



Manipulation of worker size diversity does not affect colony fitness under natural conditions in the ant *Temnothorax nylander*

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Abstract

In social insects, within-colony worker diversity has long been thought to improve colony fitness. Recent studies start to question this assertion in species with moderate diversity. However, demonstrating an absence of effect is difficult as this absence could be due to the measurement of only few life history traits or to artificial conditions. In order to circumvent these limitations, we experimentally decreased worker size diversity within colonies of the ant *Temnothorax nylander*, with or without affecting mean worker size and we reintroduced them in the field for 4 months (spring and early summer). We then thoroughly measured their fitness based on survival, growth, and reproductive success. Our results show that our manipulation did not affect colony fitness. In addition, colonies did not restore diversity to its initial level, further suggesting that worker size diversity is not a key parameter to them. We found the classically observed positive relationship between colony size, colony growth, and reproductive success. Overall, our results confirm that worker size diversity within colony is not necessarily adaptive in species where it is moderate. We discuss the alternative mechanisms that could explain the evolutionary persistence of moderate worker size diversity.

Significance statement

Organisms that live in groups can greatly benefit from the emergence of novel group-level traits. For instance, social insects show significant variability in worker size within colonies. This size diversity increases the division of labor among workers and improves colony fitness. However, in species where size diversity is moderate, this relationship may not always be verified. Here, we manipulated both worker size diversity and mean worker size within colonies of the ant *Temnothorax nylander*, we reintroduced them in the field, and we extensively measured colony fitness after 4 months. We found no impact on colony survival, growth, and reproduction. We discuss how social life and its associated cooperation and conflicts could cause variation in worker size, without any positive effect on colony fitness.

Keywords Colony size · Social insects · Mean body size · Survival · Growth · Reproductive success

Introduction

Animal societies can exhibit genetic, behavioral, physiological, and morphological diversity among individuals of the same group, and this is thought to provide benefits to the group (Sendova-Franks and Franks 1999; Robinson 2009). One of the most obvious and ecologically important traits in social insects is size diversity of individuals within colonies (bees, Brand and Chapuisat 2012; Linksvayer et al. 2011; wasps, Kovacs et al. 2010; ants, Molet et al. 2012). Worker size diversity improves division of labor and colony efficiency in many species (e.g., bumblebee, Peat et al. 2005; stingless bee, Ramalho et al. 1998). Among social insects, ants are the most morphologically diverse group and the one where the highest level of morphological diversity within colonies can be observed (Wheeler 1991). In ant species with high worker polymorphism (including discrete worker and soldier castes),

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studies support a general positive relationship between worker size diversity and colony performance (Davidson 1978; Wilson 1984; Yang et al. 2004; Evison et al. 2008; Powell 2009; Arnan et al. 2011; but see Rissing and Pollock 1984; Calabi and Traniello 1989). However, most species have moderate worker size diversity (with an intra-colonial coefficient of variation lower than 0.2 (Fjerdingstad and Crozier (2006))), and in this case, the link between worker size diversity and colony performance is less clear. Beshers and Traniello (1994, 1996) suggest that the adaptiveness of size variation in moderately polymorphic ants is unrelated to division of labor, and size could be driven by other pressures which increase survival or colony growth. Some studies show a positive relationship (Porter and Tschinkel 1985a, 1986; Heinze et al. 2003; Billick and Carter 2007; Modlmeier et al. 2013), others do not (Billick 2002; Modlmeier and Foitzik 2011; Westling et al. 2014; Yilmaz et al. 2019). In ant species with moderate worker size diversity, within-group morphological diversity could therefore be non-adaptive at the colony level.

However, it is difficult to reliably rule out any adaptive value of worker size diversity as the presence or the lack of positive effects could always result from the limited number of traits investigated or the rearing setup under artificial laboratory conditions. Indeed, some studies focus on a single trait, such as foraging (Billick and Carter 2007; Westling et al. 2014), brood production (Porter and Tschinkel 1985a, 1986; Billick 2002; Modlmeier and Foitzik 2011), thermal tolerance (Heinze et al. 2003; Yilmaz et al. 2019), or resistance to starvation (Modlmeier et al. 2013). Accordingly, trade-offs with other traits that could be negatively affected by size diversity may have been overlooked, resulting in a biased assessment of fitness. Moreover, some of these studies are correlative, so they do not prove a causal link between size diversity and colony efficiency (e.g., Modlmeier and Foitzik 2011; Westling et al. 2014). Finally, all the experimental studies were performed in the laboratory where realistic environmental pressures are lacking (e.g., Porter and Tschinkel 1985a; Colin et al. 2017). Indeed, the distinct environmental conditions (photoperiod, temperature, food availability, social environment) of laboratory and field experiments cause variation in the behavior of both vertebrates and invertebrates (Campbell et al. 2009).

