Current Zoology 60 (3): 392–400, 2014

Age and ovarian development are related to worker personality and task allocation in the ant *Leptothorax acervorum*

Stephan KÜHBANDNER[1,2], Andreas P. MODLMEIER[3,4*], Susanne FOITZIK[3]

[1] Institute of Zoology, University of Regensburg, Regensburg, Germany

[2] Department Biology II, Ludwig Maximilian University Munich, Planegg-Martinsried, Germany

[3] Institute of Zoology, Johannes Gutenberg University, Mainz, Germany

[4] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

Abstract In social insects, workers of different morphological castes and age are known to act differently. Yet, it is unclear how body size and ovarian development influence worker personalities (i.e. consistent behavioral variation) and task allocation in similar aged ant workers of monomorphic species. Behavioral variation is thought to be a key element of division of labor, but few studies have linked worker personality to task allocation. We investigated individual behavior in *Leptothorax acervorum* ant workers at two time points during the first three months of their life and in two different settings. We observed worker behavior in the nest (i.e. task allocation) and in standardized aggression, exploration and brood care experiments (i.e. personality) and found behavioral repeatability in foraging and exploration. Further, workers acted consistently across settings: workers with a more aggressive and exploratory personality type were more active in the nest. Moreover, ovarian development was associated with worker personality and task allocation: older workers with well-developed ovaries foraged less, but were more aggressive and exploratory. In accordance with the typical age-polyethism of social insects, workers became more active and foraged more as they grew older. Consequently, our study suggests that task allocation in *Leptothorax acervorum* is not only influenced by ovarian development and age, but moreover by the personalities of its workers [*Current Zoology* 60 (3): 392–400, 2014].

Keywords Personality, Behavioral syndrome, Division of labor, Ovarian development, Foraging, Social insects

Animal personalities and behavioral syndromes have drawn much attention during the last few years in the social insect literature (Jandt et al., 2013): studies focused either on collective personalities of whole colonies (e.g. Gordon et al., 2011; Wray et al., 2011; Pinter-Wollman et al., 2012; Scharf et al., 2012) or on variation in personalities between single individuals (e.g. Muller et al., 2010; Chapman et al., 2011; Modlmeier et al., 2012). Following Keiser and Pruitt (2014), we call consistent individual variation in behavior "personality types" or "behavioral types" (BTs) and correlations across different behaviors "behavioral syndromes". Although the concept of personality has only recently been used to describe consistent behavioral differences in social insects, individuals in insect societies have long been known to consistently differ in their behavior and in the tasks they undertake (Hölldobler and Wilson, 1990). A characteristic trait of social hymenopteran societies is reproductive division of labor: queens are morphologically and behaviorally specialized on reproduction, while workers are either entirely sterile or else show a much lower fertility (Robinson, 1992; Julian and Cahan, 1999). Moreover, in most social insects, and especially in ants, workers show a non-reproductive division of labor. Some workers focus on offspring care, whereas others forage or defend the nest. Task specialization is thought to increase worker efficiency, albeit recent findings indicate that this is not always the case (Dornhaus, 2008).

But how are the different tasks distributed among the work force? There are two common patterns of division of labor: temporal polyethism (i.e. age-polyethism) and morphological polyethism (for reviews on division of labor see: Gordon, 1996; Beshers and Fewell, 2001). Tasks typically change with age: young individuals perform tasks inside the nest, like brood care and nest cleaning, before they switch to foraging and other risky activities outside the nest (Seeley, 1982; Calabi et al., 1983). Morphological polyethism describes task allocation according to body size. Some ant species exhibit morphologically distinct worker castes, e.g. minors, majors, or soldiers, which strongly differ in their be-

Received Jan. 20, 2014; accepted Mar. 11, 2014.

∗ Corresponding author. E-mail: andreas.modlmeier@gmail.com

© 2014 *Current Zoology*

havior and the tasks they perform (Wilson, 1980). Larger workers (majors) are usually engaged in external defense tasks, while smaller ones (minors) show more care and foraging behavior (Waser, 1998; Fournier et al., 2008). Other important influences on behavior appear to be social interactions, experience, and genetic or environmental factors (Robinson, 1992; Gordon, 1996; Ravary et al., 2007). In honey bees, the occurrence of different worker patrilines within a single hive due to multiple mating of the queen increases variability in behavior and body size of workers (Frumhoff and Baker, 1988; Robinson and Page, 1988; Chapman et al., 2007; Oldroyd and Fewell, 2007). Similarly, workers of different patrilines of the leaf-cutting ant *Acromyrmex echinatior* are specialized in behavioral tasks like waste-management or foraging (Waddington and Hughes, 2010). Yet in *Cataglyphis* ants, behavioral caste was uncorrelated to patriline and mainly depended on body size (Fournier et al., 2008). Last but not least, physiology can influence task allocation: in honeybees, foraging behavior is regulated by their reproductive physiology (Amdam et al., 2004; Siegel et al., 2012). Further, ovarian development was connected to division of labor among workers of different ant species (e.g. Bourke, 1988; Fénéron et al., 1996; Pohl et al., 2011). In harvester ants, high levels of ovarian-produced ecdysteroids resulted in a specialization on inside tasks, demonstrating the importance of endocrine physiology for the division of labor in workers (Dolezal et al., 2012). Moreover, the influence of ovarian activity on task specialization was independent of worker age in harvester ants suggesting that endocrine mechanisms played a key role in the evolution of social behavior (Dolezal et al., 2013).

