



HAL
open science

Early social context does not influence behavioral variation at adulthood in ants

Iago Sanmartín-Villar, Raphaël Jeanson

► **To cite this version:**

Iago Sanmartín-Villar, Raphaël Jeanson. Early social context does not influence behavioral variation at adulthood in ants. *Current zoology*, 2021, 10.1093/cz/zoab063 . hal-03360576

HAL Id: hal-03360576

<https://hal.science/hal-03360576>

Submitted on 14 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Original Article

Early social context does not influence behavioral variation at adulthood in ants

Iago SANMARTÍN-VILLAR^{a,b,*} and Raphaël JEANSON^{a,†}^aCentre de Recherches Sur la Cognition Animale, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UPS, Toulouse, France and ^bUniversidade de Vigo, ECOEVO Lab, Escola de Enxeñaría Forestal, Campus A Xunqueira, Pontevedra, Spain[†]Current address: Escola de Enxeñaría Forestal, Campus A Xunqueira 36005, Pontevedra, Galiza, Spain

*Address correspondence to Iago Sanmartín-Villar. E-mail: sv.iago@gmail.com.

Handling editor: Zhi-Yun Jia (贾志云)

Received on 7 April 2021; accepted on 27 July 2021

Abstract

Early experience can prepare offspring to adapt their behaviors to the environment they are likely to encounter later in life. In several species of ants, colonies show ontogenic changes in the brood-to-worker ratio that are known to have an impact on worker morphology. However, little information is available on the influence of fluctuations in the early social context on the expression of behavior in adulthood. Using the ant *Lasius niger*, we tested whether the brood-to-worker ratio during larval stages influenced the level of behavioral variability at adult stages. We raised batches of 20 or 180 larvae in the presence of 60 workers until adulthood. We then quantified the activity level and wall-following tendency of callow workers on 10 successive trials to test the prediction that larvae reared under a high brood-to-worker ratio should show greater behavioral variations. We found that manipulation of the brood-to-worker ratio influenced the duration of development and the size of individuals at emergence. We detected no influence of early social context on the level of between- or within-individual variation measured for individual activity level or on wall-following behavior. Our study suggests that behavioral traits may be more canalized than morphological traits.

Key words: brood care, canalization, locomotion, metamorphosis, ontogeny, variability.

Early life experience can have lasting influences on the expression of phenotypic traits. Social context is among the many factors that can impact the development of individuals and the expression of their behavioral performance (Creel et al. 2013; Sachser et al. 2013; Bengston and Jandt 2014; Taborsky 2017). Social cues obtained before or after birth can prepare offspring to adjust their behaviors to the social environment they are likely to experience later in life (Taborsky 2016). Such early-life effects are widespread both in vertebrates (e.g., mammals, Guenther et al. 2014; birds, Bentz et al. 2013; fish, Chapman et al. 2008) and invertebrates (e.g., locust, Islam et al. 1994).

In social insects, the quality of care provided to the brood is an important component shaping the expression of adult phenotypic

variations. A paradigmatic example is found in honey bees where the quality of food given to larvae determines their fate in the queen or worker caste (Schwander et al. 2010; Kamakura 2011). In ants, a critical element that influences the quality of brood care is the number of brood items relative to the number of workers (Cassill and Tschinkel 1999). At different phases of their life cycle, ant colonies experience natural variations in the brood-to-worker ratio. The most dramatic changes probably occur during the earliest developmental stages of the colony, when newly mated queens initiate their colonies. The per-capita productivity, defined as the total number of workers and sexuals produced by a colony divided by colony size, often declines with colony size (Kramer et al. 2014). Therefore, this

implies a reduction in the brood-to-worker ratio (number of brood items divided by worker number) over colony ontogeny.

Colony maturation is accompanied by phenotypic changes in workers, particularly in size, a trait that has received the most attention. Indeed, incipient colonies typically produce workers of smaller size than those produced at later colony ages (e.g., Porter and Tschinkel 1985; Gibson 1989; Dejean and Lachaud 1992; Billick 2001). When the number of larvae exceeds the number of workers in the earliest stages of the colony, the brood may also be exposed to irregular and fluctuating attendance. Indeed, small nests generally perform less well in dampening the effects of external fluctuations than larger ones that offer more steady conditions for developing brood (Kleineidam and Roces 2000). Previous studies in ants suggested that a brood-to-worker ratio of 1:1 is the tipping point associated with a change in nursing behavior and an impact on worker size (Cassill and Tschinkel 1999; Purcell et al. 2012). Variations in the brood-to-worker ratio can impact the distribution of food supplied to the developing brood, for example, in the ant *Myrmica rubra* where larvae received more food when reared by many workers (Evesham 1985). Similarly, larvae of *Solenopsis invicta* experienced a substantial decline in feeding rates when reared under a high brood-to-worker ratio (Cassill and Tschinkel 1999). In a pioneering study, Brian (1953) reported that increasing the brood-to-worker ratio in experimental colonies of the ant *M. rubra* resulted in a decrease in the size of the adult progeny ultimately produced. Because then, several studies have documented similar trends in other ant species (e.g., Gray 1971; Porter and Tschinkel 1985; Purcell et al. 2012). Yet, it remains to be determined how variations in the brood-to-worker ratio experienced during larval development affect the behavior of workers in the adult stage. Unlike body size, which is definitive at the time of eclosion in ants, behavior is a labile trait that can vary during adult life. Evidence is now accumulating across taxa that individuals experiencing distinct early-life conditions can differ in their level of behavioral variation at later stages (Stamps 2016). For example, the activity and boldness patterns of adults of the beetle *Phaedon cochleariae* are influenced by the density experienced in the larval stages (Müller et al. 2016). Also, tadpoles exposed to predatory cues during early development showed greater between-individual variations in plasticity for activity (estimated by the distance traveled in a given time) than did controls (Urszán et al. 2018).

Between-individual differences in behavioral performance are the main ingredient of division of labor in social groups (Beshers and Fewell 2001; Jeanson and Weidenmüller 2014). As in other biological systems where specialization scales with system size (Bell and Mooers 1997; Bonner 2004), insect colonies typically show a general increase in division of labor as colonies grow from a few to many individuals (Thomas and Elgar 2003; Jeanson et al. 2007; Holbrook et al. 2011; Ulrich et al. 2018). A lower level of specialization could be beneficial in the incipient stages by allowing colonies to adapt more quickly to fluctuating conditions (Moritz and Page 1999). The scaling between colony size and division of labor during ontogenesis might arise incidentally via by-products of increased colony size including, for instance, a reduction in demand or a rise in task number in large colonies (Jeanson et al. 2007). The existence of individual behavioral changes as colonies mature represents a possible additional mechanism, with workers in incipient colonies being more flexible and less behaviorally canalized than those in larger colonies (Jeanne 1986; Gordon 1989; Jeanson 2019). Irregular or less intensive brood care may be used by larvae as an indication of an incipient colony stage and may prepare the newborn

workers to show flexible behavioral patterns to enable colonies to adapt effectively and rapidly to the changing needs faced by growing societies (Jeanson 2019).

