Evolutionary biology

Family-based guilds in the ant *Pachycondyla inversa*

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High relatedness promotes the evolution of sociality because potentially costly cooperative behaviours are directed towards kin. However, societies, such as those of social insects, also benefit from genetic diversity, e.g. through enhanced disease resistance and division of labour. Effects of genetic diversity have been investigated in a few complex eusocial species. Here, we show that genetically based division of labour may also be important in ‘simple societies’, with fewer individuals and limited morphological caste differentiation. The ponerine ant *Pachycondyla inversa* has small colonies, headed by several unrelated queens. We show that nest-mate workers from different matrilines engage in different tasks, have distinct chemical profiles and associate preferentially with kin in the nest, while queens and brood stay together. This suggests that genetically based division of labour may precede the evolution of complex eusociality and facilitate the existence of low relatedness societies functioning as associations of distinct families that mutually benefit from group living.

1. Introduction

Division of labour characterizes groups across levels of organization from the origins of the first genomes and multi-cellularity to human societies, and is a major mechanism for generating benefits of group living [1]. Division of labour may increase group efficiency by using variable skills of individuals, enhancing task coordination or diminishing competition over resources [2].

If division of labour had a genetic component, societies would benefit from genetic diversity [3]. Evolution of low relatedness groups may require such benefits, because of the logic of inclusive fitness theory that predicts low relatedness to select for selfishness and nepotism when groups consist of several lineages [1]. Together with other factors, such as social coercion and improved disease resistance, enhanced division of labour might counterbalance selection against genetic heterogeneity and explain the existence of cohesive societies with low relatedness.

In social insects, genetic effects on division of labour have been shown in a few complex societies, such as honeybees and leafcutter ants [4,5], where low relatedness is a derived trait that has been preceded by resolution of conflicts through coercion and acquiescence [1]. The potential to divide labour among individuals seems to be a feature of already very simple social groups, even solitary bees [6]. However, it is currently unclear whether genetically based division of labour is a consequence of or a precursor for the evolution of complex social organization.

We studied division of labour and variation in chemical profiles among matrilines in the ponerine ant *Pachycondyla inversa*, a species with solitary foraging habits, small colony sizes and little caste dimorphism [7,8]. The unusual peaceful coexistence of unrelated queens (mean relatedness $\bar{r} = 0.036 \pm 0.02$ [9]) in mature colonies of *P. inversa* means that genetic differences among lineages within
colonies are larger than in the previously investigated social insects [9]. We predicted that matriline differences in both tasks and cues could be found. Small colony size allows behavioural observations of all individuals, and investigation of behavioural, genetic and chemical heterogeneity.

2. Material and methods

_Pachycondyla inversa_ [7] colonies were collected in 2004 at Centro de Pesquisas do Cacau, Itabuna, Brazil. Five polygynous colonies (2–7 queens, 32–50 workers) were kept in the laboratory (60% humidity, 27°C, 12 L: 12 D photoperiod, ad libitum food and water), each housed in two interconnected plastic boxes (20 × 20 × 6 cm each) with a moistened plaster floor, both with two glass plate-covered cavities (5 cm diameter) as nest sites. At the beginning of the experiment, the cuticular lipids of all workers were analysed by Solid Phase Micro Extraction by rubbing the gaster for 3 min with a 7 μm polydimethylsiloxane fibre (Supelco), which was injected into a gas chromatograph (Agilent Technologies 6890N, capillary column: Rtx-5, 30 m) using the AGILENT CHEMSTATION software. Compounds were identified by comparison with published results [10] and on the basis of their mass spectra produced by an Agilent 5975 Inert MSD (70 eV electron impact ionization). The lognormal transformed proportional contributions of each peak to total peak area were used as variables in a hierarchical non-parametric MANOVA – matrices were used (implemented in an _Excel_ macro by M.R.). Correlation between pairwise genetic relatedness (calculated in GenALEX [15] according to [16]) and Euclidean chemical distance was investigated through Mantel tests. Statistical analyses were carried out in _R_ v. 2.15. The data are deposited in the Dryad repository [17].