Here, we study how body size and ovarian development are related to personality and task allocation in similar-aged workers in the monomorphic ant species, *Leptothorax acervorum*. This ant has small colonies with one to several queens and on average less than 100 workers. At two points during their early life, we analyzed whether workers of the same age differ consistently in their behavior and if yes, which parameters co-vary with the different behavioral types. To search for relationships between behaviors that are known to be part of behavioral syndromes in other species (Bell, 2007), we observed workers both in the nest setting and in standardized aggression, exploration and brood care experiments. Finally, we measured body size and ovarian development to study the associations of these morphological traits to worker behavior. *Leptothorax acervorum* is well-suited to study an association of ovary development and behavior, because worker egg-laying occurs even in queenright colonies (Bourke, 1991). Based on earlier work on other social insect species we derived the following hypotheses and predictions:

Age-polyethism also occurs in *L. acervorum* and we predict a shift in worker behavior from inactivity and social behaviors to more outside work during the first three months of the workers life.

Workers should not only show behavioral consistency over time (personality), but also suites of correlated traits (behavioral syndromes).

We expect that morphology and physiology influence worker behavior. In particular, we predict based on earlier studies on ants (e.g. Dolezal et al., 2013) that workers with more developed ovaries perform tasks inside the nest, while workers with small degenerated ovaries forage. If morphology is important for worker behavior, we would expect that larger workers take over external tasks like foraging and nest defense, while smaller ones perform more brood care.

1 Materials and Methods

1.1 Ant colonies

The *Leptothorax acervorum* colonies were collected in summer 2008 in the Reichswald near Nuremberg, Germany. We kept the ant colonies in our laboratory in three chambered quadratic nest boxes (Buschinger, 1974; Heinze and Ortius, 1991) with a side length of 9.5 cm and a height of 3 cm and a plastered floor. The chambers were connected by holes that allowed the ants to enter all three chambers of the nest box. The left chamber contained the nestsite, consisting of an inlay plastic nest with red foil cover. We offered food in the middle chamber and water in the right chamber. Ants were fed three times per week with honey and pieces of crickets. Colonies were kept in the laboratory under these standardized conditions for six months before we started the experiments. As we did not census the ant colonies, inferences on the impact of colony size on ant behavior cannot be made.

1.2 Markings with wire

Workers were individually marked with three differently colored wires with a diameter from 0.02 to 0.03 mm. Small pieces of wire were looped around the ant at three different parts of the body: head and thorax, petiole and postpetiole, or postpetiole and gaster (Mirenda and Vinson, 1979). The ends of the wires were shortened, so they did not restrict the movement of the

worker. With this method 110 randomly chosen workers (of which 66 survived until the end of the experiment) could be marked in nine colonies, one week after eclosion from the pupae. It was necessary to wait a week, because attempts to mark ants earlier were unsuccessful as the cuticle of freshly eclosed ants was too soft and nestmates showed aggressive behavior towards them. One week old ants ('callows') could be still reliably recognized by their lighter cuticle.

1.3 Scan Samplings in the nest setting

In order to record the behavior of all marked workers in the nest setting, we performed two series of scan samplings. Observations for the first series started on average 16.8 ± 0.6 days (mean ± *SE*) after eclosion and lasted for 27.0 ± 1.4 days (*n* of observations per ant: 12.0 ± 0.4). The second series started 92.6 ± 0.9 days after eclosion and lasted for 20 days (*n* of observations per ant: 34.0 ± 0.4). If an individual could not be detected during a scan, this was treated as a missing value and was not included in the total number of observations. The observed behavior was classified using the following categories: foraging (walking outside the nest), social care, and resting. Social care was defined as feeding and cleaning of nestmates, and all behaviors related to brood care (carrying, cleaning and feeding of brood). Resting was defined as being inactive, i.e. not moving or interacting with others. When more than one scan was made on a single day (range: 1–3 scans per day), observations were carried out at intervals of at least two hours. The observed behavior was categorized and the relative percentage of each behavior was calculated.

1.4 Individual behavior in standardized experiments

Experiments were carried out in two series 38.5 ± 0.6 and 67.8 ± 0.2 days after eclosion and consisted of individual aggression, brood care and exploration experiments. Both the aggression and the brood care experiments were conducted within a small test arena with a diameter of 1.5 cm and a height of 0.3 cm. Individual behavior was recorded every 30 seconds for 10 minutes. First, aggression to non-nestmates was tested using freshly frozen and defrosted workers from another, so-called opponent colony. We used dead ants to exclude potential effects of the opponents' behavior. Further, it has been shown that aggression to dead workers is positively correlated to aggression to live opponents (Modlmeier and Foitzik 2011). Aggressive interactions included mandible spreading (threat display), biting, dragging, carrying and stinging. Second, in the brood care experiment a worker was tested with a worker pupa from the same nest. Brood care behavior included cleaning, carrying and guarding of the pupa (i.e. prolonged contact). Afterwards, the relative percentages (number of times behavior × was observed / total number of observations) for each behavioral category were calculated. Third, for the exploration experiment a special test arena was constructed with one central arena (diameter 29 mm and height 3 mm) surrounded by eight side chambers which were connected with the central arena via corridors (length 32 mm and width 7 mm). To investigate exploration of novel objects, small pieces of trees and spices (spruce needle, oregano, caraway, thyme, sage, chamomile, common European oak wood, one chamber was empty) were put into the side chambers (similar to Modlmeier and Foitzik 2011). After the worker was released in the central chamber, we recorded the number of antennation events with all objects (exploration of novel objects) and calculated the percentage of chambers entered (exploration of novel environment) within 10 minutes.