This study manipulated the brood-to-worker ratio in the black garden ant *Lasius niger* to test the hypothesis that the social context experienced at pre-imaginal stages can impact the morphology of workers and the expression of behavioral variability at adulthood. We investigated activity level because earlier theoretical and experimental work has proposed the existence of changes in workers' activity patterns with colony size (Jeanson and Lachaud 2015; Waters et al. 2010 but see Holbrook et al. 2011). Moreover, this trait has been repeatedly used to assess behavioral variability in both vertebrates (e.g., Biro and Adriaenssens 2013; Bierbach et al. 2017) and invertebrates (e.g., Lichtenstein et al. 2017; Niemela et al. 2019). Recording activity in an open field also offers the advantage of allowing each individual to be tested many times (here, 10 observations per individual) in a standardized assay and of allowing several individuals to be tested (here, 273 individuals). Additionally, we considered the proportion of time workers spent on the periphery of the arena. Wall-following behavior or thigmotaxis represents an important component of the strategy used by many insects, including ants, to navigate their environment (Pratt et al. 2001; Dussutour et al. 2005). Interestingly, a link between individual variation spatial cognitive performance and thigmotaxis has been reported in the field cricket *Gryllus texensis* (Doria et al. 2019). Thigmotaxis has also been used as a measure of boldness in invertebrates (e.g., Detrain et al. 2019) and vertebrates (e.g., Sneddon 2003; Carlson and Langkilde 2013).

We compared behavioral variation by considering between-individual differences in temporal plasticity (i.e., extent of change as a function of time; Stamps and Groothuis 2010; Stamps 2016) and within-individual variability (or predictability) among colonies and treatments (Stamps et al. 2012; Westneat et al. 2015). We predicted that workers reared under a high brood-to-worker ratio, which presumably experienced less frequent brood care, would exhibit increased between and within-individual variations in behavior.

Material and Methods

Experimental procedure

Three source colonies (A–C) of *L. niger* were collected in southwest France (Canens, Haute-Garonne, France; 43°18'N, 1°15'E) in March 2019. Each source colony was installed in a plastic tray (28 cm × 28 cm × 9 cm) with Fluon[®]-coated walls that contained dark nesting tubes, water, and food ad libitum (Dussutour and Simpson 2008). After 10 days, we formed 90 experimental groups of 60 workers of unknown age in the source colony (30 groups from each source colony). The workers in each group were randomly collected from the source colonies to ensure that the composition of the groups of 60 ants was as similar as possible between treatments. Each group was introduced to an experimental nest that consisted a piece of cellular concrete (5 cm × 5 cm × 0.7 cm) with a hole drilled in the center ($\varnothing = 3.2$ cm, depth = 0.4 cm) and a lateral entrance (Figure 1).

The nest was covered with a transparent plastic lid and a red filter (E-Colour #19, Rosco Ltd, London, UK) and was placed in the center of a Petri dish ($\varnothing = 10$ cm) with Fluon[®] coated walls. Food (Dussutour and Simpson 2008) and water were provided ad libitum in plastic cups ($\varnothing = 1$ cm) placed in the Petri dish. The cellular concrete was watered every other day. The brood-to-worker ratio in 6-week-old colonies of *L. niger* is around 5:1 (Madsen and Offenberg

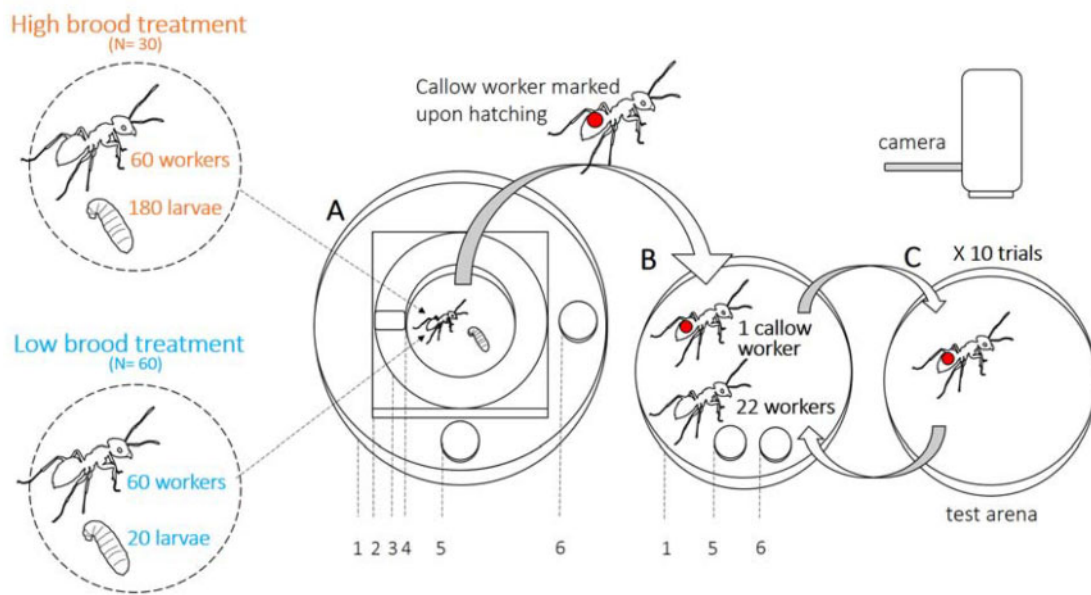


Figure 1. Schematic representation of the experimental design. Workers and larvae were introduced to experimental nests (A) according to the treatment ratios (dashed circles). Each callow worker that eclosed was marked and transferred to a dish (B) with 22 mature workers. After 48 h, each callow worker was transferred individually to a test arena (C) to record her activity for 60 min. After behavioral assay, the ant was returned to her dish (B). Each ant was tested 10 times. (1) Fluon[®] coated Petri dish. (2) Cellular concrete (nest). (3) Plastic lid and red filter. (4) Nest entrance. (5) Food container. (6) Wet cotton. Not at scale.

2017). For logistical reasons due to the number of larvae available, we used a ratio of 3:1 to simulate the ratio observed in incipient colonies. In 30 experimental nests (10 from each source colony), we introduced 180 larvae of early instar (brood-to-worker ratio = 3:1; hereafter high brood). In the remaining 60 experimental nests (20 from each source colony), we introduced 20 larvae (ratio = 1:3; hereafter low brood). We prepared twice as many experimental nests for the low brood treatment as for the high brood treatment to anticipate that the number of workers eclosion per experimental nest will be lower in the low brood treatment (due to the 9 times lower number of larvae).

The larvae introduced into each experimental nest were collected from their source colony and were of the same size. Each callow worker that eclosed in the experimental nests, during the course of experiments, was moved to another dish (see below) and 1 larva was added in replacement.

We used a photo of each experimental nest taken with a camera (Canon EOS 50D) 17 days after the start of the experiments (first quarter of total experiment duration) to estimate the number of larvae and workers. This allowed us to estimate whether the initial ratio of brood to worker was preserved despite the possible mortality of brood after the ants were installed into their experimental nests. The same picture was used to count the number of workers with a body part overlapping at least 1 larva. The proximity of the workers to the brood was used as a proxy for brood attendance because we assumed that the presence of individuals next to the larvae (and not only grooming or feeding behaviors) can provide cues to the larvae in the social context. Pilot experiments conducted on 2 other colonies revealed no significant fluctuation in the number of workers near the brood over a period of 30 consecutive days for either the low or high brood treatment.

Because our goal was to study the influence of the social environment experienced by larvae on adult behavioral variability by minimizing differences in the environment experienced as an adult, we needed to ensure that each focal worker experienced the same social

context after hatching. When a worker emerged, we placed it in a Petri dish ($\varnothing = 5.5$ mm, height = 1 cm) with 22 workers collected in its source colony. This ensured that each worker was exposed to the same condition, regardless of the treatment experienced in the larval stage. The callow individual was marked with a dot of paint (Edding 750[®]) on the abdomen the day after its emergence (callow workers can easily be distinguished on the basis of their light cuticular pigmentation which darkens several days after eclosion, Hartmann et al. 2019). Food and water were provided ad libitum.

