardlaw, 1983; Porter, 1988; Kipyatkov et al., 2005; Abril et al., 2010), this did not translate into a decrease in larvae size at pupation or in worker size as expected under the TSR (Elmes and Wardlaw, 1983, Cassill and Tschinkel, 2000, Kipyatkov et al., 2005). Only one of these experiments (Cassill and Tschinkel, 2000) investigated the effect of temperature on both mean and variation of worker size of the ant *Solenopsis invicta* and no significant effect was detected. This was in part due to compensatory behavioral mechanisms of workers regulating the homeostatic development of the larvae (Cassil and Tschinkel, 2000). In this species, worker size is an important factor in division of labor (Cassil and Tschinkel, 1999) and mean worker size does not vary among seasons in the field (Cassil and Tschinkel, 2000). The absence of temperature effect was hence probably due to the strong selective pressure maintaining an optimal mean and variation in worker size.

In our study, we investigated the effect of thermal stress during brood development on the mean size and variance of workers produced by colonies of the ant *Temnothorax nylanderi*. In contrast with *S. invicta*, workers are only slightly polymorphic within colonies, with a 1.5 fold variation in head width. Even though larger workers are more prone to forage in *Temnothorax* species (Westling et al 2014) and resist better to starvation (Modlmeier et al 2013), workers are usually assumed to be highly flexible in their behavior in monomorphic or slightly polymorphic species (Hölldobler and Wilson, 1990). Behavioral specialization was shown to be disadvantageous when colonies have to face a high risk of worker loss in *T. longispinosus* (Jongepier & Foitzik, 2016). Moreover, we recently showed that a decrease in worker size variation did not affect the efficiency of various colony level tasks in *T. nylanderi* (Colin T., Doums C., Péronnet R., Molet M., unpublished data). Interestingly, colonies of this species are known to sporadically produce phenotypes that are intermediate between workers and queens (intercastes) (Okada et al., 2013), maybe as a consequence of environmental stress (Molet et al., 2012). Because of the weaker link between worker size and division of labor in *T. nylanderi* than in *S. invicta*, we predict a larger effect of temperature on mean and variation of worker size in the former. If the social environment cannot buffer thermal stress efficiently, warmer temperatures should 1/ lead to the production of smaller workers according to the TSR and 2/ lead to increased worker size variation caused by the disruption of the behavioral regulation and even potentially to the production of intercastes. By using colonies with different numbers of workers but a fixed number of larvae, we indirectly assessed the potential regulating role of the social environment. We predicted that a higher number of workers per larva should allow colonies to cope better with stress and reduce the effect of thermal stress on mean worker size and worker size variation.

2. Materials and Methods

2.1. Colony collection and experimental rearing design

T. nylanderi is an ideal model to study the effect of external and social environments as colonies contain less than a few hundred workers and nest in acorns or twigs on the ground where they are more exposed to external environmental fluctuations than species nesting underground (Mitrus 2013). We used 50 queenright *T. nylanderi* colonies collected in the Bois de Vincennes, Paris; France (48°49’56.3624” North; 2°26’28.9252” East) in January 2014. This is a deciduous forest with oaks and ground vegetation such as brambles, ferns and ivy. Colonies were housed in artificial nests consisting of two microscope glass slides separated by a plastic layer where chambers and an opening had been cut out. Each nest was placed in a foraging area made of a 10.5x8.7x5cm plastic box under regular laboratory conditions (20/21°C under natural light) for about 15 days before the start of the experiment. In each colony, all brood was removed except 30 first instar larvae that colonies would rear to adulthood during the experiment. Accordingly, the size of workers that developed from these larvae and emerged at the end of the experiment could not be under any major influence of prior energy intake, notably from the field. They developed using almost exclusively the energy intake received over the course of the experiment. Moreover, keeping the number of larvae constant whatever the size of the colony should allow the largest colonies to better control brood development as more time and food would be potentially available per larva and the effect of social environment should therefore be more pronounced than by keeping the workers/larvae ratio constant. Before starting the experiment, all workers were counted and marked with a paint dot on the thorax so that they could be distinguished from the newly emerged workers at the end of the experiment.