1.5 Measurements of head width and ovary development

To investigate the influence of body size on worker behavior, head width was measured to the nearest 0.01 mm using a stereomicroscope (Zeiss Stemi 2000 C) with an object micrometer and 128-fold magnification. Despite being monomorphic, the measured *L. acervorum* workers still showed considerable variation in size (mean head width = 0.75 mm, range: 0.63–0.84 mm, *SD* = 0.04). The influence of reproductive state of the workers was investigated through dissection of the gaster and measurement of the ovaries. This was done under a stereomicroscope with an object-micrometer and 41-fold magnification after the end of the second trial series. *Leptothorax acervorum* workers generally have one single ovariole per ovary (2 total) and we measured the length of both and calculated a mean as a measure of ovary development. Although we also counted the number of fertile eggs, which was positively correlated to mean ovary length (Spearman's $r = 0.45$, $P = 0.0001$, $n = 66$), we decided to use mean ovary length as a proxy for ovarian development. While current fertility is important, endocrine mechanisms (e.g. influence of ecdysteroid hormones) are most likely the key regulators of behavior and ovarian physiology, even in functionally sterile workers (reviewed in Page and Amdam, 2007; Dolezal et al., 2013). Therefore, we feel confident that mean ovary length, which in this study could nevertheless be a proxy for fertility, because it was positively correlated to the number of fertile eggs,

is an appropriate measure to examine the relationship of behavior and ovarian development. Ants were frozen and killed before ovary dissection, so that it was impossible to measure ovary length at the end of the first trial series. Hence, we analyzed the influence of ovary length after the second trial series on behaviors of the first and second trial series.

1.6 Statistics

1.6.1 Changes in worker behavior with age

Comparing the individual behavior of each ant in both trial series enabled us to investigate changes in worker behavior that are due to age and/or experience. We decided to use non-parametric sign tests to investigate age-specific differences in behavior between the two trial series, because only exploration of novel environments and resting in trial series one were normally distributed (Kolmogorov–Smirnov test: $P > 0.10$), while all other behaviors were not (K-S test: $P < 0.05$).

1.6.2 Repeatability of behavior

Behaviors are considered repeatable when they show low intra-individual variation and large inter-individual variation (Bell et al., 2009). A repeatable behavior differs among individuals but is consistent across time. The repeatability score has the same function as a correlation coefficient and determines how consistent a behavior is over time. As pointed out by Bell et al. (2009), repeatability is the most widely-used measure for behavioral consistency and has been used to not only describe personality, but also as a first step to study the genetic basis of behavior (to define the upper bound of heritability). To calculate the repeatability of individual behavior between the two trial series, we used a generalized linear mixed-effects model (GLMM) with logit or log link following the guidelines outlined in Nakagawa and Schielzeth (2010), and the R package 'rptR' (http://r-forge.r-project.org/projects/rptr) for the free programming language R (R Development Core Team, 2009). As the behavioral data consisted of count (exploration of novel objects) and proportion data (all other behaviors), we calculated repeatability with multiplicative overdispersion models fitted by PQL (penalized quasi-likelihood) estimation with 1,000 permutations using the R functions 'rpt.binomGLMM.multi' (overdispersed binomial GLMM with logit link) for proportion data and 'rpt.poisGLMM.multi' (overdispersed Poisson GLMM with log link) for count data. We calculated link scale repeatability that estimates the underlying (latent) repeatability of behavior. Although workers may differ in the amount of work they performed between the two trial series, the latent repeatability would still be significant if all workers equally increase/decrease the work performed. In addition, to a discussion of the *P*-values, we also interpret the more informative 95% confidence intervals (CI) as suggested by Nakagawa and Schielzeth (2010).

1.6.3 Behavioral Syndromes (suites of correlated behavioral traits)

We investigated behavioral syndromes in similar-aged workers and also if these syndromes are consistent across time (defined as structural consistency; Stamps and Groothuis, 2010) by performing a principal component analysis (PCA) each for the first and the second trial series. We analyzed whether the principal components remained consistent over time by correlating each PC from trial 1 with its corresponding PC from trial 2 (following Pamminger et al., 2014). PCAs are frequently used to study behavioral syndromes because they circumvent multiple testing (Dingemanse et al., 2010). Prior to the PCA, we performed a z-score transformation for all individual scores, i.e. we subtracted the mean value from the individual scores and divided by the standard deviation (Ellison and Gotelli, 2004). We included all z-score transformed behaviors of the standardized experiments (aggression, brood care and both exploration measures), but only extracted factors with an eigenvalue greater than one. We did not include behaviors from the nest setting, because these behaviors are dependent on each other, i.e. a worker that is resting cannot forage or do social care. To facilitate interpretation, components were rotated using varimax rotation (Ellison and Gotelli, 2004). According to Comrey and Lee (1992), only loadings larger than 0.32 are discussed.