Size diversity and mean worker size increase with colony size (respectively, Ferguson-Gow et al. 2014; Porter and Tschinkel 1985b). Only colonies with sufficient resources can produce large workers and enhance worker size diversity. It is important to disentangle these two traits in order to properly conclude about the adaptive value of size diversity and mean worker size within colonies of social insects. Indeed, a recent study on bumblebee colonies highlighted a positive correlation between colony performance and mean worker size, but no link with worker size diversity (Herrmann et al. 2018).

In this study, we aimed at quantifying the impact of within-colony worker size diversity and worker mean size on colony

fitness using a long-lasting field-based experiment with extensive measures of fitness (survival, growth, and reproductive success). We focused on an ant species lacking discrete worker subcastes and exhibiting moderate worker size diversity, *Temnothorax nylander* (see Appendix 1). In *Temnothorax* species, worker size does not determine worker specialization (Pinter-Wollman et al. 2012); task allocation is highly flexible (Robinson et al. 2009) and it relies on physiological parameters (Robinson et al. 2012). Colin et al. (2017) manipulated worker size diversity within *T. nylander* colonies and challenged colonies in the laboratory with various tasks (nest emigration, foraging, hygienic behavior, cold shock, etc.). They did not find any differences in performance between colonies with reduced size diversity and control colonies with unmanipulated diversity. Moreover, neither mean size nor size diversity correlate with per-capita productivity (Modlmeier and Foitzik 2011). This suggests that worker size diversity is not adaptive in this species, although larger individuals survive longer to cold temperature (Heinze et al. 2003) and starvation (Modlmeier et al. 2013). We tested whether this holds true in the field by manipulating the size of workers in colonies in the laboratory. Importantly, we manipulated both within-colony worker size diversity (decrease) and mean worker size (increase, decrease, or no change) in order to disentangle their effects. We subsequently re-implanted colonies in the field for 4 months to expose them to natural biotic and abiotic selective pressures, and we subsequently measured colony fitness extensively. In *Temnothorax* species, brood production is highly synchronized (Headley 1943; Kipyatkov 1993), so, in addition to colony survival, we were able to quantify two additional fitness parameters, namely, colony growth (based on the production of new workers) and reproductive success (based on the production of sexuals). In addition, because workers control larval development (Linksvayer et al. 2011), we expected colonies to compensate for the size of the removed individuals and restore diversity by producing workers of the very same size if size diversity is somewhat important for colony fitness.

Material and methods

Ants

Colonies of *Temnothorax nylander* nest in twigs or acorns and are thus easy to pick up from the forest leaf litter. One hundred and forty-six colonies of *T. nylander* were collected in February 2019 in the “Bois de Vincennes” forest (Paris, France, 48° 50' 22.14" N, 2° 26' 51.96" E). In the laboratory, each colony was installed in an artificial nest consisting of two microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers. The nest was placed in a plastic box (11.5 × 11.5 × 5.5 cm) providing a foraging area (see Appendix 2). During their 2-week stay in the laboratory,

colonies were kept in a climatic chamber at 10–12 °C with a natural photoperiod mimicking the natural conditions at this period. Water was provided ad libitum in a tube plugged with cotton. Colonies were fed once with a freshly killed mealworm (*Tenebrio molitor*). Workers and brood were counted.

Manipulation of worker size

Out of the 146 colonies, 80 colonies were selected for the experiment based on the presence of one queen and at least 70 workers. Four treatments were performed and, in all treatments, 50% of workers were removed from the 20 colonies. In treatment 25S25L, we decreased worker diversity without changing mean worker size by removing the 25% smallest workers and the 25% largest workers. In the two other treatments, we not only decreased worker diversity but also either increased mean worker size by removing the 50% smallest workers (treatment 50S) or decreased mean worker size by removing the 50% largest workers (treatment 50L). The last treatment was the control (treatment 50R) as we removed 50% of workers randomly. The worker removal protocols were similar to Colin et al. (2017) and based on the apparent global body size: the sorting of large and small workers was done by eye under a stereoscopic microscope (Zeiss, $\times 50$ magnification) whereas the removal of random workers was done without a microscope to make sure that worker size could not be evaluated. This method was proven to be reliable (Colin et al. 2017). Colonies were assigned to the four treatments based on their number of workers in order to keep a similar distribution of colony sizes among the four treatments. Workers remaining in the colonies after manipulation constituted the initial workforce of the colonies ($67.8 \text{ workers} \pm 22.5$). Colonies initially contained $91.6 \text{ larvae} \pm 27.7$.