Behavioral assays

For each test, the marked ant was gently collected with a soft paintbrush in the dish in which was maintained with the 22 workers (Figure 1). The focal ant was then introduced individually to a circular arena ($\varnothing = 5.8$ cm, height = 1 cm) with Fluon[®]-coated walls and covered with a glass lid. The arenas were placed in a white box (65 cm × 65 cm × 65 cm) with uniform lighting to reduce any spatial heterogeneity that might influence behaviors. Each trial lasted 60 min. Tests were performed during daytime in a climate room (25°C, 35% RH). We filmed up to 45 arenas (containing each a single ant) simultaneously with 3 cameras (Sony Handycam) placed above the experimental arenas and outside the white box. The first assay for each individual took place 48 h after it is emerged from the pupa. After each test, the ant was put back in its dish. Each ant was tested every 2 days for 20 days (i.e., 10 observations per ant). The longevity of *L. niger* workers under laboratory conditions is about 400 days (Kramer et al. 2016), which means that our estimate of behavioral variability covered ~5% of a worker's life span. Each movie was analyzed with the tracking software Swistrack version 4.0 (Correll et al. 2006) with the “Nearest Neighbour Tracking” method to extract automatically the coordinates of the ant in the arena at a rate of 2.08 frames/s. We used the fraction of time the ant spent moving during the 60-min test as a proxy for individual activity level (considering that an ant moved between 2 consecutive

images if the distance covered was equal to or >1 mm). We detected no relation between head size and the distance traveled by each individual in each test (linear mixed model (LMM) with head size and source colonies as fixed variables and ant ID as a random factor: $F_{1,265} = -1.266$, $P = 0.207$). However, we used time spent moving (not the distance) as an estimate of activity to reduce any influence of size differences in the estimation of behavioral variability. For each individual, we also determined a score of thigmotaxis, which we defined as the proportion of time callow workers spent walking at 1 cm (about twice the length of an ant) from the wall of the arenas during the 60-min test. All experiments were performed in May 2019. A total of 273 individuals were tested (Colony A: 100, Colony B: 81, Colony C: 92; low brood: 138, high brood: 135). These individuals were not randomly selected but corresponded to the first individuals hatched in each experimental nest before we stopped the experiments. We tested individuals alone because testing individuals in a group may attenuate interindividual differences in behavioral profiles, as active individuals may stimulate inactive individuals and thus lead, through amplification processes, to group members synchronizing their activity patterns (Cole 1991).

After the completion of behavioral assays, we assessed the size of callow workers by measuring head width (minimum width across the eyes) to the nearest micrometer using a stereomicroscope Nikon SMZ1000 at a magnification of $\times 40$. The head of 4 corpses was lost and therefore not measured.

Statistical analyses

Statistical analyses were performed using R version 4.0.5 (www.r-project.org). For each experimental nest, we computed the actual brood-to-worker ratio on Day 17 by dividing the number of brood items counted on Day 17 by the number of workers (i.e., 60). These ratios were compared with a t -test after log-transformation. For each experimental nest, we calculated the fraction of workers that were in contact with brood. These fractions were log-transformed and compared between treatments using a 1-way ANOVA with source colony and treatments as fixed factors. We compared the coefficients of variations (CV = standard deviation/mean) of the head size of workers raised under each treatment using the statistic D'AD of Feltz and Miller (1996) implemented in the function *asymptotic_test* from the package *cvequality*, version 0.2 (Marwick and Krishnamoorthy 2019). Head width and larval development (i.e., number of days between the start of the experiment and hatching) were compared between source colonies and treatments with LMMs (*lmer* function of the *lme4* package; Bates et al. 2015) using nest ID (i.e., the ID of nests in which workers emerged) as a random effect. We used the function *glbt* implemented in the *multcomp* R package (Hothorn et al. 2008) using Holm's correction to evaluate the differences in head size of workers or larval development between colonies.

For each behavior and treatment, we computed the intraclass correlation coefficient (ICC) as a measure of repeatability, that is, the proportion of total variance that can be attributed to variation between individuals (Nakagawa and Schielzeth 2010). We included observations and source colonies as fixed effects and ant ID as the grouping factor to compute ICC using the function *rptGaussian* (package *rptR*) (Stoffel et al. 2017). Values were considered significant when 95% confidence intervals did not overlap zero. We further analyzed behavioral data using a double-hierarchical LMMs (DHGLM) in a Bayesian framework with the R package "brms" (Bürkner 2017, 2018). DHGLM allows fitting simultaneously the mean and dispersion levels by considering both fixed and random

effects (Cleasby et al. 2015; Mitchell et al. 2016, 2021; Hertel et al. 2020, 2021). DHGLM is recommended to estimate variation in intra-individual variability (*rIV*) and to compare behavioral predictability between groups or treatments because they can model the structure of residual variance (Cleasby et al. 2015; Mitchell et al. 2021). We consider observations (Tests 1–10) and source colonies as fixed factors in the mean model. The fixed factor "observations" was mean-centered before fitting the models. The random factors in the mean model were ant ID and nest ID (i.e., the ID of nests in which workers emerged). In the residual model, we specified a random intercept (ant ID) to estimate individual differences in predictability (Mitchell et al. 2021). Values of model parameters were considered significant when 95% credible intervals did not overlap zero. We applied a logit transformation ($\log[p/(1-p)]$) to the score of thigmotaxis (i.e., proportion of time spent moving on the periphery of the arena) after adding 0.01 to null values ($N = 29$ out of 2,730) or subtracting 0.01 to scores of 1 ($N = 25$ out of 2,730).

For each treatment (low and high brood-to-worker ratios) and behavior (activity and thigmotaxis), we fitted model that included (Model 1) or not (Model 2) observations as random slopes in the mean part of the model to test whether workers that experienced distinct early social context (i.e., low versus high brood-to-worker ratio) differed in temporal plasticity at adulthood:

Model 1: performance \sim observations + colony + (1|nest) + (1|ID), *sigma* \sim (1|ID)

Model 2: performance \sim observations + colony + (1|nest) + (observations|ID), *sigma* \sim (1|ID)

with the dispersion part of each model in italics.

We assessed the predictive performance of each Bayesian model with the *loo* package which performs leave-one-out (LOO) cross-validation on the posterior likelihood to give the estimated log predictive density (ELPD) and its standard error (Vehtari et al. 2017). We compared models using the function *loo_compare* (Vehtari et al. 2017) implemented in the R package *brms*. The function *loo_compare* calculates the differences in ELPD (*elpd_diff*) between 2 models and the standard error of the difference (*se_diff*) to compare the predictive performance of the models.

Models with a small ELPD_diff with respect to the SE have similar predictive performance and the simpler model is preferred. In our study, a better fit of the model with random slopes (i.e. Model 2) would indicate that individuals differed in the way they varied their level of activity or thigmotaxis between observations. We next used the best model selected for each treatment and behavior to compute the residual intra-individual variation (*rIV*) (i.e., the spread of residuals around an individual's reaction norm). We used LMMs using nest ID as a random effect to test whether *rIV* for activity or thigmotaxis varied with head width and the duration of development.

Ethical statement

This work complied with the current laws in France on the use of invertebrates in research.

