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Absence of Nepotism in Genetically Heterogeneous Colonies of a Clonal Ant

Katrin Kellner & Jürgen Heinze

Biologie I, University of Regensburg, Universitätsstraße, Regensburg, Germany

Correspondence
Katrin Kellner, Biologie I, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany.
E-mail: antkatrina@gmail.com

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Abstract

Insect societies are normally closed entities from which alien individuals are excluded. The occasional fusion of unrelated colonies of the thelytokous ant *Platythyrea punctata* is therefore puzzling, because it strongly intensifies competition among nestmates for the replacement of an old reproductive. Most colonies of *P. punctata* have only one or few reproductives, which produce female offspring from unfertilized eggs, and therefore have a clonal structure. Fusion leads to multi-clone colonies. We compared the occurrence of dominance and policing behavior between single- and double-clone colonies. We find that the frequency of aggression is higher in double-clone colonies, but that individuals do not preferentially direct attacks toward non-clonemates. This matches observations in other species that social insects perceive genetic homogeneity but are not capable of reliable discrimination among nestmates of different degree of relatedness.

Introduction

Non-reproducing workers in insect societies obtain fitness only indirectly through caring for the sexual offspring of related reproductives. Insect societies therefore are usually closed systems, and an efficient system of discrimination among non-nestmates protects the workers from being exploited by unrelated individuals (Hamilton 1964, 1972; Trivers & Hare 1976). It is therefore surprising that a growing number of studies have documented the occasional merging of unrelated colonies in ants, wasps, and termites (Foitzik & Heinze 1998, 2000; Fisher et al. 2004; Prezoto & Santos-Prezoto 2005; Johns et al. 2009; Kellner et al. 2010; Kronauer et al. 2010). Colony fusion may lead to the puzzling phenomenon that non-relative individuals inherit colony resources. It is therefore expected to intensify conflict among nestmates about the division of reproductive labor and to lead to increased rates of dominance, policing, or punishment among nestmates.

Such conflict should be particularly prominent when new reproductives start to supersede the old reproductives, e.g., when replacement queens eclose from the brood, or when all individuals are potentially capable of becoming fertile. In the ponerine ant *Platythyrea punctata*, workers produce female offspring from unfertilized eggs by thelytokous parthenogenesis (Heinze & Hölldobler 1995). Dominance interactions and policing against surplus reproductives ensure that in each colony only one, rarely a few, workers lay eggs. This normally leads to a clonal colony structure (Schilder et al. 1999). Nevertheless, a considerable percentage of colonies contain workers with incompatible genotypes, suggesting the frequent occurrence of colony fusion or usurpation (Hartmann et al. 2005; Kellner et al. 2010). Indeed, unrelated colonies readily merge when they are forced to do so by experimental destruction of one of their nests (Kellner et al. 2010). After a short period of aggression and the elimination of one of the two original egg layers, workers from the different lineages form a stable,

mixed society. During the elimination of reproductives, workers apparently do not favor their clonemates. Instead, they might simply choose the more fertile reproductive, as suggested for regulation of queen numbers in foundress associations (Forsyth 1980).

However, nothing is known about the selection of a new reproductive in colonies consisting of different clone lineages. Several social insects have been shown to indirectly recognize the overall genetic structure of the society they live in and adaptively respond to it, e.g., in sex allocation (Boomsma et al. 2003; Hannonen & Sundström 2003) and facultative policing in a wasp species (Foster & Ratnieks 2000) and a leaf-cutting ant (Dijikstra et al. 2010). In contrast, unambiguous direct evidence for nepotism has so far only been found in drywood termites (Korb 2006), whereas evidence for the absence of nepotism has been found in numerous species (DeHeer & Ross 1997; Keller 1997; Holzer et al. 2006; Ratnieks et al. 2006; Goodisman et al. 2007; Atkinson et al. 2008; Zinck et al. 2009). If nepotistic behavior was present in the parthenogenetic ponerine ant *P. punctata*, we would expect a) workers of *P. punctata* in fused colonies compete more intensively for replacement of a removed reproductive and b) that worker policing against surplus reproductives is predominantly directed against unrelated nestmates.