To investigate whether the behaviors from the standardized experiments are related to behaviors in the nest setting, we used Spearman rank correlations.

1.6.4 Influence of head width and ovary length

To study how morphology and physiology are related to the studied behavioral traits, we correlated the PCs of the first and second trial series and the behaviors from the nest setting against head width and ovary length with Spearman rank correlations. Unless otherwise stated, statistical analyses were performed with Statistica 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA). To control for multiple testing, we calculated the q-value for all Spearman rank correlations. The q-value is the minimum value at which a test should be rejected due to false discovery (Storey, 2002). Hence, a test can be considered significant if the p-value is below the q-value.

2 Results

Of the 110 workers that were originally marked, 66 survived until the end of the experiment evenly spread across the 9 colonies with 7.33±1.41 (mean±standard deviation, *SD*) workers per colony (range 5–9 workers per colony). For statistical analyses, we used only workers that survived until the end of the experiment, because we were interested in individual development of personalities and not in colony averages.

2.1 Changes in worker behavior with age

In the nest setting, workers rested more and foraged less during the first trial series compared to the second series (Sign test; resting: Z = 3.32, n = 66, $P < 0.001$; foraging: $Z = 2.91$, n = 66, $P = 0.004$). All other behaviors did not change with age ($P > 0.16$).

2.2 Repeatability of behavior

In the nest setting, foraging was highly repeatable (GLMM: $R = 0.71$, $P = 0.01$; Table 1). Resting (i.e. how active an individual was) possessed very low repeatability ($R = 0.02$, $P = 0.02$). Social care was not repeatable ($P = 0.65$).

Table 1 Summary of behavioral repeatability estimates of 66 *L. acervorum* workers displaying the results of GLMMs using multiplicative overdispersion models fitted to PQL

Behavior	*R*	95% CI	*P*
Aggression	0.14	0.08–0.35	0.13
Brood Care	0.00	0.00–0.03	0.75
Exploration (object)	0.29	0.10–0.53	0.10
Exploration (environment)	0.04	0.00–0.09	**0.03**
Foraging	0.71	0.00–0.92	**0.01**
Resting	0.02	0.00–0.05	**0.02**
Social Care	0.00	0.00–0.21	0.65

Individual workers were tested in two trial series in standardized experiments (aggression, brood care and exploration) and in the nest setting (foraging, resting, social care). Presented are link (underlying) scale repeatabilities. Significant *P*-values are given in bold.

In the standardized experiments, exploration behavior (environment) was repeatable, although repeatability was very low and the confidence intervals included zero ($R = 0.04$, 95% $CI = 0.00$–0.09, $P = 0.03$). In contrast, exploration (object) showed a weak trend with a much higher repeatability ($R = 0.29$; $P = 0.10$) supported by confidence intervals that were well above zero (95% $CI = 0.10$–0.53). Aggression was not repeatable considering the p-value ($R = 0.14$, $P = 0.13$), but the confidence intervals suggest that the majority of workers showed at least some consistency in their behavior (95% $CI = 0.08$–0.35). Brood care behavior was not repeatable ($P = 0.75$).

2.3 Behavioral Syndromes

Principal component analysis of the first series of experiments (~ 1 month after worker eclosion) gave two PCs with an eigenvalue larger than one explaining 60.3% of the total variation in behavior (Table 2). The first PC revealed that both exploratory behaviors, i.e. exploration of novel objects and of novel environments, are tightly linked in an overall exploration syndrome. The second PC suggested that aggressive workers perform less brood care in the standardized tests.

Table 2 Component loadings from PCAs of the first and second trial series

Trait	1. Trial		2. Trial	
	PC 1	PC 2	PC 1	PC 2
Aggression	0.064	**-0.711**	**0.522**	**0.336**
Brood care	0.078	**0.723**	-0.038	**0.927**
Exploration (object)	**0.811**	-0.195	**0.775**	-0.264
Exploration (environment)	**0.790**	0.235	**0.726**	0.148
% variation explained	32.27	28.04	35.01	26.61

Only components with an eigenvalue greater than one were extracted. All behavioral data from the standardized experiments were included. Components were rotated using varimax rotation. Loadings larger than 0.32 are shown in bold.

The second series of experiments (~2 months after worker eclosion) resulted in two PCs explaining 61.6 % of the total behavioral variation (Table 2). Similar to the first series of experiments PC 1 showed an overall exploration syndrome positively linking exploration of novel objects and environments. In addition, aggression loaded positively on PC 1 suggesting that very exploratory workers are also more aggressive. PC 2 was dominated by a high positive loading of brood care behavior, but also contained a weak positive loading of aggression. Notably, principal component scores did not differ between colonies (one-way ANOVAs: $P > 0.12$).