Results

Proximity of workers to the brood

First, we checked that the brood-to-worker ratio measured 17 days after the start of the experiment was, as expected, lower in the low brood (mean \pm SD: 0.30 ± 0.09 , $N = 60$) than in the high brood

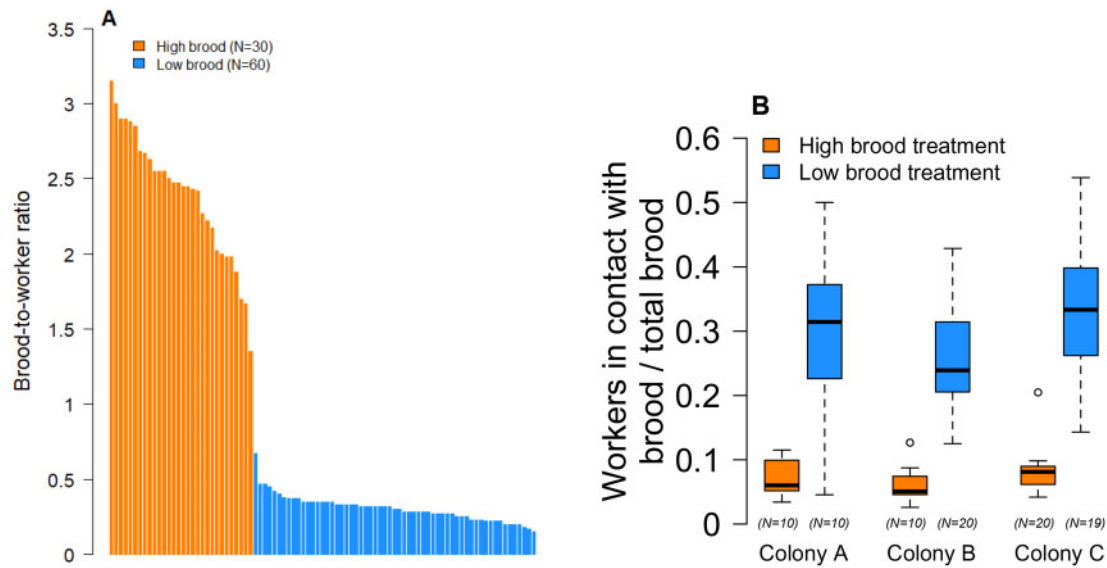


Figure 2. (A) Actual brood-to-worker ratio measured on Day 17. (B) Boxplots of the fraction of larvae in contact with workers in experimental nests from the high and low brood-to-worker treatments on Day 17. Horizontal line in each box represents the median, and the lower and upper hinges indicate the first and third quartiles. Lower and higher whiskers extend to the most extreme values within 1.5 interquartile ranges from the first and third quartiles, respectively.

treatment (mean \pm SD: 2.39 ± 0.43 , $N = 30$) (t -test: $t_{87} = 37.25$, $P < 0.001$; Figure 2A). The observed ratio differed slightly from the initial ratio due to brood mortality, but importantly, the average observed ratio was still almost 8 times higher for the high brood treatment than for the low brood treatment. The number of workers in contact with brood divided by the total number of larvae was >4 times greater in low-brood than in high brood treatment (ANOVA: $F_{1,83} = 183.94$, $P < 0.001$) and differed marginally between colonies (ANOVA: $F_{1,83} = 3.05$, $P = 0.05$; Figure 2B).

Larval development and size of callow workers

Workers that emerged in nests from the low brood treatment were larger than ants that developed in the high brood treatment (LMM: $F_{1,188.70} = 26.14$, $P < 0.001$). Source colonies also influenced body size (LMM: $F_{1,50.34} = 17.26$, $P < 0.001$) with workers from source Colony C being larger than those from colonies A (post-hoc glht: estimate = 0.014, $SE = 0.003$, $z = 4.90$, $P < 0.001$) or B (estimate = 0.011, $SE = 0.003$, $z = 3.67$, $P < 0.001$; Figure 3A). The duration of larval development was almost 2 days shorter in nests from the high brood than in the low brood treatment (LMM: $F_{1,52.92} = 4.76$, $P = 0.03$; Figure 3B). There was an influence of the source colony on development (LMM: $F_{1,57.68} = 42.27$, $P < 0.001$) with larvae from Colony B emerging faster than larvae from colony A (post-hoc glht: estimate = -4.06 , $SE = 0.66$, $z = -6.14$, $P < 0.001$), which themselves developed faster than those from Colony C (estimate = 2.02, $SE = 0.66$, $z = 3.05$, $P < 0.001$; Figure 3B).

In both treatments, body size at adulthood increased with the duration of larval development (LMM: $F_{1,188.70} = 26.13$, $P < 0.001$; Figure 3C). The coefficient of variation (CV) for head size was lower for workers reared under the low brood ($CV_{low} = 0.031$) than under the high brood condition ($CV_{high} = 0.044$) for Colony A (Feltz-Miller test: $D'AD = 5.87$, $df = 1$, $P = 0.015$) but not for Colonies B ($CV_{low} = 0.031$, $CV_{high} = 0.038$; $D'AD = 1.50$, $df = 1$, $P = 0.22$) or C ($CV_{low} = 0.040$, $CV_{high} = 0.037$; $D'AD = 0.21$, $df = 1$, $P = 0.65$).

Behavioral variations

A total of 2,730 tracks of 60 min each were analyzed (Figure 4). We found no differences in repeatability for activity between workers reared under a low (ICC [95% confidence intervals]: 0.31 [0.24, 0.37]) or a high (0.23 [0.17, 0.29]) brood-to-worker ratio. Similarly, workers from each treatment showed no difference in repeatability for thigmotaxis (low ratio: 0.11 [0.07, 0.15]; high ratio: 0.06 [0.02, 0.10]). Repeatability was 3 times lower for thigmotaxis than for activity.

The model with random slopes (Model 2) for activity provided a better fit than the model without random slopes (Model 1) both for workers of the low (ELPD diff = -19.7 , $SE = 6.7$) and high (ELPD diff = -22.4 , $SE = 6.74$) brood-to-worker ratio. Regardless of early social context, this indicated that workers exhibited interindividual differences in variations in activity level over the course of the trials (Figure 3). Results were more mixed for thigmotaxis, as models with random slopes only marginally improved fitting for workers from the low (ELPD diff = -11.9 , $SE = 5.9$) and high (ELPD diff = -8.8 , $SE = 5.1$) brood-to-worker treatment. We detected no influence of the early social context on the behavioral patterns for activity or thigmotaxis (Table 1, Figure 1). The level of activity decreased during successive trials but no similar trend was observed for thigmotaxis (Table 1). Workers from Colony C were more active but spent less time close to the wall of the arena than workers from Colonies A and B (Table 1). Overall, the early social context thus seemed to play no role in the expression of between-individual differences in plasticity.

Values of rIV for activity overlapped between workers from the low (median [first and third quartiles]: 0.09 [0.08, 0.11]) and high ratio (median [first and third quartiles]: 0.10 [0.09, 0.12]). Similarly, no difference in the values of rIV for thigmotaxis was evidenced between workers from each treatment (median [first and third quartiles]; low ratio: 1.14 [0.89, 1.49], high ratio: 1.15 [0.88, 1.44]). Accordingly, we found no difference in variation in behavioral predictability between workers from each treatment for activity (CVp [95% credible intervals]: low ratio: 0.35 [0.29, 0.43], high