The experiment started when the 50 colonies were equally distributed in two temperature-controlled chambers with glass door (reference CTS TP10/600). Colonies of similar size were randomly assigned to one of the two treatments. The location of nests within the climate chamber was random but kept similar in the two climate chambers for colonies of similar size. A preliminary study using temperature sensors placed at few centimeters underground in forest populations around Paris showed that late Spring ground temperatures were about 20°C during the day and 15°C at night (Jarrosson S., Péronnet R., Doums C. and Molet M., unpulished data). Accordingly, one chamber was set to a medium daily temperature cycle typical of what colonies experience in late Spring in Bois de Vincennes (12h day at 20°C and 12h night at 15°C) while the other chamber was set to a warmer and potentially stressful daily temperature cycle without changing the degree of fluctuation (12h day at 27°C and 12h night at 22°C). Preliminary experiments showed that rearing colonies under higher temperatures impacts brood development negatively and leads to a decreased production of workers. Even though daily fluctuation cycles in temperature occur in many environments, studies of development under fixed temperature greatly outnumber studies performed under daily fluctuation cycles in temperature (Niehaus et al., 2012). However setting a fixed daily temperature for species that evolved under daily fluctuating temperature can in some way represent a thermal stress (Niehaus et al., 2012). We therefore decided to test for an effect of warming under a similar level of daily fluctuation in temperature. Humidity and access to water were similar in all boxes: a tube containing water and plugged with paper was placed in the foraging area.

Colonies were fed diluted honey and pieces of dried crickets *ad libitum*. These resources were placed in the foraging area twice a week. Every time about 10 larvae had turned into young adults (recognizable by their pale brown color and lack of paint mark) in a nest, these workers were taken out of the colony and put into 95% alcohol. Colonies were removed from the climate chambers once they had produced more than 15 workers or that no more pupae or large larvae were alive. The duration of the experiment for each colony provides in some way an indirect estimate of the developmental time of the larvae. We decided not to count the larvae and newly emerged workers on a daily basis since removing the glass roof of the nest and taking the box out of the climate chamber for extended periods would have disturbed the colony. Any dead marked workers were collected at the same time as young workers and were used to estimate the mortality rate as the ratio of the total number of dead workers to the initial number of workers in the colony. Moreover, given that the duration of the experiment was not the same for each colony, we divided this ratio by the number of weeks the colony stayed in the climate chamber to obtain a weekly mortality rate.

2.2. Measurements and statistical analyses

For each colony, we measured all newly emerged (unmarked) workers, hereafter called 'laboratory-born workers' as well as 15 randomly selected original marked workers hereafter called field-born workers (i.e. workers present as adult in the field colonies; these workers developed from larvae that experienced field conditions only). The ant heads were cut off and stuck on a piece of double-sided tape in a petri dish. Heads were photographed under a Discovery.V12 Zeiss stereomicroscope connected to a computer via a Zeiss AxioCam ICc 5 video camera. Head width was measured just posterior to the eyes with ImageJ (available at http://imagej.nih.gov/ij/ ; Abràmoff et al., 2004). This measurement in ants is known to be highly correlated with body size (Araujo and Tschinkel, 2010, Spagna et al., 2008).

Data were analyzed with R (R Core Team 2014) using the package nlme (Pinheiro et al., 2016) for mixed effect models, and graphs were built using the package ggplot2 (Wickham, 2009). To compare the duration of the experiment, the weekly mortality rate of adult workers and the number of emerged workers between the two temperature conditions, we used non parametric Wilcoxon tests as these variables did not follow a normal distribution.

We tested the effect of rearing temperature on mean worker size using laboratory-born workers. We tested for an effect of social parameters (colony size but also the mean size and coefficient of variation of the field-born nestmate workers) and their interactions with temperature rearing conditions (warm, medium) on mean worker size. We used the mean size and/or the size variation of field-born workers as covariates because both could affect the size of the workers produced in the laboratory. For instance, larger workers or a higher variance in worker size could provide some benefits to the colony and allow for the production of larger workers. We used a mixed effect model with colony as random factor and we allowed for heterogeneous variance between the two levels of temperature regimes using the weight function (varIdent). We tested for heterogeneous variances by comparing the full model (containing all fixed and random factors) with a reduced model that did not allow for heterogeneous variance using a log likelihood ratio test. When heterogeneous variance significantly improved the model, it was kept in the model for all the other subsequent tests and parameters estimations. Fixed effects were tested by comparing the models fitted using maximum likelihood method with and without the effect of interest using a log likelihood ratio test. The fixed and random parameters were estimated using a restricted maximum likelihood method.