Colony rearing in the field

We manufactured artificial nests to make them match natural nests as closely as possible, so that colonies could easily live in them after reintroduction in nature. For that purpose, we used 2.5×2 cm truncated cone corks. We drilled a 4-, 5- or 6-mm-wide chamber from the larger side of the cone cork, and plugged this side with a glue gun to seal the chamber. Then, a 1-mm-wide entrance gallery was pierced from the smaller side of the cone cork using a pointed plier in order to connect the chamber to the outside. In the laboratory, six corks (two of each size) were placed inside each plastic box, and we induced the emigration of colonies into the corks of their choice by removing the cover glass of the original nest. Then, the six corks from each plastic box (one containing the colony) were reintroduced in the “réserve ornithologique du Bois de Vincennes” in a semi-buried (10-cm deep) 40×35 cm bucket with a pierced bottom (for water draining) and containing local soil (see Appendix 3). The bucket lid was cut off into a ring shape and the bottom side was covered with Fluon®, a slippery coating, to prevent ant

escape while retaining a wide entrance. The six corks were placed randomly. This allowed colonies to relocate to the nest of their choice (size and location) inside the bucket whenever they wanted to. Indeed, *T. nylanderi* colonies readily switch nest depending on environmental conditions (Foitzik et al. 2003). Because the 80 buckets had been in place for a year, the soil and litter in the buckets were very similar to those of the surrounding forest; many arthropods and soil organisms could come in and out of the buckets. A preliminary food-supplementation experiment showed that adding food into the buckets did not increase colony survival and growth, so the 40-cm-wide foraging area provided enough food for the colonies. Colonies were left in the buckets from 26th of March to 16th of July, and subsequently collected and brought back to the laboratory. Corks were collected in the early morning, when workers were inactive because of cool temperature, in order to make sure that complete colonies were collected. Cork nests were destroyed, and colonies were forced to move to artificial microscope-slide nests. Workers, sexual and worker pupae, and larvae were counted. There were no sexual adults yet. Brood production is highly synchronized during summer (July–August) in *Temnothorax* (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011); therefore, the individuals that we recovered as pupae most likely reflected the total production of the colonies. We allowed pupae to molt into adults in order to assess the sex of the brood. To do so, colonies were kept in the laboratory at room temperature (21 ± 2 °C) with a natural photoperiod. Water was provided ad libitum, and colonies were fed once a week with half a freshly killed mealworm. Emerging adults were killed by freezing and then stored in 96° ethanol. The workers, males, and gynes counted as “newly produced” by colonies were individuals that emerged in the laboratory after developing into pupae in the field. All adults had emerged by 16th of August, and all remaining individuals were killed.

We considered that colonies had survived if they were recovered in July and if their queen was still alive. This loose definition of survival is an over-estimate relative to the proper life-history trait “colony survival,” as some colonies may actually have escaped from the bucket (entirely or only a fragment containing the queen, although this is highly unlikely because the bottom side of the lid was covered with a slippery coating—see “Material and methods”), and some colonies could have recovered from orphanage by later adopting a new fertilized queen (Foitzik and Heinze 2000). Queenless colonies were excluded from the analyses. Mortality rate of adult workers was computed as $1 - (\text{number of workers retrieved on 16th of July} / \text{initial workforce})$. Indeed, it is very unlikely that any workers were produced during this period (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011). We also counted and measured the workers and sexuals (gynes and males) produced out of the pupae brought back to the laboratory. Traits related to colony growth were the mortality rate of adult workers, the number of workers

Table 1 Models and statistics related to colony growth

Predictors	Response variables			
	Mortality rate of adult workers	Produced workers	CV of produced workers	Size of produced workers
Treatment	Minimum model $F_{57:66} = 1.94$, $F_{66:63} = 0.38$, $P = 0.8$ $P = 0.063$		$F_{63:60} = 0.39$, $P = 0.8$	$\chi^2_3 = 3.25$, $P = 0.3$
Initial workforce	Minimum model $F_{57:61} = 2.31$, $P = 0.069$		Minimum model $F_{66:67} = 51.81$, $P < 0.001$	$F_{63:62} = 0.02$, $P = 0.9$ $\chi^2_1 = 0.002$, $P = 0.9$
Treatment—initial workforce interaction	Minimum model $F_{57:60} = 2.52$, $P = 0.067$		$F_{66:63} = 0.50$, $P = 0.7$ $F_{63:59} = 0.43$, $P = 0.8$	$\chi^2_4 = 4.24$, $P = 0.4$
Initial larvae	Minimum model $F_{57:61} = 2.28$, $P = 0.07$		$F_{66:65} = 1.94$, $P = 0.2$ $F_{63:62} = 0.22$, $P = 0.6$	Minimum model $\chi^2_1 = 3.79$, $P = 0.05$
Treatment—initial larvae interaction	Minimum model $F_{57:60} = 2.95$, $P = 0.04$		$F_{66:62} = 0.55$, $P = 0.7$ $F_{63:59} = 0.54$, $P = 0.7$	$\chi^2_3 = 4.42$, $P = 0.22$
Mortality rate of adult workers		Minimum model $F_{66:67} = 4.44$, $P = 0.04$	$F_{63:62} = 0.05$, $P = 0.8$	$\chi^2_1 = 0.49$, $P = 0.5$
Treatment—mortality rate of adult workers interaction		$F_{66:63} = 0.40$, $P = 0.7$	$F_{63:59} = 0.33$, $P = 0.8$	$\chi^2_4 = 4.95$, $P = 0.3$
Produced workers			$F_{63:62} = 0.26$, $P = 0.6$	Minimum model $\chi^2_1 = 38.51$, $P < 0.001$
Treatment—produced workers interaction			$F_{63:59} = 1.01$, $P = 0.4$	$\chi^2_3 = 3.30$, $P = 0.3$