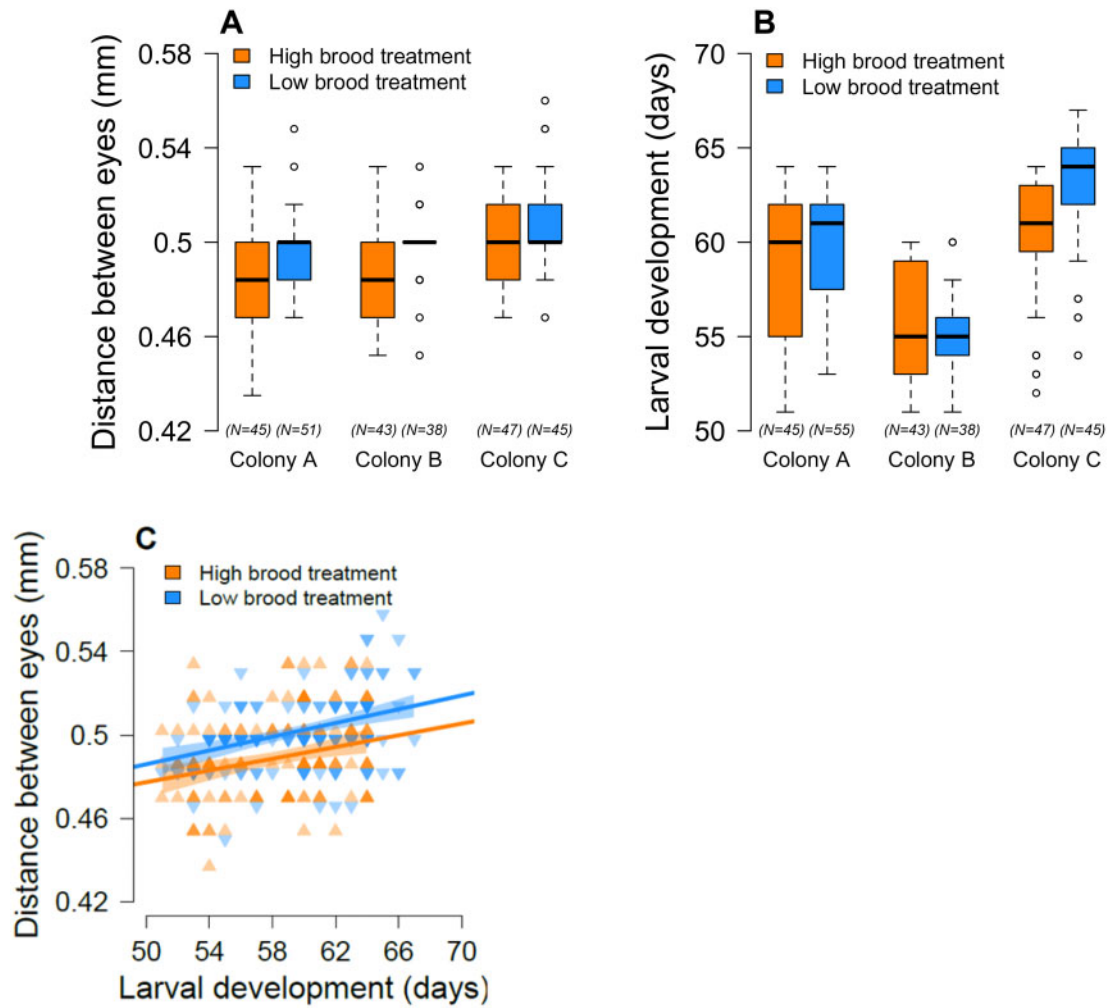


Figure 3. (A) Callow worker head size and (B) duration of larval development for each source colony and brood-to-worker ratio. Sample size is given within brackets. (C) Relationship between larval development and head size for each treatment. The color bands give the 95% confidence intervals of linear models.

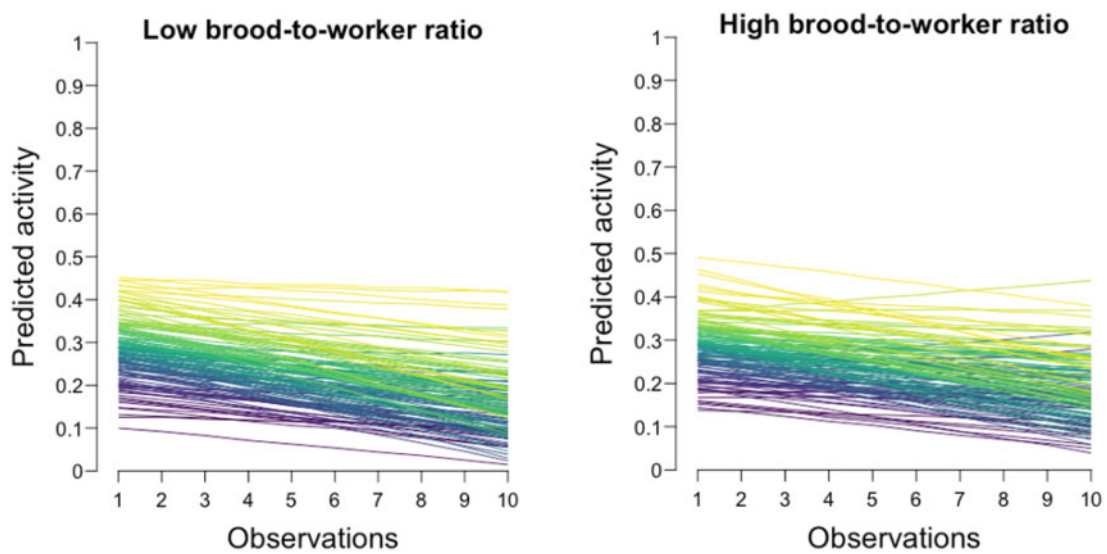


Figure 4. Predicted level of activity for workers reared under a low or high brood-to-worker ratio. Warm (yellow) to cool (purple) colors code for the level of activity measured during the first test. Predicted values were obtained from models using mean-centering for "observations," but raw values for "observations" are used on the plots for clarity.

Table 1. Estimates [95% credible intervals] of fixed and random effects on activity and thigmotaxis (mean model) and residual variations (dispersion model) for workers reared under a low or high brood-to-worker ratio

Mean model	Activity		Thigmotaxis	
	Brood-to-worker ratio		Brood-to-worker ratio	
	Low	High	Low	High
<i>Fixed effects</i>				
Intercept	0.226 [0.199, 0.254]	0.266 [0.243, 0.290]	2.631 [2.438, 2.826]	2.346 [2.155, 2.533]
Observations	-0.013 [-0.016, -0.011]	-0.010 [-0.013, -0.008]	0.008 [-0.016, 0.031]	0.020 [-0.006, 0.047]
Colony B	0.037 [-0.001, 0.077]	-0.002 [-0.036, 0.031]	-0.313 [-0.605, -0.025]	0.006 [-0.262, 0.276]
Colony C	-0.046 [-0.086, -0.008]	-0.082 [-0.116, -0.050]	0.397 [0.113, 0.690]	0.582 [0.313, 0.851]
<i>Random effects</i>				
ant ID				
sd _{intercept}	0.068 [0.055, 0.081]	0.059 [0.049, 0.070]	0.522 [0.426, 0.626]	0.370 [0.274, 0.467]
sd _{observations}	0.009 [0.006, 0.012]	0.010 [0.007, 0.013]	0.064 [0.033, 0.097]	0.091 [0.059, 0.122]
nest ID				
sd _{intercept}	0.027 [0.003, 0.050]	0.014 [0.001, 0.033]	0.138 [0.006, 0.319]	0.153 [0.013, 0.313]
Dispersion model				
ω_{ID}	0.342 [0.281, 0.410]	0.282 [0.222, 0.349]	0.430 [0.364, 0.505]	0.396 [0.333, 0.467]

Parameters with credible intervals that include 0 are nonsignificant. A logit transformation was used on the thigmotaxis data before fitting the models.

ratio: 0.29 [0.22, 0.36]) or thigmotaxis (CVp [95% credible intervals]: low ratio: 0.45 [0.37, 0.53], high ratio: 0.41 [0.34, 0.45]).