To investigate the effect of the rearing temperature on the distribution of worker size, we computed three parameters for each colony: the coefficient of variation (variance/mean*100), the kurtosis and the skew. We performed the same analyses as above but we used a linear regression model instead of a mixed effect model because the dependent variable was measured at the colony level.

Finally, we compared the mean and distribution of worker size between field-born worker and laboratory-born workers under the two temperature conditions. For the mean worker size, we used a mixed effect model with rearing conditions (three levels: warm, medium, field) as fixed factor and colony as random factor. We also allowed heterogeneous variance in individual worker size among rearing conditions. We tested the heterogeneous variance and the effect of rearing conditions as explained above. For the distribution of worker size (CV, kurtosis and skew), even though the variables were estimated at the colony level, we still used a mixed effect model to control for the fact that field-born and laboratory-born workers belonged to the same colonies. If the effect of rearing conditions was significant, we performed multiple comparisons of means using Tukey contrasts after the mixed model using the glht function of the multcomp package (Torsten et al. 2008).

The underlying assumptions of the mixed and linear models regarding the residuals were checked visually following Pinheiro and Bates (2000); no transformation of the data was necessary.

3. Results

3.1. Effects of temperature on offspring production

All 50 colonies survived the experiment and produced a total of 891 workers (average = 9; range 4-38) and 27 males. Eight colonies produced a total of 23 males at medium temperature whereas only four colonies produced a total of 4 males at warm temperatures. No female sexuals emerged during our experiment and no intercastes were produced. Most of the emerged workers probably originated from the 30 first instar larvae present at the beginning of the experiment even though we cannot exclude that some of the workers originated from eggs produced during the experimental rearing. This clearly did occur at least in the six colonies reared at medium temperature for which more than 30 workers emerged (31, 32, 33, 35, 36, 38.). The other 19 colonies produced less than 30 workers. We did not notice many small or medium larvae in colonies towards the end of the experiment, so resource investment into the original larvae was probably barely affected and mostly directed towards the 30 original larvae. Any larva that would have developed into an adults from an egg laid during the experiment would have been reared under the same treatment as the 30 original larvae, so this would not bias our results. It would merely compensate for some of the mortality among the 30 original larvae. The duration of the experiment (time that the colony spent in the climate chamber until at least 15 workers emerged) was longer at medium temperature (median = 22 weeks; range: 19-25) than at warm temperature (median = 10 weeks; range: 7-11) (Wilcoxon test: W = 625, *P*< 0.001). The weekly mortality rate (ratio of dead workers over the total number of workers divided by the duration of the experiment) was significantly higher at warm (median = 0.017; range: 0.003-0.052) than at medium temperature (median = 0.010; range: 0.001-0.057) (Wilcoxon test: *W*= 154, *P*= 0.004). The number of emerged workers was also significantly lower at warm (median = 18; range: 11-27) than at medium temperature (median = 26; range: 5-38) (Wilcoxon test: *W*= 462, *P*= 0.0012). We measured a total of 531 and 360 laboratory-born workers under medium and warm temperatures respectively. For the field-born workers, we measured a total of 734 individuals, i.e. 15 workers per colony except for four small colonies for which less than 15 workers were available for measurement at the end of the experiment (1 colony with 10 workers, 2 colonies with 11 and one colony with 12).

3.2. Effect of temperature and social parameters on mean head size of laboratory-born workers

We tested for an effect of temperature on the head size of workers born in the laboratory controlling for three colony-level parameters that are colony size, mean head-size and coefficient of variation of head size. We found a significant variance heterogeneity in head size between the two temperatures (L-ratio=6.22, *P*=0.008) with variance under medium temperature being 77% lower than at warm temperature (estimated coefficient at medium temperature = 0.88, warm temperature being set to one). Including the interaction terms between the three covariates and the temperature effect did not significantly improve the model (L-ratio=0.24; *P*=0.97) and these interactions were therefore removed. The head size of workers produced in the laboratory increased with colony size (L-ratio=20.24, *P*< 0.001; Figure 1) and with the mean head size of the field-born nestmate workers (L-ratio=14.49, *P*< 0.001; Figure 2) but it was not affected by their coefficient of variation (L-ratio=1.98, *P*= 0.16). The head size of lab-born workers was significantly smaller at warm temperature than at medium temperature (L-ratio=10.69, *P*=0.001; Figure 1 and 2). Warm temperature led to a 4.6% decrease in worker size (estimated from the model coefficient).