Materials and Methods

Setting Up Experimental Colonies

Colonies of *Platythyrea punctata* were collected in Puerto Rico (Oct. 2005), Dominican Republic (Nov. 2006), and Barbados (June 2007). Prior to this experiment, colonies were genotyped with five polymorphic microsatellite markers developed for this species (Schilder et al. 1999). As source colonies for this experiment, we chose genetically homogeneous colonies from different populations. Setting up mixed colonies with workers originating from different populations presumably maximized the difference in genetic odor cues and was thus expected to facilitate nepotism.

Four single-clone colonies (SC1, SC2, SC4, and SC8) were established by placing 10–12 freshly eclosed callows and 5–10 cocoons into plastic boxes (20cm × 10cm × 6cm) with a plaster floor and a preformed cavity in the plaster (7 cm × 5 cm × 0.5 cm) covered by a glass plate and red foil serving as a nest. In each experimental single-clone colony, all individuals originated from a single source colony. In contrast, four double-clone colonies (DC1, DC2, DC7, and DC8) were established in similar nest boxes by placing 2–5 callows and 4–5 cocoons from one source colony together with 4–7 callows and 4–5 larvae from another source colony from a different population. The mean size of colonies found in the field is 34.8 (measured from 189 collected colonies; standard deviation: 52.26; range 2–475 individuals) (Kellner et al. unpublished data). The number of individuals used for setting up the experimental colonies was determined by the availability of callows and brood in the source colonies. To avoid distinct behaviors because of different age cohorts, only callows and brood items with approximately the same age were chosen. As freshly eclosed callows do not show aggression toward non-nestmates, this method allowed us to obtain colonies with two different clone lineages (in the following referred to as 'A' and 'B'). Furthermore, using callows instead of fusing mature colonies minimized the effect of different age structures per colony on behavior and division of reproductive labor. All ants were marked individually with Edding markers.

Experimental colonies were reared in the laboratory over a period of 1 mo under near-natural conditions in incubators (27°C, 60% humidity, and 12-h:12-h light/dark cycles) and fed three times per week a mixed diet of honey, frozen *Drosophila* flies, and pieces of crickets and cockroaches. The plaster was regularly wetted and a tube with water and cotton served as a permanent water supply.

Behavioral Assay

The behavioral assay was started after the first eggs had appeared in each colony, confirming the existence of an established egg layer. An overview over colony composition at the time of the start of the experiment is given in Table 1. Dead workers were recorded throughout the experiment. No biased mortality was observed, and none of the double-clone colonies turned into a single-clone colony because of the extinction of one clone lineage. The number of workers at the end of the experiment is shown in Table 1. Behavioral observations were carried out in a climate chamber with near-natural conditions (27°C, 60% humidity, and 12-h: 12-h light/dark cycles), under a stereomicroscope.

Colonies were observed by opportunistic sampling in individual sessions of 5 min each. For each session and individual, we recorded whether it moved inside or outside the nest or engaged in brood care, foraging, allogrooming, and aggression (ritualized

Table 1: Colony composition of experimental colonies at the start and the end of the experiment

			Start of experiment		End of experiment
Experimental colony	Source colony	Clone lineage	Workers	Brood	Workers
SC1	Barbados-Turner's Hall 8	–	13	10 cocoons	11
SC2	Barbados-Turner's Hall 1	–	21	One cocoon	16
SC4	Dominican Republic-Rancho Wendy 6	–	12	One cocoon	8
SC8	Puerto Rico-Sabana 4	–	8	One cocoon	6
DC1	Puerto Rico-Sabana 4	A	6	Four cocoons	4
	Dominican Republic-Monte Plata	B	5	Five larvae	4
DC2	Dominican Republic-Miches 9	A	6	Two cocoons	5
	Puerto Rico-Sabana 9	B	6	Five larvae	3
DC7	Dominican Republic-Rancho Wendy 7	A	5	Two cocoons	4
	Barbados-Turner's Hall 12	B	7	Four larvae	5
DC8	Dominican Republic-Rancho Wendy 12	A	7	Three cocoons	6
	Barbados-Turner's Hall 2	B	5	Five larvae	6

SC, single-clone colonies; DC, double-clone colonies; Whereas single-clone colonies represent true clone colonies with within-colony relatedness of $R = 1$, double-clone colonies containing two different clone lineages 'A' and 'B' exhibit relatedness values of $R = 1$ within and $R = 0$ between lineages.

aggression: antennal boxing, gaster flexing; violent aggression: biting; biting and dragging the opponent; stinging attempts). The behavior of ants was observed during four different phases, following a protocol used in previous studies (e.g., Brunner et al. 2009). In phase I, we observed the colonies over a period of 5 d (three sessions per day, 5 min per session, the total observation time was 75 min per colony) to identify the egg-laying individual by its specific behavioral profile (Hartmann et al. 2003). When we could not directly observe egg laying during this time span, we separated the supposed reproductive from the colony for one night (12 h). Finding freshly laid eggs the next morning confirmed the individual's status as reproductive.