Discussion

In this study, we manipulated the brood-to-worker ratio (1:3 versus 3:1) to examine the influence of social context experienced in early ontogeny on the expression of individual variation in activity level and thigmotaxis at adulthood in ants. We hypothesized that exposure of larvae to a high brood-to-worker ratio during early ontogeny would result in more developmental noise and increased behavioral variability at adulthood. Our experimental design allowed us to examine the influence of the early social context whereas avoiding the possible confounding influence of maternal effects and adult's social experience on worker phenotypes. We first showed that larvae developed more slowly and produced larger workers when exposed to a low brood-to-worker ratio during pre-imaginal stage. We also found greater variation in head size between workers in the high brood than in the low treatment for 1 colony. Overall, this confirmed that our treatment was able to affect the phenotypes of workers. In our experiments, the nest surface was equal in both treatments, which led to a 9-fold increase in larval density in the high brood treatment, whereas keeping the number of workers constant. However, this increase in density and thus proximity between larvae did not result in an equivalent rate of brood attendance as shown by the number of contacts between workers and larvae being 4 times lower for the high brood treatment than for the low brood treatment (Figure 2). Similar patterns were observed in the ant *S. invicta* where larvae were tended at reduced rates when brood outnumbered workers (Cassill and Tschinkel 1999).

The manipulation of the brood-to-worker ratio triggered detectable changes in morphological traits and development time. This confirmed previous studies that reported a reduction of body size of about 5%, which is of the same order of magnitude as that found in our study, when the brood-to-worker ratio was high (Brian 1953; Purcell et al. 2012). The smaller body size observed in the high brood-to-worker ratio was likely the result of the workers' reduced ability to meet all the larvae's needs as documented in previous work in *S. invicta* where the rate of trophallaxis to feed brood was lower in colonies composed of an excess of larvae (Cassill and Tschinkel 1999). Interestingly, the experimental changes of the brood-to-worker ratio reproduce, albeit to a lesser extent, the differences in worker size observed in ant colonies during their development with the existence of nanitic or minim workers in societies at the initial stages of their development when the brood size greatly exceeds the number of workers (Tschinkel 1988). In honey bees, manipulation of the brood-to-worker ratio also resulted in changes in body weight, with bees being heavier at birth when reared in the presence of many workers (Eischen et al. 1983). The influence of the early social context on the development time offers more contrasted results because our study showed that larvae developed faster in the high brood-to-worker ratio condition whereas an opposite tendency seems to exist in the ant *Formica selysi* (Purcell et al. 2012), the reason for this discrepancy remaining unexplained.

Ants in each treatment showed decreasing activity over successive trials. It is unclear whether this reduction resulted from maturational processes associated with aging, as the ants were tested for 20 days immediately after emerging from the cocoon, and/or from habituation to the environment in which they were exposed 10 consecutive times. Further studies using older ants carried out under the same experimental conditions should make it possible to decide. This pattern of decreased activity contrasts with that found in the

Argentine ant *Linepithema humile* where individuals that were tested 5 consecutive times in a setup similar to that used in this study showed increased activity in workers (Sanmartín-Villar et al. 2021). Too few studies so far have tested individuals 5 or more times, so it is difficult to offer a plausible explanation for differences in activity patterns.

We expected callow workers from the high brood-to-worker ratio treatment to show higher between-individual differences in plasticity and also lower behavioral predictability (i.e., lower within-individual variance) than conspecifics reared under the low brood-to-worker ratio. We found that the level of activity was repeatable, meaning that individuals showed consistent individual differences in activity and contrasted with the lower level of repeatability observed for thigmotaxis. The absence of differences in repeatability between treatments for either behavior suggested that neither the colony nor early social context had a detectable influence on the expression of behavioral variations. Also, we detected no influence of the early social context on the expression of behavioral plasticity. Overall, our results lead us to reject our initial prediction that workers reared under a high brood-to-worker ratio would display greater individual variation. Although caution is required in drawing a conclusion based on 2 behavioral traits, our results suggest that the expression of behavioral variation in ants might be less vulnerable to differences in the early developmental context, and thus be possibly more canalized, than the production of a physical trait (i.e., body size). It might not be unexpected that effective regulatory mechanisms control the expression of the degree of behavioral variation, an uncontrolled level of which could have adverse consequences on a range of behavioral traits and jeopardize the stability of the organization of social groups.

Holometabolous insects, such as ants, undergo massive internal changes during metamorphosis (Consoulas et al. 2000) but previous studies have nevertheless shown that pre-imaginal experience can have lasting consequences on a range of behaviors (Amat et al. 2018). For instance, adult workers that experienced high colony-level aggressiveness during their preimaginal stages behave more aggressively than siblings exposed to low-aggression environment in the honeybee *Apis mellifera* (Rittschof et al. 2015). Similarly, the thermal response of adult brood-tenders in the ant *Camponotus rufipes* depends on the temperature experienced at pupal stages (Weidenmüller et al. 2009). Our study focused on brood care because ontogenic or seasonal changes in the worker-to-brood ratio could represent a simple mechanism requiring no explicit coding to adjust the behavioral diversity within the worker caste and allow colonies to produce optimal patterns of division of labor (Jeanson 2019). However, changes in the brood-to-worker ratio alone may not be sufficient to influence behavioral variability in callow workers, and the quality of nursing (e.g., amount of time that a larva is licked by a worker), an important component of brood care which has not been considered here, should be considered in future studies. This dimension could be more important as young workers, which are relatively abundant in incipient colonies, are less efficient brood tenders than older individuals (Muscedere et al. 2009). The impact of the quality of nursing, possibly in combination with changes in the brood-to-worker ratio, on the expression of behavioral variations deserves further attention.

Despite being used repeatedly across studies, the level of individual activity or thigmotaxis may not represent an appropriate measure to reveal behavioral variation between treatments. Although intra-individual variability reflects the existence of unpredictable

fluctuations resulting supposedly from spontaneous fluctuations in internal state (Stamps 2016), this does not necessarily imply that all behavioral traits are equally concerned. For instance, *Rana dalmatina* tadpoles reared in presence of predator olfactory cues showed higher predictability for activity level (distance traveled) than control animals, but no difference was observed in terms of risk-taking behavior (latency to resume activity after a simulated predator attack) between treatments (Urszán et al. 2018). Also, the effects of the environment on behavioral variability do not always go in the same direction. In the fruit fly *Drosophila melanogaster*, for example, Akhund-Zade et al. (2019) manipulated the enrichment of the environment and then introduced individuals to a Y-maze to collect several behavioral measures. They found that flies exposed to an enriched environment displayed increased variability for some behaviors (e.g., inter-choice intervals, number of turns) but reduced variability for others (e.g., turn bias, switchiness) in comparison to conspecifics that experienced standard rearing conditions. A high-throughput experimental pipeline developed to capture dozens of behaviors in *Drosophila* confirmed the existence of many independent dimensions of behavioral variations (Werkhoven et al. 2019).

Determining which measures are relevant to reveal changes in behavioral variability in response to differences in the early environment is important but challenging. The identification of the sources of variability often requires the use of controlled and standardized conditions that are potentially disconnected from natural conditions (e.g., Watanabe et al. 2012; Turza and Miler 2021). However, working in more natural environments is difficult because it may not be possible to determine whether the level of variability observed in adulthood is the result of specific developmental conditions or the context experienced at the time of observation. Further reflections on these aspects are necessary to better understand the role of the early social environment on the expression of behavioral variations in adulthood and to study its consequences on social organization in insect colonies.

Acknowledgments

We are grateful to Abel Bernadou, Violette Chiara, Adolfo Cordero-Rivera, and Cristian Pasquaretta for comments and Gérard Latil for assistance in ant maintenance. We thank anonymous reviewers for the comments.