3.3. Effects of temperature and social parameters on the within colony variation of head size of laboratory-born workers

We next tested for an effect of temperature on the dependent variables describing the distribution of head size (coefficient of variation, kurtosis and skew) of workers born in the laboratory controlling for three colony level covariates that are colony size, mean and coefficient of variation of head size of the field born nestmate workers. The interaction terms between the covariates and temperature effect did not significantly improve the models (Table 1). None of the covariates had an effect on the worker size variation parameters (Table 1). In agreement with the previous analysis, temperature only had a significant effect on the coefficient of variation of head size of laboratory-born workers, the coefficient of variation being higher under warm temperature than under medium temperature (Table 1, Figure 3).

3.4. Comparison of laboratory-born and field-born workers

Finally, we compared the mean size of laboratory-born and field-born workers. We allowed for heterogeneous variance in head size among the three levels of rearing conditions (field-born; laboratory-born at medium temperature and laboratory-born at warm temperature) as it significantly improved the mixed model (L-ratio=19.84, *P*<0.0001). Not surprisingly, we found a significant effect of rearing condition on mean worker size (L-ratio=190.92, *P*< 0.0001). Multiple comparisons (using Tukey constrasts) showed that field-born workers were significantly larger than laboratory-born workers whatever their rearing conditions (for medium temperature: difference estimate=0.018±0.002, z=9.13, *P*<0.001; for warm temperature: difference estimate=0.03±0.002, z=11.40, *P*<0.001). Regarding the distribution of worker size, the rearing conditions had a significant effect on the coefficient of variation only (CV : L-ratio=16.14, *P*<0.001; skew : L-ratio=1.52, *P*=0.47; kurtosis : L-ratio=4.43, *P*=0.109). Multiple comparisons (using Tukey contrasts) showed that the CV of laboratory-born workers under medium temperature conditions was not different from field-born workers (estimate difference=0.017±0.02, z=0.89, *P*=0.65) whereas under warm temperature, the CV of laboratory-born workers was significantly higher than the CV of field-born workers (estimate difference=0.08±0.02, z=4.09, *P*<0.001; Figure 3). These results are in agreement with the heterogeneous variance found in the previous models.

4. Discussion

Although the effect of rearing temperature on insect brood development and adult body size is well documented, few studies have experimentally investigated the effect of temperature on worker body size in ants (Cassil and Tschinkel, 2000; Heinze et al., 2003; Kipyatkov et al., 2005), and only one considered within-colony variation in worker size (Cassil and Tschinkel, 2000). Because worker size and its variation within colonies are important colony-level parameters that potentially influence colony fitness, assessing how thermal stress affects these parameters and whether colonies can buffer these effects is of crucial importance. Using the small ant *T. nylanderi*, we found an effect of thermal environment on both mean and within colony variation of worker size. Colonies reared under warm laboratory conditions produced smaller but more variable workers compared to medium laboratory conditions. The importance of the social environment on worker size was indirectly shown by the positive relationship observed between colony size and worker size under laboratory conditions, a relationship already observed in other studies (e.g. Wood and Tschinkel, 1981; Clémencet and Doums, 2007). Moreover, there was a positive correlation between the mean size of field born workers and that of laboratory-born workers. This could be due to some level of heritability of worker size and/or to the fact that colonies with large workers are more efficient and can afford the production of large workers. Finally, worker size variation was not higher in field-born workers than in laboratory-born workers under medium temperature whereas *T. nylanderi* colonies face seasonal temperature fluctuations as they nest on the ground surface in acorns or twigs. This indirectly suggests that workers are able to buffer the effect of external seasonal variations in the field. These results confirm the role of the social environment on the determination of worker phenotypic variation (Linksvayer, 2006; Linksvayer, 2007; Linksvayer et al., 2011; Purcell et al., 2012; Shpigler et al., 2013).