In phase II, we split colonies into two equal parts, one with the reproductive ('queenright') and one without ('queenless'). This allowed one worker to establish itself as replacement reproductive. Individuals were separated haphazardly into the two parts; however; in the double-clone colonies, we made sure that each half had a 50:50 distribution of the two clone lineages. We observed behavior in the part without reproductive until the first eggs indicated the presence of a new reproductive (9-day observation period with three sessions per day, 5 min per session, total observation time 135 min, except colony DC7: 6 d of observation, 90-min total observation time). During this phase, observations of double-clone colonies allowed determining whether the establishment of a new reproductive was associated with nepotistic aggression.

To confirm reproductive status, individuals were separated in small boxes for 12 h over night, and the boxes were scanned for the presence of freshly laid eggs thereafter.

In phase III, we reunited the two split parts and observed continuously for the next 20 min each and thereafter in observation sessions of 5-min duration over several days until aggression had ceased (SC2: 3 d, SC1: 4 d, all others: 5 d, total observation time 80 min and 70 min, respectively). During this phase, observations of double-clone colonies allowed determining whether policing against the new reproductive was nepotistic.

Finally, we removed the old and new reproductives and all eggs from each colony in phase IV. The now orphaned colonies were again observed over the following 3 d (3 sessions per day, 5 min per session, total observation time 45 min) until new eggs were discovered in the colonies (already after 3 d). Observations during phase IV were made to determine the fate of police workers, which previously have been shown to have an increased likelihood of replacing the reproductive (Brunner et al. 2009).

State of Ovaries

After the experiment, all individuals (including the reproductives) were frozen and dissected to measure ovary length and record the status of their ovaries (I: undeveloped ovaries; n = 66 individuals; median, quartiles ovary length 0.87, 0.00, 2.52 mm, II: slightly elongated ovaries; n = 5; median, quartiles 6.96, 4.52, 7.83 mm, III: fully elongated mature ovaries with yellow bodies suggesting former egg-laying activity, n = 22; median, quartiles 10.35, 8.70, 10.70 mm).

Statistics

For statistical analysis, the frequencies of behaviors were quantified by standardizing each behavior per the number of individuals per 10 min (behavior/number of individuals/10 min). All statistical analyses were conducted in STATISTICA 6.0 (Statsoft 2003).

Note that for comparing single-clone and double-clone colonies the low sample size (4 vs. 4 colonies) means that even with large differences among treatments, the lowest p-value possible would be 0.02, which makes correction for multiple testing futile. For comparing aggression against reproductives and non-reproductive individuals, we chose to perform permutation unpaired *t*-tests as implemented in the software PAST (Hammer et al. 2001) because of the unbalanced sample size (e.g., comparing two reproductives against six or more non-reproductives). Because we were testing the absence or presence of nepotism (H_0: nepotism is absent), in the case of insignificant test results, we would accept the null hypotheses. However, a non-significant outcome can occur for two reasons: (1) the null hypothesis of no effect is actually true or (2) the null hypothesis of no effect is false, but small sample sizes prevent the test from detecting this effect. The latter option is the probability of encountering a type 2 error. To account for this problem, we calculated, as *post hoc* Power Analyses, the effect size measured as Cohen's d (Cohen 1988; Nakagawa & Cuthill 2007) and the 95% confidence intervals, as recommended by Colegrave & Ruxton (2002). If the statistical test was truly non-significant, then the confidence interval for the effect size would span 0 (Colegrave & Ruxton 2002). To calculate the minimum effect size, which we defined as the effect size that has to be present to achieve an α level of at least 0.049 (the sensitivity of a test), we used G*Power 3.1.2 (Faul et al. 2007).