Funding

Funding was provided by CNRS (www.cnrs.fr) and Université Toulouse III (www.univ-tlse3.fr) to R.J. I.S.-V. was funded by a post-doctoral fellowship of the Galician government (Xunta de Galicia; Axudas de apoio á etapa posdoctoral 2017; ref: ED481B-2017/034). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' Contributions

I.S.-V. contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, software, validation, visualization, writing—original draft, writing—review and editing. R.J. contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft, and writing—review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

Data and Material Availability Statement

All relevant data are within the paper and its Supporting Information files.

Code Availability Statement

Authors agree to make code supporting the results or analyses presented in this article available upon request.

References

- Akhund-Zade J, Ho S, O'Leary C, de Vivort B, 2019. The effect of environmental enrichment on behavioral variability depends on genotype, behavior, and type of enrichment. *J Exp Biol* 222:jeb202234.
- Amat I, Desouhant E, Gomes E, Moreau J, Monceau K, 2018. Insect personality: what can we learn from metamorphosis? *Curr Opin Insect Sci* 27: 46–51.
- Bates D, Maechler M, Bolker B, Walker S, 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48.
- Bell G, Mooers AO, 1997. Size and complexity among multicellular organisms. *Biol J Linn Soc* 60:345–363.
- Bengston SE, Jandt JM, 2014. The development of collective personality: the ontogenetic drivers of behavioral variation across groups. *Front Ecol Evol* 2:1–13.
- Bentz AB, Navara KJ, Siefferman L, 2013. Phenotypic plasticity in response to breeding density in tree swallows: an adaptive maternal effect? *Horm Behav* 64:729–736.
- Beshers SN, Fewell JH, 2001. Models of division of labor in social insects. *Annu Rev Entomol* 46:413–440.
- Bierbach D, Laskowski KL, Wolf M, 2017. Behavioural individuality in clonal fish arises despite near-identical rearing conditions. *Nat Commun* 8:15361.
- Billick I, 2001. Density dependence and colony growth in the ant species *Formica neorufibarbis*. *J Anim Ecol* 70:895–905.
- Biro PA, Adriaenssens B, 2013. Predictability as a personality trait: consistent differences in intraindividual behavioral variation. *Am Nat* 182:621–629.
- Bonner JT, 2004. Perspective: the size-complexity rule. *Evolution* 58: 1883–1890.
- Brian MV, 1953. Brood-rearing in relation to worker number in the ant *Myrmica*. *Physiol Zool* 26:355–366.
- Bürkner PC, 2017. brms: an R package for Bayesian multilevel models using Stan. *J Stat Softw* 80:1.
- Bürkner PC, 2018. Advanced Bayesian multilevel modeling with the R package brms. *R J* 10:395, 411.
- Carlson BE, Langkilde T, 2013. Personality traits are expressed in bullfrog tadpoles during open-field trials. *J Herpetol* 47:378–383.
- Cassill DL, Tschinkel WR, 1999. Effects of colony-level attributes on larval feeding in the fire ant *Solenopsis invicta*. *Insectes Soc* 46:261–266.
- Chapman BB, Ward AJW, Krause J, 2008. Schooling and learning: early social environment predicts social learning ability in the guppy, *Poecilia reticulata*. *Anim Behav* 76:923–929.
- Cleasby IR, Nakagawa S, Schielzeth H, 2015. Quantifying the predictability of behaviour: statistical approaches for the study of between-individual variation in the within-individual variance. *Methods Ecol Evol* 6:27–37.
- Cole BJ, 1991. Short-term activity cycles in ants: generation of periodicity by worker interaction. *Am Nat* 137:244–259.
- Consoulas C, Duch C, Bayline RJ, Levine RB, 2000. Behavioral transformations during metamorphosis: remodeling of neural and motor systems. *Brain Res Bull* 53:571–583.
- Correll N, Sempo G, De Meneses YL, Halloy J, Deneubourg JL et al. 2006. SwisTrack: a tracking tool for multi-unit robotic and biological systems. *IEEE Int Conf Intell Robots Syst; 2006 October 9–15; Beijing, China*. 2185–2191.
- Creel S, Dantzer B, Goymann W, Rubenstein DR, 2013. The ecology of stress: effects of the social environment. *Funct Ecol* 27:66–80.
- Dejean A, Lachaud JP, 1992. Growth-related changes in predation behavior in incipient colonies of the ponerine ant. *Ectatomma tuberculatum* (Olivier). *Insectes Soc* 39:129–143.
- Detrain C, Pereira H, Fourcassié V, 2019. Differential responses to chemical cues correlate with task performance in ant foragers. *Behav Ecol Sociobiol* 73:107.
- Doria MD, Morand-Ferron J, Bertram SM, 2019. Spatial cognitive performance is linked to thigmotaxis in field crickets. *Anim Behav* 150:15–25.
- Dussoutour A, Deneubourg JL, Fourcassié V, 2005. Temporal organization of bi-directional traffic in the ant *Lasius niger* (L.). *J Exp Biol* 208:2903–2912.
- Dussoutour A, Simpson SJ, 2008. Description of a simple synthetic diet for studying nutritional responses in ants. *Insectes Soc* 55:29–333.
- Eischen FA, Rothenbuhler WC, Kulinčević JM, 1983. Brood rearing associated with a range of worker-larva ratios in the Honeybee. *J Apic Res* 22: 163–168.
- Evesham EJM, 1985. The interaction of food distribution and the caste composition of an ant colony (*Myrmica rubra* L.). *J Zool* 207:241–250.
- Feltz CJ, Miller GE, 1996. An asymptotic test for the equality of coefficients of variation from k populations. *Stat Med* 15:647–658.
- Gibson RL, 1989. Soldier production in *Camponotus novaeboracensis* during colony growth. *Insectes Soc* 36:28–41.
- Gordon DM, 1989. Dynamics of task switching in harvester ants. *Anim Behav* 38:194–204.
- Guenther A, Kowalski G, von Engelhardt N, 2014. Prenatal social conditions shape offspring adult phenotype and reproductive success. *Behav Ecol Sociobiol* 68:1661–1667.
- Gray B, 1971. A morphometric study of the ant species, *Myrmecia dispar* (Clark)(Hymenoptera: formicidae). *Insectes Soc* 18:95–109.
- Hartmann C, Heinze J, Bernadou A, 2019. Age-dependent changes in cuticular color and pteridine levels in a clonal ant. *J Insect Physiol* 118:103943.
- Hertel AG, Niemelä PT, Dingemans NJ, Mueller T, 2020. A guide for studying among-individual behavioral variation from movement data in the wild. *Mov Ecol* 8:30.
- Hertel AG, Royauté R, Zedrosser A, Mueller T, 2021. Biologging reveals individual variation in behavioural predictability in the wild. *J Anim Ecol* 90: 723–737.
- Holbrook CT, Barden PM, Fewell JH, 2011. Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behav Ecol* 22: 960–966.
- Hothorn T, Bretz F, Westfall P, 2008. Simultaneous inference in general parametric models. *Biom J* 50:346–363.
- Islam MS, Roessingh P, Simpson SJ, McCaffery AR, 1994. Effects of population density experienced by parents during mating and oviposition on the phase of hatching desert locusts *Schistocerca gregaria*. *Proc Royal Soc Biol Sci* 257:93–98.
- Jeanne RL, 1986. The organization of work in *Polybia occidentalis*: costs and benefits of specialization in a social wasp. *Behav Ecol Sociobiol* 19: 333–341.
- Jeanson R, 2019. Within-individual behavioural variability and division of labour in social insects. *J Exp Biol* 222:jeb190868.
- Jeanson R, Lachaud JP, 2015. Influence of task switching costs on colony homeostasis. *Sci Nat* 102:1–4.
- Jeanson R, Weidenmüller A, 2014. Interindividual variability in social insects: proximate causes and ultimate consequences. *Biol Rev* 89:671–687.
- Jeanson R, Fewell JH, Gorelick R, Bertram SM, 2007. Emergence of division of labor as a function of group size. *Behav Ecol Sociobiol* 62:289–298.
- Kamakura M, 2011. Royalactin induces queen differentiation in honeybees. *Nature* 473:478–483.