At a first glance, the shorter developmental time and smaller body size found at warm temperature are in agreement with the temperature size rule (TSR) shown in many ectotherms (Atkinson, 1994; Angilletta and Dunham, 2003; Lee et al., 2015) and with the positive correlation observed in the field between worker size and latitude in *Temnothorax* ants (Heinze et al. 2003). However, even though the mechanisms underlying the TSR are still unclear, the range of temperatures used to determine the reaction norm should not encompass extreme stressful temperatures (Atkinson and Sibly, 1997, Angilletta and Dunham, 2003). Our result might not therefore simply support the TSR as the warm temperature used in our study was chosen to be stressful for the ants. Indeed, the higher mortality rates of field-born workers under warm temperature associated with the fewer emerged workers support the idea that the warm temperature induced a stress. Smaller body size in response to stress might simply results from a decrease in the energy intake (Partridge et al., 1994; Chapman, 1998; Dodson et al., 2011) or a disturbance in neurohormonal secretion that accelerates development (Perić-Mataruga et al., 2006). In this context, the study that revealed a lower worker size under low temperature (Heinze et al., 2003) is not necessarily in contradiction with our result provided that the low temperature used (0°C-10°C cycle) was outside the normal range and potentially also stressful. The finding that workers reared under medium temperature were also smaller than field-born workers also supports the possibility that laboratory rearing conditions are themselves stressful even though we paid attention to allow daily regular temperature fluctuation. This is also suggested by the near absence of sexual production in our colonies. Even though food is given ad-libitum in the laboratory, it might simply not be as diverse and as nutrionally rich as in nature. A lower size of laboratory-born workers was commonly reported for different social insect species (Knee and Medler, 1965; Wood and Tschinkel, 1981; Bragança et al., 2002; Purcell et al., 2016; but see Linksvayer, 2006).

Interestingly, warm temperature also affected the coefficient of variation of nestmate worker size whatever the size of colonies. Given that we did not observe any effect on skew or kurtosis, this suggests that warm temperature just increased the worker size variation without affecting the shape of the distribution. This result is in accordance with the fact that, in most solitary insects, the size of adults in a population tends to be more variable under stressful conditions (Teder et al., 2008). The reasons underlying this trend are not clear. It might result from individual differences in resource allocation choice that only need to be done under energetic constraints (Tammaru and Teder, 2012). Extreme or novel environments can also reveal cryptic genetic variation (genetic variation that is not expressed in the usual environment) and thereby increase phenotypic variance (Schlichting, 2008; McGuiguan et al., 2011). These two hypotheses rely on the presence of genetic variation among individuals. Even though *T. nylanderi* is supposed to be monogynous and monoandrous, within-colony genetic diversity can still be high due to colony fusion (Foitzik and Heinze, 2001). More work is needed to test whether high genetic diversity could occur in some of our colonies and impact worker size variation. Another hypothesis based on the social environment can explain this higher variation in worker size under warm temperatures. Indeed, the ability of nestmate workers to regulate larval development could be reduced under stressful conditions. A parallel can be done with solitary organisms: stress decreases the ability of their internal environment to control cell growth rates, it induces developmental instability and it leads to higher fluctuating asymmetry (Dongen, 2006). A colony of social insects can be viewed as a superorganism that has to control the internal nest environment to avoid developmental instability. This social environment could be disrupted under stressful conditions and generate phenotypic variance among workers, which could be viewed as a sort of colony-level equivalent of fluctuating asymmetry. Under this hypothesis, removing all workers should lead to the production of workers with high phenotypic variation. In honey bees, rearing larvae without workers indeed led to the production of a large and continuous variation in worker size instead of two discrete queen and worker castes (Linksvayer et al., 2011). More experiments are needed to test whether stressful conditions lead to social developmental instability and to a subsequent increase in within-colony morphological variation. It is commonly conjectured in literature that higher phenotypic variation among workers provides colony-level benefits through a better division of labour (Hölldobler and Wilson, 1990; Bourke and Franks, 1995; Londe et al., 2016). For instance, Modlmeier and Foitzik (2011) showed that colony productivity increased with intracolonial variation in worker aggressiveness. Even though the effect of worker size on division of labor is not well known in *Temnothorax* species, large workers are more likely to forage than small workers in *T. rugatulus* (Westling et al., 2014). This suggests that division of labor can be linked with worker size even in such small species with continuous but limited worker size variation. Whether the higher phenotypic variation produced under stressful condition could provide some benefits for colonies and whether workers can actively increase variation above background levels to face stress is far from clear and would merit more investigation.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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Fig. 1. Mean head size of workers per colony is represented as a function of colony size. Each circle is a colony reared in the field (green circles), in the laboratory under medium temperature regime (blue triangles) and in the laboratory under warm temperature regime (red squares). The regression lines are drawn from the coefficients of the mixed model with the interaction between colony size (X) and rearing conditions. Mean within colony head size (± s.d.) was 0.56 ± 0.02 for field-born workers, 0.55 ± 0.02 for lab-born workers under medium temperature and 0.53 ± 0.02 for lab-born workers under warm temperature. The mean worker size is based on 21.2 ± 1.6, 14.4 ± 1 and 14.7 ± 0.2 workers per colony (± sd) for field-born, lab-born under medium and lab-born under warm temperature respectively.