“Minimum model” means the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the size of produced workers in order to control for the colony of origin of workers

produced, the mean size of the workers produced and its coefficient of variation (CV). Traits related to reproductive success were the number of produced sexuals (gynes and males), the mean size of the sexuals and the sexual investment (number of sexuals/number of workers produced). All three main fitness components of colonies were thus measured.

Morphological measurements

The workers and sexuals that emerged in the laboratory were measured. Heads were separated from bodies. Heads were stuck on double-sided tape and photographed under a Discovery.V12 Zeiss stereomicroscope connected to a computer via a Zeiss AxioCam ICc 5 video camera. Head width, a standard measurement of body size in ants (Tschinkel et al. 2003), was measured with ImageJ 1.8 (Abramoff et al. 2004), and measurements were blind to treatment.

Statistical analysis

We investigated the effect of treatments (predictor variable, four levels) on colony survival, traits related to colony growth (mortality rate of adult workers, number of new workers produced, size of the produced workers, and CV of the size of produced workers) and reproductive success (number of produced sexuals, size of the sexuals and its CV, sexual investment). The mortality rate of adult workers and the number of produced individuals were also used as predictor variables in some models (Tables 1 and 2). So, in addition to treatments, we considered four other predictor variables, as follows: the initial

workforce (number of workers in a colony after the experimental removal of 50% of workers), the initial number of larvae in March, the mortality rate of adult workers, and the number of produced workers and sexuals. All first-order interactions between treatment and these variables were also included.

All statistical analyses were carried out with R v3.6.1 (www.r-project.org). All plots were generated using ggplot2 (Wickham 2009). First, we tested the effect of predictor variables on colony-level-dependent variables (mortality rate of adult workers, number of produced workers and males, sexual investment, and size and CV of the produced individuals) using generalized linear models (GLMs). Counting data (number of produced individuals and sexual investment) were analyzed using quasi-Poisson distribution. Mortality rate of adult workers and CV were analyzed using Gaussian distribution. Finally, we tested the effect of predictor variables on the head size of emerged individuals, an individual-level-dependent variable, using linear mixed models (LMMs, package *nlme*; Pinheiro et al. 2016), with colony as random factor. All models are summarized in Tables 1 and 2.

For each analysis, the minimum adequate model was selected using a backward stepwise approach where predictor variables were removed one by one from a full model based on a log likelihood ratio test. We used log likelihood ratio tests, i.e., F tests for Gaussian distribution or χ^2 tests for quasi-Poisson distribution, to obtain the P values for each predictor variable by comparing the minimum adequate model with a model excluding or including the variable of interest (according to whether the variable was present in or absent from the minimal adequate model respectively). Normality of