Examining the structural consistency of the behavioral syndromes revealed that PC 1 was consistent over time (Pearson's $r = 0.31$, $P = 0.01$, n = 66; Fig. 1). This is in line with a comparison of the loadings: PC 1 from trial 1 and 2 both showed a general exploration syndrome. In contrast PC 2 was not consistent over time ($P = 0.52$): PC 2 in trial one suggested a negative relationship between aggression and brood care, whereas PC 2 in trial two indicated a very weak positive relationship between both behaviors.

Correlating the principal component scores from the standardized experiments with the behaviors from the nest setting demonstrated that during the first series of

experiments, behaviors were not consistent across different settings ($P > 0.26$). However, during the second series of experiments resting was negatively correlated to PC 1 (Spearman's $r = -0.272$, $P = 0.03$, $q = 0.13$): workers that rested less, i.e. were more active in the nest setting, were also more exploratory and aggressive. No other behaviors from the nest setting were correlated with the PC scores.

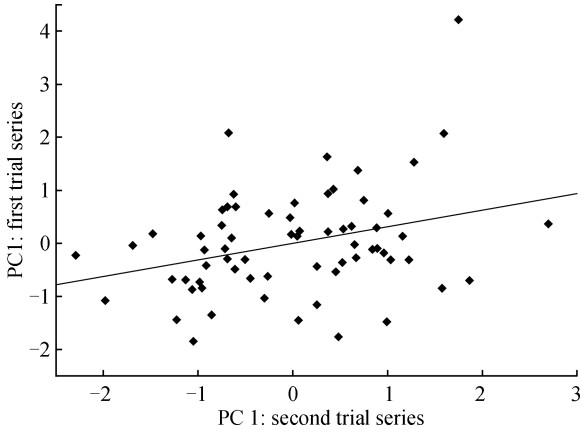


Fig. 1 Scatterplot describing the structural consistency of a suite of correlated behaviors described by the first principal component (PC 1)

Pearson's $r = 0.31$, $P = 0.01$. The first trial series involved a strong positive loading of both exploratory behaviors, while the second trial series also included a positive loading of aggressiveness. Aggressiveness, exploratory behavior and brood care of 66 same-aged *L. acervorum* workers was measured about one month (first trial series) and two months (second trial series) after eclosion. Experiments were performed separately for every worker.

2.4 Influence of head width and ovary length

Most of the principal components of the first trial series were not significantly related to head width or ovary length (Spearman rank correlation: $P > 0.21$). However, PC 2 was positively related to head width (Spearman's $r = 0.271$, $P = 0.03$, $q = 0.13$) suggesting that larger workers perform more brood care but are less aggressive. We also found that foraging was negatively related to ovary length (Spearman's $r = -0.251$, $P = 0.04$, $q = 0.16$). The second trial series revealed a positive relationship between ovary length and the first PC, i.e. aggressive and exploratory workers had larger ovaries (Spearman's $r = 0.269$, $P = 0.03$, $q = 0.13$; Fig. 2). Further, foraging was again negatively correlated to ovary length (Spearman's $r = -0.598$, $P < 0.0001 < q$).

3 Discussion

Our study revealed that same-aged workers of the ant species *L. acervorum* exhibit clear associations between

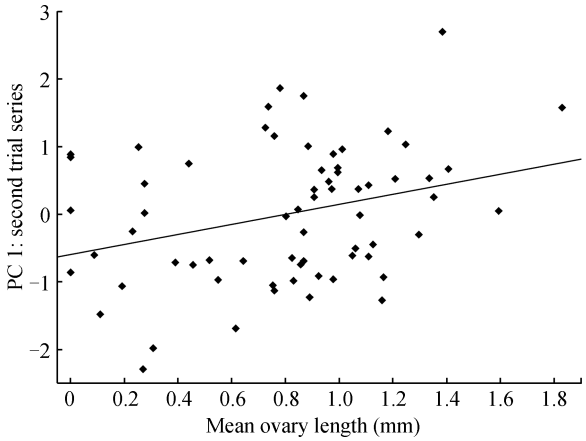


Fig. 2 Positive relationship between mean ovary length and the first principal component (PC 1 was dominated by aggressiveness and both exploratory behaviors) of 66 same-aged individuals in the second trial series

Workers with longer ovaries behaved more aggressively and exploratory (Spearman's $r = 0.269$, $P = 0.03$).

behavioral syndromes, task allocation and physiological traits early in their life. In accordance with the typical age-polyethism in social insects, we also found changes in worker behavior with age: individuals became more active and foraged more. We found an overall exploration syndrome that persisted during both trial series demonstrating that workers that are fast in exploring novel environments are also more interested in novel objects. In contrast, aggressiveness changed its association with brood care during the study period from strongly negative to weakly positive. Finally, ovary length was negatively associated with foraging behavior during our study period, that is, foragers possessed smaller (less developed or degenerated) ovaries. In addition, ovary length was positively associated with exploratory and aggressive behavior in the second trial series (Fig. 2).