Results

Aggressive policing was observed in both single- and double-clone colonies. While aggression rates were low in phases I and II (unmanipulated and split state), the frequency of attacks increased significantly in phase III (occurrence of two reproductives) and decreased again in phase IV (establishment of a new reproductive) (comparison of total aggression: Friedmann ANOVA, $\chi^2 = 13.5000$, df 3, $p < 0.004$; see Fig. 1). In phases I and II, only ritualized aggression was observed, whereas in phases III and IV, both ritualized and violent aggression occurred.

During the policing phase (phase III), in six of eight colonies, the two reproductives were involved in more than 50% of the observed aggressive interactions. (In colonies DC7 and DC8, the reproductives were involved only in 33% and 40%, respectively, of the observed total aggression.) As previously observed (Brunner et al. 2009), reproductive individuals were on average more frequently attacked than non-reproductives. In this study, reproductives in six of eight colonies (DC1, DC2, SC2, SC8 $p < 0.0001$, permutation *t*-test; SC1, SC4 $p < 0.05$) were more attacked than non-reproductives (non-significant results in DC7: $p = 0.4365$ and DC8: $p = 0.0642$). Workers did not show preference for either reproductive, because old reproductives and new reproductives were attacked at a similar rate (Mann–Whitney *U*-test, $U = 26.000$, $p = 0.528$). Reproductives were similarly attacked by workers which were separated from them during phase II and together with them (Wilcoxon matched pairs test: $T = 7.000$, $p = 0.463$; see Fig. 2). In all colonies, new reproductives were expelled from the nest by old reproductives and vice versa, and in one case, this leads to the death of the old reproductive SC2. In all other

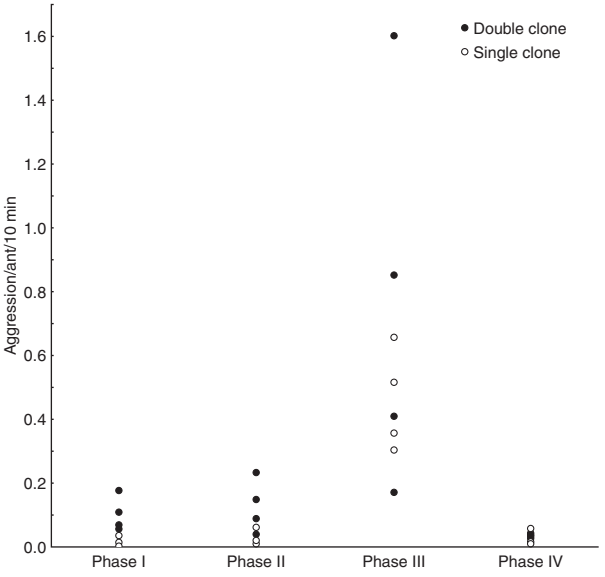

Fig. 1: Aggression profile during the different phases of the experiment. Phase I: non-manipulated colonies; Phase II: after splitting the colonies, observation of the parts without reproductives; Phase III: reunion of both parts, now each containing a reproductive; Phase IV: orphaned colonies after removing both reproductives; Each mean aggression in each colony is represented by a dot (solid dots: double-clone colonies, empty dots: single-clone colonies). The mean aggression rate (averaged over all colonies) is significantly higher in phase III than in the other phases (Friedmann ANOVA, $\chi^2 = 13.5000$, df = 3, $p < 0.004$).

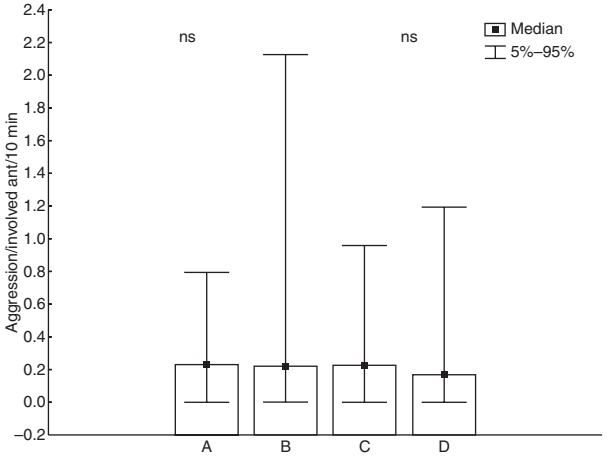

Fig. 2: Aggression toward the two reproductives during policing phase. Reproductives were similarly attacked by workers which were separated from them during phase II and together with them [Wilcoxon matched pairs test: T = 7.000, p = 0.463; effect size d = 0.33 (95%CI −1.32 to 0.65)]. Concerning clone lineage membership, reproductives were similarly attacked by workers from their own and from the other clone lineage [Wilcoxon matched pairs test: T = 14.000, p = 1.000; effect size d = 0.39 (95%CI −0.94 to 1.02)]. Legend on *X*-Axis refers to: A attacks against the reproductive from ants from the separated part, B attacks against the reproductive from ants from the same part, C attacks against the reproductives from members of a different clone lineage, D attacks against the reproductive from members of the same clone lineage.