Table 2 Models and statistics related to reproductive success

Predictors	Response variables			
	Males produced	Sexual investment	CV of produced males	Size of produced males
Treatment	$F_{67:64} = 1.22, P = 0.3$	$F_{65:62} = 0.23, P = 0.9$	$F_{45:42} = 0.32, P = 0.8$	$\chi^2_3 = 0.22, P = 0.9$
Initial workforce	Minimum model $F_{67:68} = 20.11, P < 0.001$	$F_{65:64} < 0.001, P = 0.3$	$F_{45:44} = 0.20, P = 0.6$	$\chi^2_1 = 0.38, P = 0.5$
Treatment—initial workforce interaction	$F_{67:64} = 1.11, P = 0.3$	$F_{65:61} = 0.39, P = 0.9$	$F_{45:41} = 0.37, P = 0.8$	$\chi^2_4 = 1.15, P = 0.9$
Initial larvae	$F_{67:66} = 0.19, P = 0.7$	$F_{65:64} < 0.001, P = 0.9$	$F_{45:44} = 2.54, P = 0.1$	$\chi^2_1 = 0.02, P = 0.9$
Treatment—initial larvae interaction	$F_{67:63} = 1.28, P = 0.3$	$F_{65:61} = 0.24, P = 0.9$	$F_{45:41} = 1.08, P = 0.4$	$\chi^2_4 = 0.46, P = 0.9$
Adult worker mortality rate	$F_{67:66} = 0.07, P = 0.8$	$F_{65:64} = 0.36, P = 0.3$	$F_{45:44} = 0.08, P = 0.8$	$\chi^2_1 = 0.72, P = 0.4$
Treatment—adult worker mortality rate interaction	$F_{67:63} = 0.51, P = 0.7$	$F_{65:61} = 0.73, P = 0.7$	$F_{45:41} = 0.08, P = 0.9$	$\chi^2_4 = 1.78, P = 0.8$
Males produced			$F_{45:44} = 1.32, P = 0.3$	Minimum model $\chi^2_1 = 4.03, P = 0.04$
Treatment—males produced interaction			$F_{45:41} = 0.43, P = 0.8$	$\chi^2_3 = 0.12, P = 0.9$

“Minimum model” means the predictor was retained in the minimum model selected. A mixed effect model with colony as random factor was used for the size of produced males in order to control for the colony of origin of males

the residuals and homogeneity of variances were checked visually following Pinheiro and Bates (2000); no transformation of the data was necessary.

Results

Effect of treatments on colony survival

Only five of the 80 manipulated colonies could not be recovered during colony collection in July (three colonies 50S, one colony 50L, and one colony 25L25S). Six of the 75 recovered colonies had lost their queen (three colonies 50R and three colonies 50L). These queenless colonies were excluded from the statistical analysis. Our final dataset consisted of 69 queenright colonies.

Effect of treatments on growth

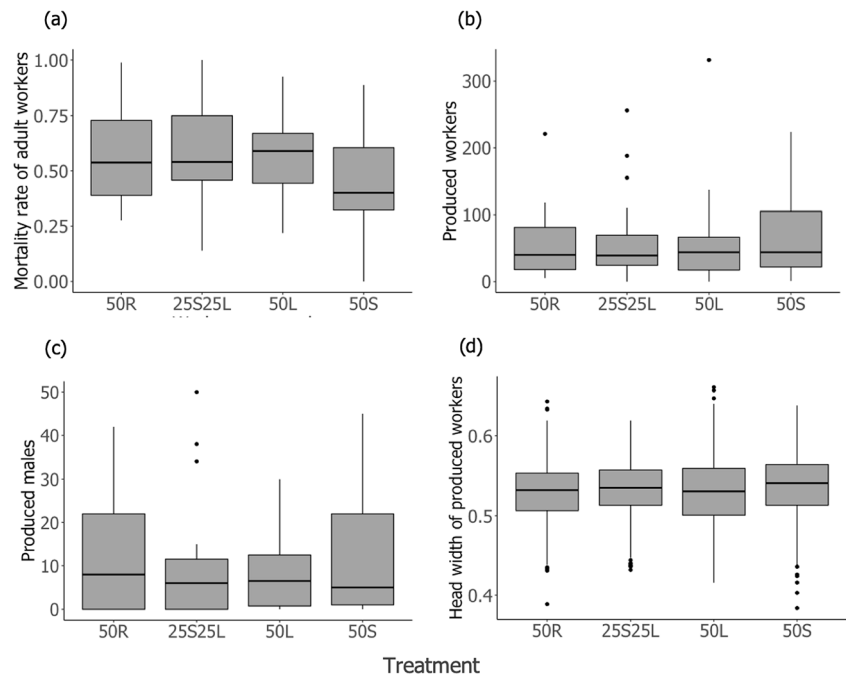
The mortality rate of adult workers depended on the initial number of larvae in interaction with the treatment (treatment \times initial larvae interaction, $F_{57:60} = 2.95, P = 0.04$). The mortality rate of adult workers decreased with the initial number of larvae with a steeper slope in treatment 50S (Fig. 2a). The mortality rate of adult workers did not differ among the treatments ($F_{57:66} = 1.94, P = 0.063$; Fig. 1a). Predictors that had a marginal effect ($0.05 < P$ value < 0.07) and could therefore slightly affect the dependent variable were retained in the minimum adequate model.