As predicted by our first hypothesis, we found indications for the classical temporal polyethism in *L. acervorum*. The findings from the scan samplings in the nest setting matched the expectations that the workers' activity and foraging frequency would rise over time. Conversely, other behaviors like aggressiveness and brood care did not change with age. Sendova-Franks and Franks (1993) had shown that in the related *Temnothorax unifasciatus* (formerly *Leptothorax*) division of labor is only weakly age-related and more based on workers 'foraging for work': workers finding work in their current role will remain in their role, while workers repeatedly not finding work will move to a different task (Tofts and Franks, 1992). Hence, even young

workers could become dedicated foragers, while older ones remain nurses. In our study, older workers foraged more and rested less. In general, behavioral differences are probably due to a complex interplay of genetic, environmental and experiential factors, which are difficult to disentangle.

According to the response threshold models behavioral differences in the nest are due to differences in response thresholds among workers (reviewed in Beshers and Fewell, 2001). An individual will only perform a task if the stimulus exceeds its internal threshold (Bonabeau et al., 1996). Different threshold values for distinct tasks within a colony may result from genetic differences (Gordon, 1996). Surprisingly, the only repeatable behaviors were foraging, resting and exploration of novel environments. However, only foraging had a high repeatability score. Resting and exploration of novel environments had very low repeatability scores indicating strong developmental effects and/or high plasticity. In addition, the confidence intervals for both resting and exploration of novel environments included zero suggesting that many workers did not behave consistently. In contrast, the confidence intervals for aggressiveness and exploration of novel objects were well above zero indicating that these behaviors showed a rather strong repeatability.

In accordance with our second hypothesis we found several suites of correlated behaviors. The PCA revealed that aggressive workers undertake less brood care early in life. Later in life (second trial series) aggressive workers were more exploratory. Hence, this could characterize a distinct guard/patroller caste that does only little brood care (at least early in life), but is scrutinizing every change in its environment and responds aggressively to intruders. Interestingly, the same workers were also more active in the nest setting. Our analyses also revealed an overall exploration syndrome between exploration of novel objects and environments. Workers that were very fast in discovering their surroundings also inspected more novel objects. Although this relationship might in part be due to the experimental set-up of testing both behaviors in one experiment, earlier experiments with *Temnothorax* workers showed that exploration of novel objects and environments do not always co-vary when tested in the same experiment (Modlmeier et al., 2012). A positive relationship between both exploratory behaviors has also been found in early exploratory behavior in male great tits (Verbeek et al., 1994). Accordingly, individuals were either quick and superficial, or slow and thorough explorers. As in our study these differences in exploratory behavior were not age-related.

In accordance with our third hypothesis, we found an association between worker physiology and behavior. Body size in *L. acervorum* workers was with one exception unrelated to behavior: during the first series of experiments, larger workers performed more brood care, but were less aggressive. However, this relationship disappeared in the second trial series. Many other studies have been able to find an association of behavior and morphology not only in polymorphic, but also in monomorphic species (e.g. Herbers and Cunningham, 1983; Waser, 1998; Fournier et al., 2008). Why we did not find a lasting association of behavior and morphology is unclear, but possibly worker behavior in the small monomorphic colonies of *L. acervorum* is more influenced by the development of the ovaries, which are an important source of hormones in many insects (e.g. Dolezal et al., 2012; Dolezal et al., 2013). Indeed, our study demonstrated that workers with well-developed ovaries foraged less suggesting that foraging activity is tightly linked to ovary length. This is in line with numerous studies demonstrating that ovarian development leads to a specialization on tasks inside the nest (e.g. Bourke, 1988; Fénéron et al., 1996; Pohl et al., 2011; Pamminger et al., 2014). Interestingly, in our study division of labor between foragers and intranidal workers (that stay in the nest) also occurred within same-aged workers. This is in line with a study by Fénéron et al. (1996) that had demonstrated that physiological differences (i.e. different ovarian development) can lead to division of labor within an age class in ponerine ants.

In general, anatomical and physiological differences between ovary size and the animal's behavior may depend on the workers age and on social interactions with other workers and the queen (Bonavita-Cougourdan and Morel, 1985; Muscedere et al., 2009). Further, mean ovary length was positively related to aggressiveness and exploration in the second trial series. In *Temnothorax allardycei* (formerly *Leptothorax*) high ranking workers (in queenright colonies) dominate co-workers (if necessary by fighting), have larger ovaries, lay more eggs and receive food from subordinates without reciprocation (Cole, 1981). Accordingly, the formation of dominance hierarchies among freshly eclosed worker in *L. acervorum* could help explain our results: young workers striving for a higher rank will be more aggressive, perform less brood care and have better developed ovaries. Conversely, Bourke (1991) found that fights between workers within a nest were not related to

worker reproduction in *L. acervorum*. Nevertheless, studies in honey bees and harvester ants have found an association of non-reproductive ovarian activity and behavior (reviewed in Page and Amdam, 2007; Dolezal et al., 2013). Hence, endocrine mechanisms could be involved in the regulation of aggressiveness of not only reproductive but also non-reproductive workers. Further, a study on ant queens also revealed a strong link between individual aggressiveness and fertility status in polygynous nests (Ortius and Heinze, 1999). If queens and workers follow a common 'ovarian ground plan' (as suggested by West-Eberhard, 1996) these results could explain a positive relationship between aggressiveness and ovarian development in workers.