Twelve-three peaks were detected and their area integrated using the AGILENT CHEMSTATION software. Compounds were identified by comparison with published results [10] and on the basis of their mass spectra produced by an Agilent 5975 Inert MSD (70 eV electron impact ionization). The lognormal transformed proportional contributions of each peak to total peak area in that sample were used in statistical analyses.

After chemical sampling, all ants were marked individually with enamel paint and observed by scan sampling. The regularly occurring behaviours of foraging, brood care and patrolling, and their location in a nest cavity were recorded three times per day at 3 h intervals from 10.00 to 16.00 (69 observations per colony). For each colony, the total numbers of occasions out of the 69 observation events when each individual was carrying out different activities, and/or located in different nest cavities, were variables for the statistical analysis. As queens in mature colonies do not engage in tasks other than egg laying, only their location was recorded, together with that of the brood pile(s).

Queens and workers were then genotyped at five microsatellite loci (according to [12]), and workers were assigned to matrilines by comparing queen and worker allele sharing patterns. The loci were highly variable (see the electronic supplementary material, table S1) and visual inspection of allele sharing patterns allowed unambiguous assignment of 96% per cent of workers. Individuals not unambiguously assigned to a matriline and individuals from matrilines with less than three workers were excluded from analyses of matriline differences. The number of variables in the chemical data was decreased to principal components analysis (PCA). Euclidean distances between individuals based on the principal components (PC) axes were used as variables in a hierarchical non-parametric MANOVA (‘adonis’ in _R_-package ‘vegan’ [13]) with colonies and matrilines nested within colonies as variables, to assess the proportions of variance explained by colony and matriline differences. Significance was tested against the distribution of values obtained by 9999 permutations of the dataset. PCA and MANOVA were also performed within each colony separately. Differences among matrilines were also assessed with canonical discriminant analysis. Task and nest cavity preferences were investigated separately within each colony using MANOVA and canonical discriminant analysis. Furthermore, we investigated temporal patterns in the data through a resampling scheme (see the electronic supplementary material, figure S2). To investigate correlations of chemical and behavioural profiles, RV-coefficients [14] between the data matrices were used (implemented in an _Excel_ macro by M.R.). Correlation between pairwise genetic relatedness (calculated in GenALEX [15] according to [16]) and Euclidean chemical distance was investigated through Mantel tests. Statistical analyses were carried out in _R_ v. 2.15. The data are deposited in the Dryad repository [17].

3. Results

Microsatellite analyses showed that in four of the five colonies, multiple queens contributed to the worker-force, and that within these colonies relatedness was low (average relatedness among nest-mate workers _r_ _w_ < 0.26; electronic supplementary material, table S2). Colony 5 consisted of only one matriline and was not used in analyses of matriline differences. Each pair of queens had at least one locus where they shared no alleles, confirming they were not full sisters.

The cuticular chemical profiles of workers consisted of saturated and unsaturated hydrocarbons, as observed previously [10]. The first five PC axes explained 51 per cent of the variation (see the electronic supplementary material, table S3) and were used in further analyses. Both colonies and matrilines within colonies differed in their chemical composition (hierarchical MANOVA, colonies _F_ _1,147_ = 33.9, _r_ ^2_ _ = 9.8% , _p_ < 0.001, matrilines within colonies _F_ _1,147_ = 15.4, _r_ ^2_ _ = 48% , _p_ < 0.0001). MANOVAs within colonies confirmed clear matriline differences (all _p_-values < 0.01, _r_ ^2_ _ > 38%). Discriminant analyses within colonies correctly classified 75–84 per cent of workers to their matrilines (figure 1).