A total of 4341 workers emerged in the laboratory from 66 queenright colonies (3 colonies did not produce any worker). The number of produced workers was positively correlated with the initial workforce ($F_{66:67} = 51.81, P < 0.001$; Fig. 2b) and negatively correlated with the mortality rate of adult workers ($F_{66:67} = 4.44, P = 0.04$), without significant interaction with the treatments (see Table 1). The number of produced workers did not differ among the treatments ($F_{66:63} = 0.38, P = 0.8$; Fig. 1b). The coefficient of variation of the size of produced workers did not differ among the treatments ($F_{63:60} = 0.39, P = 0.8$), and it was not explained by any other predictor (see Table 1). The size of produced workers increased with the number of produced workers (mixed effect model: $\chi^2_1 = 38.51, P < 0.001$; Fig. 3a) and with the initial number of larvae ($\chi^2_1 = 3.79, P = 0.05$). The treatments had no effect on the size of produced workers ($\chi^2_3 = 3.25, P = 0.3$; Fig. 1d). Detailed statistics are presented in Table 1.

Effect of treatments on reproductive success

No gynes were produced except for three in one colony of treatment 50S. However, 57 queenright colonies produced 809 males. The number of males was positively correlated with the initial workforce ($F_{67:68} = 20.11, P < 0.001$; Fig. 2c) but was not affected by the treatment ($F_{67:64} = 1.22, P = 0.3$). The size of produced males was positively correlated with the number of males ($\chi^2_1 = 4.03, P = 0.04$; Fig. 3b). The sexual investment (number of sexuals/number of produced workers) and the CV of the size of produced males were not explained by any predictor. Detailed statistics are presented in Table 2.

Fig. 1 Boxplots comparing the effects of the four treatments (50R, 50% of randomly removed workers; 25S25L, the 25% smallest and the 25% largest removed workers; 50L, the 50% largest removed; 50S, the 50% smallest removed) on four life history traits, as follows: **a** mortality rate of adult workers, **b** number of produced workers, **c** number of produced males, and **d** head size of produced workers (mm). Boxes show median, quartiles, and extremes. Statistics are presented in Table 1 for workers and Table 2 for males



Discussion

In this study, we aimed at quantifying the impact of worker size and size diversity within colony on colony fitness using a laboratory manipulation followed by a reintroduction in the field. Because brood production is synchronized in *Temnothorax nylanderii* (Headley 1943; Kipyatkov 1993), we were able to measure both colony growth and sexual investment in addition to survival, and thus to extensively quantify fitness. In agreement with a previous laboratory study (Colin et al. 2017), reducing worker size diversity did not affect colony fitness. Colony survival, reproductive success (the number of males and the investment in sexuals) and colony growth (the number of produced workers) did not differ among the four treatments (removal of large, small, large and small, or random workers). Surprisingly, treatments that reduced or increased mean worker size did not affect fitness either. We also found that colonies did not compensate for the size of the removed workers by producing workers of the same size, which further suggests that worker size diversity has no strong effect on colony fitness.

The initial workforce of colonies had a major impact on colony fitness in our experiment, which is not the case in other species of the *Temnothorax* genus (Dornhaus et al. 2008; Bengtson and Dornhaus 2013). Indeed, we found a positive relationship between the initial workforce and the number of produced workers and males. This confirms that in *T. nylanderii*, large groups grow and reproduce better (Foitzik and Heinze 2000; Foitzik et al. 2003). Only one colony produced gynes. This might be a consequence of the experimental removal of half of the workers from each colony, which resulted in a loss of workforce and a

redirection of resources towards workers and males, as already known in *T. nylanderii* (Foitzik and Heinze 2000; Foitzik et al. 2003).

Our results are in line with Colin et al. (2017), who found no impact of reducing worker size diversity on colony performance in the laboratory in the same species, and Modlmeier and Foitzik (2011) who found no correlation between worker size diversity and per capita productivity in the field in *T. longispinosus*. Modlmeier and Foitzik (2011) also found no effect of the mean worker size on colony productivity. Our experimental manipulation of mean worker size confirms that it does not affect colony fitness. Moreover, colonies do not compensate for the size of the removed workers, reinforcing the idea that worker size diversity is not of primary value to them. Billick (2002) found a similar pattern in *Formica neorufibarbis*. He collected 45 workers from field colonies, and removed the 15 largest workers (7% of colony size). He followed colony development for 35 days and found no consequence on the number of pupae produced per worker. In addition, although a positive relationship between initial workforce and both mean worker size and worker size diversity is usually found in ants (Porter and Tschinkel 1985a; Ferguson-Gow et al. 2014), we did not find such a correlation. Our results hence confirm that this trait is not of primary importance for colonies. For the first time, our study proves the absence of effect of both mean worker size and worker size diversity in the field, over a long period of time, and with an extensive measure of colony fitness (three main life-history traits), making it a highly reliable result. We discuss three non-mutually exclusive hypotheses that could explain why within-colony worker size diversity still occurs.