To conclude, we could show that there are clear behavioral differences between workers of the ant *Leptothorax acervorum*, which are consistent over time and across different settings. Albeit we found a general increase in activity and foraging with age, individuals showed high repeatability in foraging and rather low repeatability in resting and how fast they explored novel environments. Notably, both aggressiveness and exploration of novel objects demonstrated strong repeatability when we took confidence intervals into account. A general exploration syndrome was present in both trial series indicating that exploration of novel environments and novel objects have a common regulatory basis. Across settings, a positive relationship was found between activity in the nest and exploration/aggressiveness in standardized experiments. Our results also emphasize the importance of investigating physiological traits (like ovarian development) when studying behavioral syndromes. In our *L. acervorum* workers foraging was negatively correlated to ovary length. Furthermore, aggressive/exploratory workers possessed longer ovaries, which could indicate that these behaviors are regulated by endocrine mechanisms. Hence, as shown in honeybees and other ant species ovarian development could be an important component regulating personalities and division of labor in ants.

Acknowledgements We thank Sebastian Pohl, who made the ant colonies available to us and the entire Munich and Mainz ant groups for their help. In particular, we want to thank Inon Scharf for his comments on a previous version of this manuscript and Niels Dingemanse and Evelien Jongepier for statistical advice. We further want to thank Jonathan Pruitt, Nick DiRienzo and one anonymous reviewer for their valuable comments that helped us improve this paper. This study was funded by the Deutsche Forschungsgemeinschaft (Fo 298/8 and Fo 298/11). A previous version of this manuscript constitutes a segment of the doctoral dissertation of A. P. Modlmeier submitted to the Johannes-Gutenberg University of Mainz, Germany.

Ethical standards The experiments presented herein comply with the current laws of the country in which they were performed.

References

Amdam GV, Norberg K, Fondrk MK, Page RE, 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proc. Natl. Acad. Sci. U.S.A. 101: 11350–11355.

Bell AM, 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B: Biological Sciences 274: 755–761.

Bell AM, Hankison SJ, Laskowski KL, 2009. The repeatability of behaviour: A meta-analysis. Anim. Behav. 77: 771–783.

Beshers SN, Fewell JH, 2001. Models of division of labor in social insects. Annu. Rev. Entomol. 46: 413–440.

Bonabeau E, Theraulaz G, Deneubourg J-L, 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. Proceedings of the Royal Society of London. Series B: Biological Sciences 263: 1565–1569.

Bonavita-Cougourdan A, Morel L, 1985. Polyethism in social interactions in ants. Behav. Process. 11: 425–433.

Bourke AFG, 1988. Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis*. Behav. Ecol. Sociobiol. 23: 323–333.

Bourke AFG, 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. Anim. Behav. 42: 295–310.

Buschinger A, 1974. Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). Insect. Soc. 21: 381–406.

Calabi P, Traniello JFA, Werner MH, 1983. Age polyethism: Its occurrence in the ant *Pheidole hortensis*, and some general considerations. Psyche 90: 395–412.

Chapman BB, Thain H, Coughlin J, Hughes WHO, 2011. Behavioural syndromes at multiple scales in *Myrmica* ants. Anim. Behav. 82: 391–397.

Chapman NC, Oldroyd BP, Hughes WO, 2007. Differential responses of honeybee *Apis mellifera* patrilines to changes in stimuli for the generalist tasks of nursing and foraging. Behav. Ecol. Sociobiol. 61: 1185–1194.

Cole BJ, 1981. Dominance hierarchies in *Leptothorax* ants. Science 212: 83–84.

Comrey AL, Lee HB, 1992. A First Course in Factor Analysis. Hillsdale: Lawrence Erlbaum Associates.

Dingemanse NJ, Dochtermann NA, Wright J, 2010. A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. Anim. Behav. 79: 439–450.

Dolezal AG, Brent CS, Hölldobler B, Amdam GV, 2012. Worker division of labor and endocrine physiology are associated in the harvester ant *Pogonomyrmex californicus*. The Journal of Ex-