colonies, aggression calmed down after 3 d of observation. Again as described previously (Brunner et al. 2009), non-reproductives, which had been most aggressive during phases III and IV, were likely to begin laying eggs after removal of the two former reproductives in phase IV (comparison of aggression/ant/10 min of workers with non-developed ovaries and developed ovaries during phase III: *U*-test: U = 53.000, p = 0.0007).

The frequency of aggression showed similar trajectories in double- and single-clone colonies, but in phases I and II, double-clone colonies generally showed a higher frequency of aggression than single-clone colonies (*U*-test, phase I, U = 0.00, p = 0.021; phase II, U = 1.00, p = 0.043; Table 2). In phases I and IV, double-clone colonies showed significantly more allogrooming than single-clone colonies (phase I, U = 0.50, p = 0.03; phase IV, U = 0.00, p = 0.021).

To test for nepotism and preferences of individuals for members of their own clone lineage, we compared behaviors within and between clone lineages in the double-clone colonies. The frequency of aggression and grooming between members of clone lineages did not differ from those within lineages (see Table 3), and no differences were found in the amount of aggression directed toward reproductives from the own and the other clone lineage (Wilcoxon matched pairs test, T = 14.000, p = 1.000; see Fig. 2). Because all confidence intervals for the calculated effect sizes span 0, we assume that accepting our null hypothesis is correct, and a false decision based on a large type 2 error can be excluded. To obtain a significant test result on a minimum level of p = 0.05, the required minimum effect size would have been d = 1.66 in the case of comparing aggression toward reproductives from individuals from the same part and the split part, and d = 3.71 in testing aggression toward the reproductive from members of the same compared with the different clone lineage. Reproductives of different clone lineages did not fight more against each other than reproductives of the same clone lineage (*U*-test, U = 2.00 p = 1.00). In only one of four double-clone colonies did old and new reproductives belong to the same lineage (Table 4), suggesting that dominance of one lineage

Table 2: Aggressive and allogrooming behavior in single-clone (SC) and double-clone (DC) colonies

Phase	Behavior	SC	DC	U	p-value	
Phase I	Aggression	0.0218 (0.0050–0.0357)	0.0879 (0.0610–0.1434)	0.00	**0.021**	**DC > SC**
	Grooming	0.1865 (0.1587–0.1944)	0.2444 (0.2056–0.2783)	0.50	**0.03**	**DC > SC**
Phase II	Aggression	0.0134 (0.0070–0.0389)	0.1173 (0.0617–0.1914)	1.00	**0.043**	**DC > SC**
	Grooming	0.1717 (0.1574–0.1920)	0.1914 (0.1451–0.2407)	7.00	0.0773	DC = SC
Phase III	Aggression	0.4364 (0.3301–0.5872)	0.6291 (0.2893–1.2250)	6.00	0.564	DC = SC
	Grooming	0.5988 (0.4138–0.6983)	0.4149 (0.3655–0.4688)	4.00	0.248	DC = SC
Phase IV	Aggression	0.0093 (0.0056–0.0188)	0.0111 (0.0054–0.0236)	6.50	0.665	DC = SC
	Grooming	0.0250 (0.0198–0.0299)	0.0516 (0.0522–0.0145)	0.00	**0.021**	**DC > SC**

Frequencies of aggression and allogrooming were calculated by behavior/ant/10 min. Medians and lower and upper quartiles are given. Significant p-values are given in bold (Mann–Whitney *U*-test). Effect sizes for non-significant aggression tests are d = 0.65 (95%CI −2.08 to 0.77) in phase III and d = 0 (−1.39 to 1.39) in Phase IV.