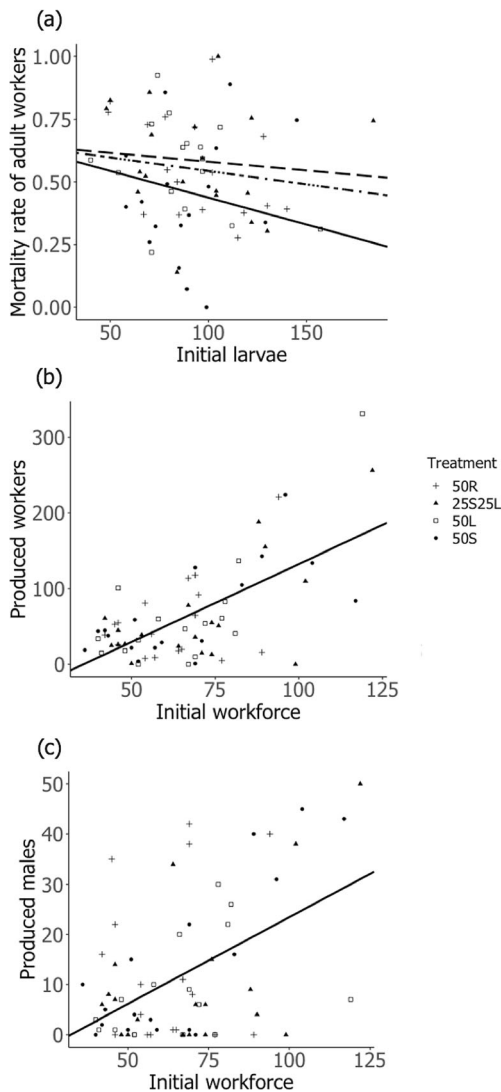
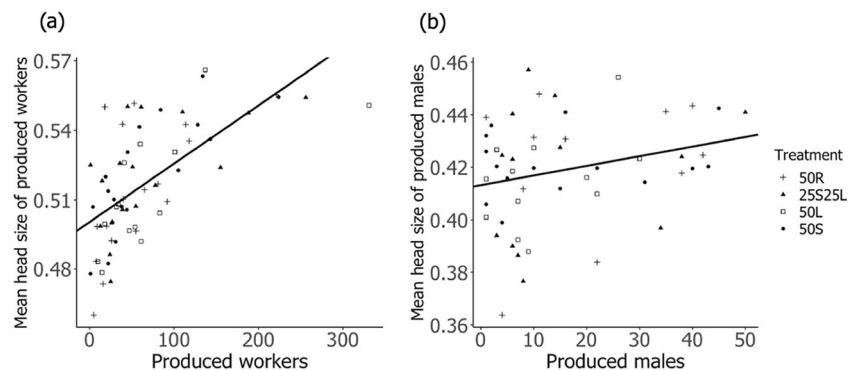


Fig. 2 Correlation between the initial larvae and workforce with three different traits. When the initial larvae increased, **a** the mortality rate of adult workers (50S: solid line type, estimate = $-2.141 \times 10^{-3} \pm 1.14 \times 10^{-3}$; 50R: dotdash, estimate = $-1.072 \times 10^{-3} \pm 1.08 \times 10^{-3}$; 50L: dotted, estimate = $-1.074 \times 10^{-3} \pm 1.16 \times 10^{-3}$; 25L25S: longdash, estimate = $-0.701 \times 10^{-3} \pm 1.03 \times 10^{-3}$). When the initial workforce increased, **b** the number of produced workers increased similarly for all treatments, and **c** the number of produced males increased similarly for all treatments. The regression lines are drawn from the coefficients of the different models. The statistics are presented in Table 1 for workers and Table 2 for males

Fig. 3 Correlation between the mean head size (mm) and the number of **a** produced workers and **b** produced males. The regression lines are drawn from the coefficients of the models and did not differ among treatments. Statistics are presented in Table 1 and Table 2



First, worker size diversity could only bring advantages in the context of harsher environmental conditions. In *T. nylanderii*, large workers resist better to starvation (Modlmeier et al. 2013). In the closely related *Leptothorax acervorum*, larger individuals survive longer in cold temperature (Heinze et al. 2003). Based on our results, such individual-level response (individual survival) does not seem to translate to the group level (colony survival). Further experiments are needed to fully explore the role of size diversity in the context of harsher environmental conditions (e.g., during overwintering) or over a full life cycle.

Second, size diversity could result from relaxed selection. Group living can buffer selective pressures on individual development because (1) phenotypes can diverge and still be viable under the protection of the social environment (Molet et al. 2012) and (2) workers themselves can control the environment of developing larvae and adjust their development if needed (Smith et al. 2008; Segers et al. 2015). This could thus decrease the level of canalization (the ability of development to remain unchanged despite environmental or genetic variation; Debat and David 2001), and thereby increase worker size diversity. The cost of maintaining developmental canalization (Van Buskirk and Steiner 2009) could be outbalanced by the lack of benefits that it brings in a social context. Accordingly, size diversity, in species where it is moderate, could be not adaptive per se but just economical to avoid the costs of canalization (Hunt et al. 2011; Colin et al. 2017).