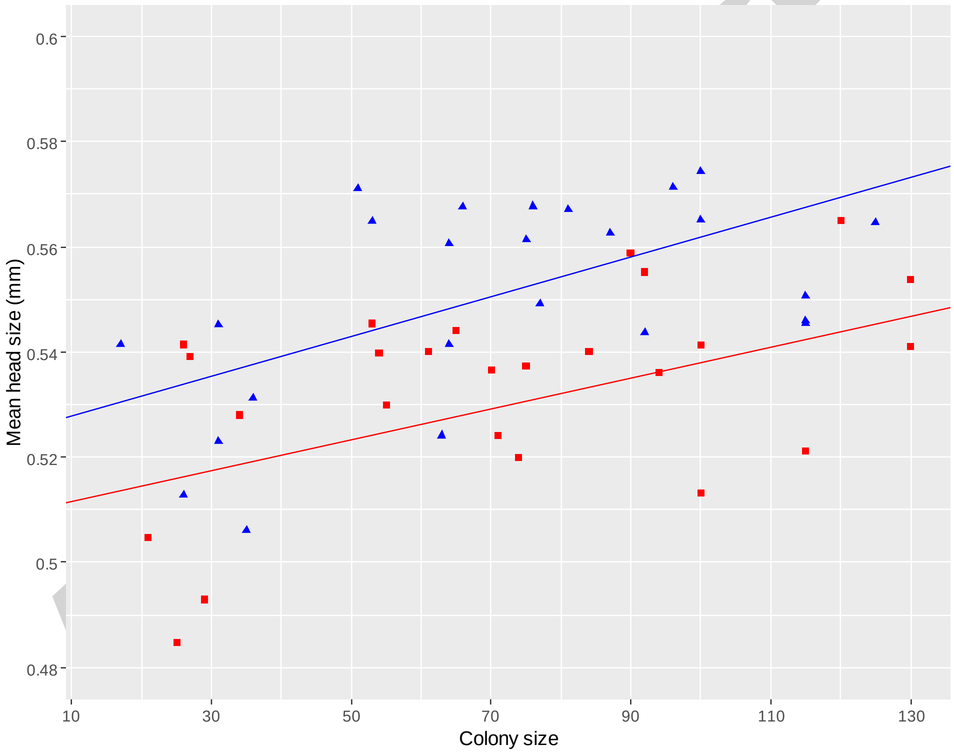

Fig. 2. Mean head size per colony of workers born in the laboratory as a function of the mean head size of workers born in the field from the corresponding colony. Each point represents a colony reared at medium temperature (blue triangle) or at warm temperature regime (red circle). The regression lines for both temperatures are drawn from the fixed effect coefficients of the minimal adequate mixed model (colony size was set to 70, i.e. the average size of colony used in the experiment, to draw the regression line).