Table 3: Comparisons of frequencies of aggression and allogrooming within clone lineages (directed from clonemate toward clonemate) and between clone lineages (directed from members of one clone lineage toward members of the other clone lineage)

	Total aggression		Effect size	Ritualized aggression		Effect size	Violent aggression		Effective size	Allogrooming		Effect size
Phases	U	p	d	U	p	d	U	p	d	U	p	d
I	–	–		5.000	0.39	0.88 (−0.56 to 2.35)	–	–		3.000	0.14	1.13 (−2.62 to 0.36)
II	–	–		2.000	0.08	1.31 (−0.21 to 2.85)	–	–		4.500	0.31	0.85 (−2.30 to 0.60)
III	8.000	1.00	0.27 (−1.66 to 1.12)	7.000	0.77	0.23 (−1.16 to 1.62)	6.000	0.56	0.50 (−1.91 to 0.91)	3.500	0.19	1.31 (−2.84 to 0.21)
IV	5.000	0.39	0.60 (−0.76 to 2.09)	6.000	0.56	0.30 (−1.09 to 1.70)	5.000	0.39	0.36 (−1.04 to 1.76)	1.000	0.04	1.39 (−2.93 to 0.15)

No significant differences were found in phase I–III, but in phase IV, allogrooming was significantly higher within clone lineages than between clone lineages (Mann–Whitney *U*-test). Violent aggression (e.g., biting) between individuals was only observed in phases III and IV. The effect size is given as Cohen's d (Cohen 1988) with 95% confidence intervals.

Table 4: Clone lineage membership of reproductives in the double-clone colonies (DC)

Colonies	OR Phase I	NR Phase II	FR Phase IV
DC1	A	A	A
DC2	A	B	A
DC7	B	A	A
DC8	A	A	B

In colony DC1, all three reproductives stem from the same clone lineage, whereas in the other three colonies, frequent turnovers of clone lineages were observed. OR, old reproductive in Phase I; NR, new reproductive, established in the part without OR in Phase II; FR, future reproductive, established in the orphaned colony after taking out OR and NR in Phase IV.

over the other is absent and frequent turnovers are common.

Discussion

Our data clearly demonstrate the occurrence of dominance interactions and aggressive policing toward reproductive individuals in both single-clone colonies and genetically heterogeneous double-clone colonies of the thelytokous ant *Platythyrea punctata*. As expected from the increased genetic heterogeneity, the level of aggression was generally higher in double-clone colonies. However, individuals did not appear to discriminate between nestmates from their own and another clone lineage, i.e., there does not appear to be evidence of nepotistic behavior.

Encounters among old and new reproductives often resulted in violent fights, which were later joined by non-reproductive individuals. However, fights among clonemates did not differ from fights among individuals belonging to different clone lineages. Moreover, non-reproductives involved in the fights supported neither the reproductive they had accompanied during phase II nor the reproductive belonging to their own clone lineage. We therefore conclude that nepotism is absent at least in the context of the regulation of reproduction and supersedure of the reproductive in fused colonies. As we set up colonies consisting of callow workers from different populations, we can safely assume that nepotism will be similarly absent from natural, mixed colonies, which contain workers from more similar genetic lineages (see Kellner et al. 2010).

Our research corroborates previous studies showing that social insects perceive and react to genetic heterogeneity of their colonies but fail to distinguish between related and unrelated nestmates. For example, workers differently manipulate the sex ratio of

the brood in colonies with singly mated or multiply mated queens (e.g., Sundström et al. 1996). Workers might obtain a crude estimate of genetic variation from genetically encoded odor cues, but apparently lack the information enabling them to distinguish reliably between full and half sisters (Boomsma et al. 2003). Nepotistic behavior is also absent in another thelytokous ant, *Pristomyrmex punctatus* (Tsuji & Ito 1986; Tsuji 1988, 1990). Although on a first glance the absence of nepotism appears to be at odds with inclusive fitness theory, group-level costs of nepotism and the risks of making mistakes might constrain the evolution of kin recognition (e.g., Carlin 1988; Wenseleers 2007) and the evolution of kin-informative recognition cues (Nehring et al. 2010; van Zweden et al. 2010).

Given that dominance and policing in *P. punctata* appear to help to maintain an optimal balance between the numbers of workers and brood items (Hartmann et al. 2003), workers might support the reproductive with the highest actual or at least potential fecundity, regardless of the clone lineage membership. Similar findings have been shown in fire ants (DeHeer & Ross 1997) and wood ants (Holzer et al. 2006) and indicate that the proximate mechanisms of recognition constrain kin discrimination.