- Kleineidam C, Roces F, 2000. Carbon dioxide concentrations and nest ventilation in nests of the leaf-cutting ant *Atta vollenweideri*. *Insectes Soc* 47: 241–248.
- Kramer BH, Schaible R, Scheuerlein A, 2016. Worker lifespan is an adaptive trait during colony establishment in the long-lived ant *Lasius niger*. *Exp Gerontol* 85:18–23.
- Kramer BH, Scharf I, Foitzik S, 2014. The role of per-capita productivity in the evolution of small colony sizes in ants. *Behav Ecol Sociobiol* 68:41–53.
- Lichtenstein JLL, Chism GT, Kamath A, Pruitt JN, 2017. Intraindividual behavioral variability predicts foraging outcome in a beach-dwelling jumping spider. *Sci Rep* 7:18063.
- Madsen NEL, Offenberg J, 2017. Effect of pleometrosis and brood transplantation on colony growth of the black garden ant, *Lasius niger*. *Asian Myrmecol* 9:e009003.
- Marwick B, Krishnamoorthy K, 2019. *Cvequality: Tests for the Equality of Coefficients of Variation from Multiple Groups*. R package version 0.2.0. <https://CRAN.R-project.org/package=cvequality>
- Mitchell DJ, Beckmann C, Biro PA, 2021. Understanding the unexplained: the magnitude and correlates of individual differences in residual variance. *Ecol Evol* 11:7201–7210.
- Mitchell DJ, Fanson BG, Beckmann C, Biro PA, 2016. Towards powerful experimental and statistical approaches to study intraindividual variability in labile traits. *R Soc Open Sci* 3:160352.
- Moritz RA, Page R, Jr, 1999. Behavioral threshold variability: costs and benefits in insect societies. In: Detrain C, Deneubourg J, Pasteels J, editors. *Information Processing in Social Insects*. Basel, Switzerland: Birkhauser. 203–215.
- Müller T, Küll CL, Müller C, 2016. Effects of larval versus adult density conditions on reproduction and behavior of a leaf beetle. *Behav Ecol Sociobiol* 70:2081–2091.
- Muscudere ML, Willey TA, Traniello JFA, 2009. Age and task efficiency in the ant *Pheidole dentata*: young minor workers are not specialist nurses. *Anim Behav* 77:911–918.
- Nakagawa S, Schielzeth H, 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev* 85:935–956.
- Niemelä PT, Niehoff PP, Gasparini C, Dingemans NJ, Tunj C, 2019. Crickets become behaviourally more stable when raised under higher temperatures. *Behav Ecol Sociobiol* 73:81.
- Porter SD, Tschinkel WR, 1985. Fire ant polymorphism: the ergonomics of brood production. *Behav Ecol Sociobiol* 16:323–336.
- Pratt SC, Brooks SE, Franks NR, 2001. The use of edges in visual navigation by the ant *Leptothorax albipennis*. *Ethology* 107:1125–1136.
- Purcell J, Brüttsch T, Chapuisat M, 2012. Effects of the social environment on the survival and fungal resistance of ant brood. *Behav Ecol Sociobiol* 66: 467–474.
- R Development Core Team. 2018. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>.
- Rittschof CC, Coombs CB, Frazier M, Grozinger CM, Robinson GE, 2015. Early-life experience affects honey bee aggression and resilience to immune challenge. *Sci Rep* 5:15572.
- Sachser N, Kaiser S, Hennessy MB, 2013. Behavioural profiles are shaped by social experience: when, how and why. *Philos Trans R Soc Lond B Biol Sci* 368:20120344.
- Sanmartín-Villar I, Csata E, Jeanson R, 2021. Variability in activity differs between castes in the ant *Linepithema humile*. *Ecol Entomol* <https://doi.org/10.1111/een.13075>
- Schwander T, Lo N, Beekman M, Oldroyd B, Keller L, 2010. Nature versus nurture in social insect caste differentiation. *Trends Ecol Evol* 25:275–282.
- Sneddon LU, 2003. The bold and the shy: individual differences in rainbow trout. *J Fish Biol* 62:971–975.
- Stamps JA, 2016. Individual differences in behavioural plasticities. *Biol Rev* 91:534–567.
- Stamps JA, Grootuis TGG, 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev* 85:301–325.
- Stamps JA, Briffa M, Biro PA, 2012. Unpredictable animals: individual differences in intraindividual variability (IIV). *Anim Behav* 83:1325–1334.
- Stoffel MA, Nakagawa S, Schielzeth H, 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol Evol* 8:1639–1644.
- Taborsky B, 2016. Opening the black box of developmental experiments: behavioural mechanisms underlying long-term effects of early social experience. *Ethology* 122:267–283.
- Taborsky B. 2017. Developmental plasticity: preparing for life in a complex world. In: Naguib M, Podos J, Simmons LW, Barrett L, Healy SD, editors. *Advances in the Study of Behavior*. Vol. 49. Cambridge (MA): Academic Press. 49–99.
- Thomas ML, Elgar MA, 2003. Colony size affects division of labour in the ponerine ant *Rhytidoponera metallica*. *Sci Nat* 90:88–92.
- Tschinkel WR, 1988. Colony growth and the ontogeny of worker polymorphism in the fire ant *Solenopsis invicta*. *Behav Ecol Sociobiol* 22: 103–115.
- Turza F, Miler K, 2021. Comparative analysis of experimental testing procedures for the elicitation of rescue actions in ants. *Curr Zool* zoab052.
- Ulrich Y, Saragosti J, Tokita CK, Tarnita CE, Kronauer DJC, 2018. Fitness benefits and emergent division of labour at the onset of group living. *Nature* 560:635–638.
- Urszán TJ, Garamszegi LZ, Nagy G, Hettyey A, Török J et al. 2018. Experience during development triggers between-individual variation in behavioural plasticity. *J Anim Ecol* 87:1264–1273.
- Vehtari A, Gelman A, Gabry J, 2017. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Stat Comput* 27: 1413–1432.
- Watanabe NM, Stahlman WD, Blaisdell AP, Garlick D, Fast CD et al. 2012. Quantifying personality in the terrestrial hermit crab: different measures, different inferences. *Behav Proc* 91:133–140.
- Waters JS, Holbrook CT, Fewell JH, Harrison JH, 2010. Allometric scaling of metabolism, growth, and activity in whole colonies of the seed-harvester ant *Pogonomyrmex californicus*. *Am Nat* 176:501–510.
- Weidenmüller A, Mayr C, Kleineidam CJ, Roces F, 2009. Preimaginal and adult experience modulates the thermal response behavior of ants. *Curr Biol* 19:1897–1902.
- Werkhoven Z, Bravin A, Skutt-Kakaria K, Reimers P, Pallares L et al. 2019. The structure of behavioral variation within a genotype. *bioRxiv* 779363.
- Westneat DF, Wright J, Dingemans NJ, 2015. The biology hidden inside residual within-individual phenotypic variation. *Biol Rev* 90:729–743.