Third, larvae could exhibit selfish strategies (Amor et al. 2011, 2016). Larvae may seek to receive more resources in order to become larger individuals, and increase their direct fitness by laying male eggs (Stroeymeyt et al. 2007). Heinze and Oberstadt (1999) showed that large workers of the ant *Leptothorax gredleri* (closely related to *T. nylanderii*) have higher social ranks than small ones, so they are more likely to be the ones that lay male eggs. It would be interesting to compare larval development in the presence or absence of workers to shed light on this potential conflict. In *Ectatomma tuberculatum*, larval begging does not fully reflect the nutritional status, suggesting that larvae do cheat to receive more food (Sauvaget 2017).

The ecological success of *T. nylanderi* (up to 10 colonies m^{-2} , Heinze et al. 1996; with a wide distribution throughout Western European forests, woods, and parks, Csoz et al. 2015; Khimoun et al. 2020) probably originates from unassessed traits other than mean worker size or worker size diversity, such as behavioral diversity. Indeed, *Temnothorax* ants demonstrate highly flexible and context-dependent task specialization (Pinter-Wollman et al. 2012), and they can quickly respond to changes in the required tasks (Robinson et al. 2009). Colonies with high within-colony behavioral diversity are more productive than colonies with less variation (Modlmeier et al. 2012). This may apply more generally to social insects. Indeed, in a ponerine ant lacking size diversity, age-associated division of labor is highly flexible. Foragers can revert to nursing and egg laying (Bernadou et al. 2015). Moreover, the functioning of honeybee societies is based on the coexistence of physiologically specialized individuals that perform fixed functions such as nectar processing, and physiologically plastic individuals that are also more flexible behaviorally (Johnson 2003). To summarize, the ecological success of social insects with high size diversity could come from their sharp division of labor in relation with workers morphology (with the exception of *Veromessor pergandei* and *Pheidole dentata*, respectively Rissing and Pollock 1984; Calabi and Traniello 1989), whereas the success of species with moderate size diversity could result from their behavioral ability to quickly adapt to specific contexts.

Fig. 4 Worker size diversity within one colony of *Temnothorax nylanderi*. In our experiment, the head width of the smallest worker produced was 0.384 mm and that of the largest was 0.661 mm



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Author's contributions RH designed the study, collected and reared ants, performed the experiment and statistical analyses, and wrote the manuscript. CD designed the study, contributed to statistical analyses, and wrote the manuscript. MM designed the study, wrote the manuscript, and supervised the project. All authors read and approved the final manuscript.

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Data availability The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Compliance with ethical standards

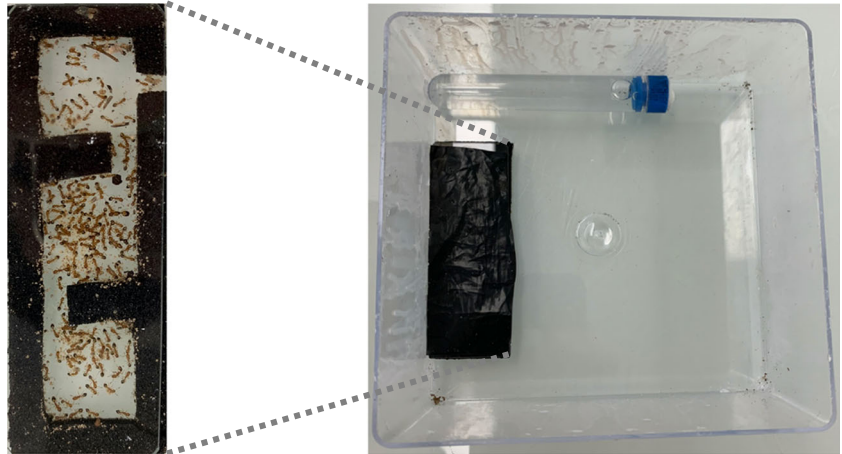
Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Appendix 1

Appendix 2

Fig. 5 Laboratory rearing setup. The nest consisted of two microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers, covered with black paper for darkness. It was placed in a plastic box (foraging area) with water (tube plugged with cotton) and food (mealworm)



Appendix 3

Fig. 6 Field rearing setup. Eighty semi-buried buckets were installed in the “réserve ornithologique du Bois de Vincennes.” Each bucket contained local soil and was pierced at the bottom for water draining. Six nests (drilled corks) were available for the colony to choose. The foraging area was restricted to the bucket. Cut-off lids are not present on the photos



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