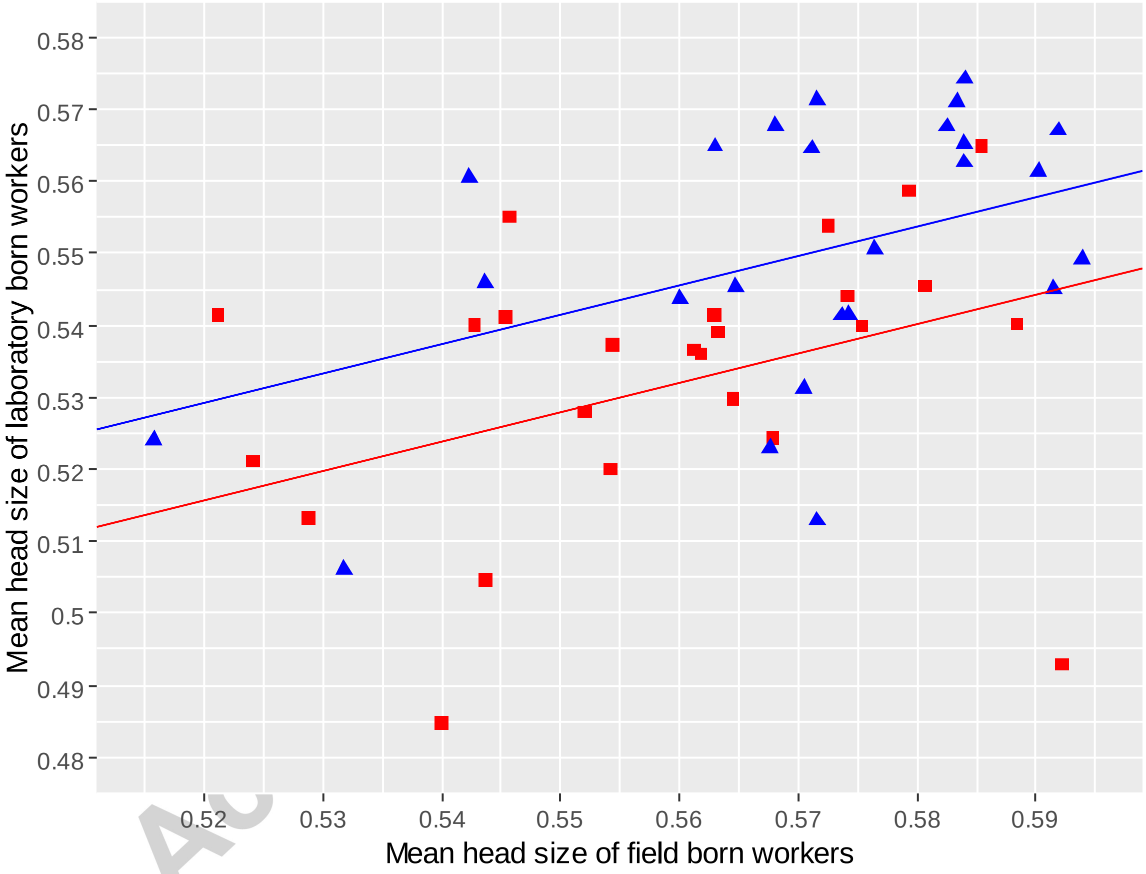

Fig. 3. Boxplot of the coefficient of variation of head size within colony for each rearing condition. The raw values of CV per colony are represented by the black dots.