perimental Biology 215: 454–460.
Dolezal AG, Johnson J, Hölldobler B, Amdam GV, 2013. Division of labor is associated with age-independent changes in ovarian activity in *Pogonomyrmex californicus* harvester ants. Journal of Insect Physiology 59: 519–524.
Dornhaus A, 2008. Specialisation does not predict individual efficiency in an ant. PLoS Biol. 6: 2368–2375.
Ellison GN, Gotelli N, 2004. A Primer of Ecological Statistics. Sunderland: Sinauer.
Fénéron R, Durand J-L, Jaisson P, 1996. Relation between behaviour and physiological maturation in a ponerine ant. Behaviour 133: 791–806.
Fournier D, Battaille G, Timmermans I, Aron S, 2008. Genetic diversity, worker size polymorphism and division of labour in the polyandrous ant *Cataglyphis cursor*. Anim. Behav.75: 151–158.
Frumhoff PC, Baker J, 1988. A genetic component to division of labour within honey bee colonies. Nature 333: 358–361.
Gordon DM, 1996. The organization of work in social insect colonies. Nature 380: 121–124.
Gordon DM, Guetz A, Greene MJ, Holmes S, 2011. Colony variation in the collective regulation of foraging by harvester ants. Behavioral Ecology 22: 429–435.
Heinze J, Ortius D, 1991. Social organization of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae). Psyche 98: 227–240.
Herbers JM, Cunningham M, 1983. Social organization in *Leptothorax longispinosus* Mayr. Anim. Behav. 31: 759–771.
Hölldobler B, Wilson EO, 1990. The Ants. Cambridge: Harvard University Press.
Jandt JM, Bengston S, Pinter-Wollman N, Pruitt JN, Raine NE et al., 2013. Behavioural syndromes and social insects: Personality at multiple levels. Biological Reviews 89: 48–67.
Julian GE, Cahan S, 1999. Undertaking specialization in the desert leaf-cutter ant *Acromyrmex versicolor*. Anim. Behav. 58: 437–442.
Keiser CN, Pruitt JN, 2014. Spider aggressiveness determines the bidirectional consequences of host-inquiline interactions. Behavioral Ecology 25: 142–151.
Mirenda JT, Vinson S, 1979. A marking technique for adults of the red imported fire ant (Hymenoptera: Formicidae). The Florida Entomologist 62: 279–281.
Modlmeier AP, Foitzik S, 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. Behavioral Ecology 22: 1026–1032.
Modlmeier AP, Liebmann JE, Foitzik S, 2012. Diverse societies are more productive: A lesson from ants. Proceedings of the Royal Society B–Biological Sciences 279: 2142–2150.
Muller H, Grossmann H, Chittka L, 2010. 'Personality' in bumblebees: Individual consistency in responses to novel colours? Anim. Behav. 80: 1065–1074.
Muscedere ML, Willey TA, Traniello JF, 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. Biological Reviews 85: 935–956.
Oldroyd B P, Fewell JH, 2007. Genetic diversity promotes homeostasis in insect colonies. Trends Ecol. Evol. 22: 408–413.
Ortius D, Heinze J, 1999. Fertility signaling in queens of a North American ant. Behav. Ecol. Sociobiol. 45: 151–159.
Page RE, Amdam GV, 2007. The making of a social insect: Developmental architectures of social design. Bioessays 29: 334–343.
Pamminger T, Foitzik S, Kaufmann KC, Schützler N, Menzel F, 2014. Worker personality and its association with spatially structured division of labor. Plos One 9(1): e79616.
Pinter-Wollman N, Gordon DM, Holmes S, 2012. Nest site and weather affect the personality of harvester ant colonies. Behavioral Ecology 23: 1022–1029.
Pohl S, Witte V, Foitzik S, 2011. Division of labor and slave raid initiation in slave-making ants. Behav. Ecol. Sociobiol. 65: 2029–2036.
Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P, 2007. Individual experience alone can generate lasting division of labor in ants. Curr. Biol. 17: 1308–1312.
Robinson GE, 1992. Regulation of division of labor in insect societies. Annu. Rev. Entomol. 37: 637–665.
Robinson GE, Page RE, 1988. Genetic determination of guarding and undertaking in honey-bee colonies. Nature 333: 356–358.
Scharf I, Modlmeier AP, Fries S, Tirard C, Foitzik S, 2012. Characterizing the collective personality of ant societies: Aggressive colonies do not abandon their home. Plos One 7: e33314
Seeley T, 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behav. Ecol. Sociobiol. 11: 287–293.
Sendova-Franks A, Franks NR, 1993. Task allocation in ant colonies within variable environments (A study of temporal polyethism: Experimental). Bull. Math. Biol. 55: 75–96.
Siegel AJ, Kaftanoglu O, Fondrk MK, Smith NR, Page Jr RE, 2012. Ovarian regulation of foraging division of labour in Africanized backcross and pollen-hoarding honeybees. Anim. Behav. 83: 653–658.
Stamps J, Groothuis TG, 2010. The development of animal personality: Relevance, concepts and perspectives. Biological Reviews 85: 301–325.
Storey JD, 2002. A direct approach to false discovery rates. Journal of the Royal Statistical Society Series B-Statistical Methodology 64: 479–498.
Tofts C, Franks NR, 1992. Doing the right thing: Ants, honeybees and naked mole-rats. Trends Ecol. Evol. 7: 346–349.
Verbeek MEM, Drent PJ, Wiepkema PR, 1994. Consistent individual differences in early exploratory behaviour in male great tits. Anim. Behav. 48: 1113–1121.
Waddington SJ, Hughes WO, 2010. Waste management in the leaf-cutting ant *Acromyrmex echinatior*: The role of worker size, age and plasticity. Behav. Ecol. Sociobiol. 64: 1219–1228.
Waser NM, 1998. Task-matching and short-term size shifts in foragers of the harvester ant *Messor pergandei* (Hymenoptera: Formicidae). J. Insect Behav. 11: 451–462.
West-Eberhard MJ, 1996. Wasp societies as microcosms for the study of development and evolution. In: Turillazzi S, West-Eberhard MJ ed. Natural History and Evolution of Paper Wasps. New York: Oxford University Press.
Wilson EO, 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). I. The overall pattern in *Atta sexdens*. Behav. Ecol. Sociobiol. 7: 143–156.
Wray MK, Mattila HR, Seeley TD, 2011. Collective personalities in honeybee colonies are linked to colony fitness. Anim. Behav. 81: 559–568.