Matrilines specialized on different behaviours (figure 2; MANOVA, _p_-values for matriline differences: Col 1: _p_ = 0.0004, _r_ ^2_ _ = 59% ; Col 2: _p_ = 0.001, _r_ ^2_ _ = 35% ; Col 3: _p_ = 0.0005, _r_ ^2_ _ = 42% ; Col 4: _p_ = 0.0004, _r_ ^2_ _ = 26% ; 52–67% of individuals classified correctly by discriminant analysis). The largest and most consistent differences among matrilines were found in the time spent tending brood (_p_ < 0.01 for difference among matrilines in every colony in univariate ANOVAs). Matrilines were non-randomly distributed among nest cavities (MANOVA _p_-values for matriline differences: Col 1: _p_ = 0.0005, _r_ ^2_ _ = 56% ; Col 2: _p_ = 0.0004, _r_ ^2_ _ = 42% ; Col 3: _p_ = 0.0005, _r_ ^2_ _ = 53% ; Col 4: _p_ = 0.02, _r_ ^2_ _ = 15% ; 37–56% of individuals classified correctly), suggesting association with kin. Queens and brood were found together on most of the occasions (see the electronic supplementary material, table S4), and no temporal dependencies were found (see the electronic supplementary material, figure S2).

Chemical profiles of individuals were correlated to their tasks in the total data, and the correlation was significant in three out of five colonies when tested separately (RV-coefficient for combined data = 0.08, _p_ < 0.001; Col 1: _RV_ = 0.20, _p_ = 0.085; Col 2: _RV_ = 0.24, _p_ = 0.029; Col 3: _RV_ = 0.16, _p_ = 0.26; Col 4: _RV_ = 0.27, _p_ = 0.001; Col 5: _RV_ = 0.4, _p_ < 0.001). The higher the relatedness between individuals in colonies with multiple matrilines, the shorter was their pairwise chemical distance (Col1: Mantel’s _r_ = 0.58, _p_ < 0.001; Col2: _r_ = 0.35, _p_ < 0.001; Col3: _r_ = 0.30, _p_ < 0.001; Col4: _r_ = 0.30, _p_ < 0.001), confirming the kin informative component in chemical profiles.

4. Discussion

Matrilines showed division of labour, i.e. formed family-based guilds, in _P. inversa_ colonies. Surface chemicals of
workers had matriline-specific components, and workers accordingly associated with their kin when choosing nest cavities. Thus, despite a shared colony environment, genetic heterogeneity results in behavioural heterogeneity. Potential benefits of division of labour among matrilines could at least partly explain why matrilines stay together despite potentially disruptive kin informative cues.

While factors such as colony stage, individual state and experience affect division of labour in simple societies [2], genetic effects have previously been observed only in complex...
societies. Our results are the first example that suggests that genetically based division of labour may precede the evolution of complex social organization and facilitate the existence of low relatedness societies. Benefits of division of labour have been notoriously difficult to demonstrate [2,8], but faster growth of multiple queen societies in P. inversa [18] could counterbalance the relatedness losses of sharing a nest with non-kin [9].

Matriline-specific cues reflect behaviour and could play a role in coordinating tasks [19]. The current data cannot resolve whether chemical differences among matrilines reflect underlying variation in cue genotypes per se, or whether matriline biases in tasks indirectly affect cue phenotypes. Maintenance of kin informative cues is an evolutionary puzzle [20], and it is an interesting prospect that effects of tasks on cues could help maintain genetically determined variation in cues that could be otherwise selected against. Similarly, matriline associations might arise indirectly through factors such as shared microhabitat preferences rather than genotypic differences in cues, but nevertheless individuals are provided information about the underlying genotypes.

Matriline differences in behaviour might reflect age-dependent task preferences [2]. However, no temporal trends were observed in our data over an observation period of one month, which suggests that such effects are minor. Furthermore, correlations among worker matriline and age would require temporal division of reproduction among queens, which appears to be absent in this species, at least based on egg laying rates of co-foundresses [8].

Matriline-specific odour variation also raises the question about nepotistic discrimination [1]. In our study, chemical differences among matrilines were reflected only in non-aggressive interactions among workers. The fact that queens and brood occupied the same nest cavities suggests that cue scrambling might hamper discrimination by workers. Detailed observations of matriline-specific worker–brood and worker–queen interactions are needed to unravel this.

Our work suggests that the interplay of costs and benefits of genetic diversity in social insects may be seen in a different light if we investigate associations with large genetic differences and low physical integration within colonies. We suggest that they may be seen more fruitfully as societies based on mutually benefiting groups of distinct families, more analogous to duck creches [21] and primitive human societies [22], than as tightly integrated colonies where all lineages share ancestry.

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References