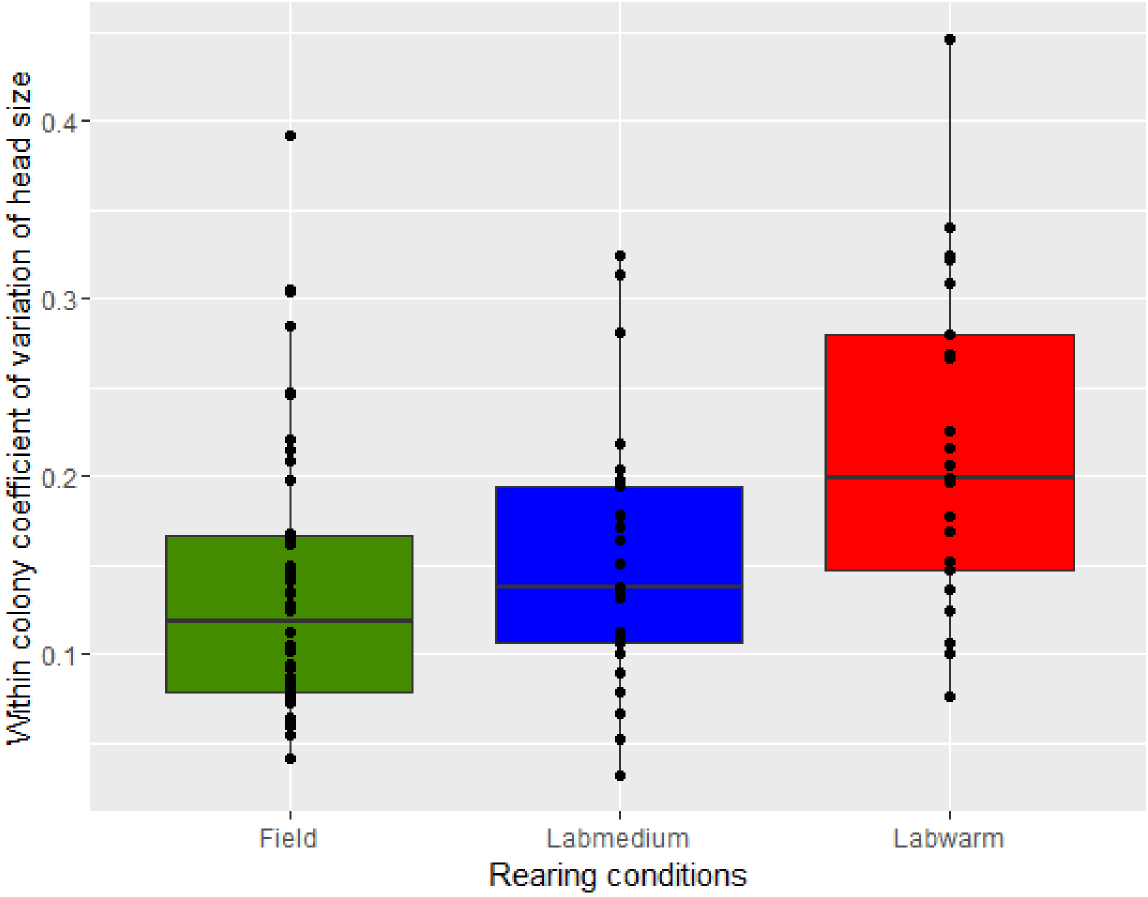

Table 1. Results of the general linear model testing the effect of temperature (warm and medium) and three social environment covariates (colony size, mean (meanF) and coefficient of variation (cvF) of head size of their field-born nestmate workers) for the three parameters describing the distribution of worker head size of laboratory-born workers within colonies (coefficient of variation, skew and kurtosis). The significance of each factor was tested by comparing the models with and without the factor of interest using Fisher ratio tests that are provided in the table with their *P*-values between parentheses. The two way interactions between temperature and the three covariates were removed from the model as they did not improve it (see the F-test for the interaction terms).

	Coefficient of variation	Skew	Kurtosis
Temperature	$F_{[1;45]}$ = 8.74 (0.005)	$F_{[1;45]}$ = 0.24 (0.63)	$F_{[1;45]}$ = 2.04 (0.16)
MeanF	$F_{[1;45]}$ = 1.04 (0.31)	$F_{[1;45]}$ = 2.36 (0.13)	$F_{[1;45]}$ = 0.08 (0.78)
cvF	$F_{[1;45]}$ = 0.80 (0.38)	$F_{[1;45]}$ = 1.36 (0.25)	$F_{[1;45]}$ = 3.21 (0.08)
Colony size	$F_{[1;45]}$ = 0.40 (0.53)	$F_{[1;45]}$ = 0.29 (0.59)	$F_{[1;45]}$ = 0.67 (0.42)
Interaction terms	$F_{[3;42]}$ = 1.09; (0.36)	$F_{[3;42]}$ = 1.52 (0.22)	$F_{[3;42]}$ = 2.59 (0.07)

Vitae

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Highlights

- Temperature affects the size and size variance of workers produced by ant colonies.
- Under warmer temperature, colonies produce smaller workers.
- Under warmer temperature, colonies produce workers that are more variable in size.
- Stressful warm temperatures can disrupt the social regulation of worker size.