Despite the increased level of aggression in fused colonies of *P. punctata*, genetic data suggest that fusion regularly occurs in natural populations (Kellner et al. 2010). At present, it is unknown whether heterogeneous colony composition has a positive impact on colony performance regarding parasite defense (e.g., Liersch & Schmid-Hempel 1998; Reber et al. 2008). Alternatively, regular fusion might be a consequence of limited variability in genetic odor cues, as suggested for another ant with regular fusion, *Themnothorax nylanderi* (Heinze et al. 1996). Nestmate discrimination in the thelytokous ant *Pristomyrmex punctatus* similarly appears to rely on transferable, presumably environmentally derived odor cues, which makes colonies vulnerable to socially parasitic lineages (e.g., Dobata et al. 2009, 2011).

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Literature Cited

Atkinson, L., Teschendorf, G. & Adams, E. S. 2008: Lack of evidence for nepotism by workers tending queens of the polygynous termite *Nasutitermes corniger*. Behav. Ecol. Sociobiol. **62**, 805—812.

Boomsma, J. J., Nielsen, J., Sundström, L., Oldham, N. J., Tentschert, J., Petersen, H. C. & Morgan, E. D. 2003: Informational constraints on optimal sex allocation in ants. Proc. Natl Acad. Sci. USA **100**, 8799—8804.

Brunner, E., Kellner, K. & Heinze, J. 2009: Policing and dominance behaviour in the parthenogenetic ant *Platythyrea punctata*. Anim. Behav. **78**, 1427—1431.

Carlin, N. F. 1988: Discrimination between and within colonies of social insects: two null hypotheses. Neth. J. Zool. **39**, 86—100.

Cohen, J. 1988: Statistical Power Analysis for the Behavioural Sciences, 2nd edn. Erlbaum, Hillsdale, NJ.

Colegrave, N. & Ruxton, G. D. 2002: Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. Behav. Ecol. **14**, 446—450.

DeHeer, C. J. & Ross, K. G. 1997: Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. **40**, 27—33.

Dijikstra, M. B., van Zweden, J. S., Dirchsen, M. & Boomsma, J. J. 2010: Workers of *Acromyrmex echinatior* leafcutter ants police worker-laid eggs, but not reproductive workers. Anim. Behav. **80**, 487—495.

Dobata, S., Sasaki, T., Mori, H., Hasegawa, E., Shimada, M. & Tsuji, K. 2009: Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. Proc. Biol. Sci. **276**, 567—574.

Dobata, S., Sasaki, T., Mori, H., Hasegawa, E., Shimada, M. & Tsuji, K. 2011: Persistence of the single lineage of transmissible 'social cancer' in an asexual ant. Mol. Ecol. **20**, 441—455.

Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. 2007: G*Power: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods **39**, 175—191.

Fisher, M. L., Gold, R. E., Vargo, E. L. & Cognato, A. I. 2004: Behavioral and genetic analysis of the colony fusion in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology **44**, 565—576.

Foitzik, S. & Heinze, J. 1998: Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. Behav. Ecol. **9**, 367—375.

Foitzik, S. & Heinze, J. 2000: Intraspecific parasitism and split sex ratios in a monogynous and monandrous ant (*Leptothorax nylanderi*). Behav. Ecol. Sociobiol. **47**, 424—431.

Forsyth, A. 1980: Worker control of queen density in Hymenopteran Societies. Am. Nat. **116**, 895—898.

Foster, K. R. & Ratnieks, F. L. W. 2000: Facultative worker policing in a wasp. Nature **401**, 692—693.

Goodisman, M. A. D., Kovacs, J. L. & Hoffman, E. A. 2007: Lack of conflict during queen production in the social wasp *Vespula maculifrons*. Mol. Ecol. **16**, 2589—2595.

Hamilton, W. D. 1964: The genetical evolution of social behaviour. I, II. J. Theor. Biol. **7**, 1—52.

Hamilton, W. D. 1972: Altruism and related phenomena, mainly in social insects. Annu. Rev. Ecol. Syst. **3**, 193—232.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. 2001: PAST: Palaeontological Statistics software package for education and data analysis. Palaeontologia Electronica **4**, 9.

Hannonen, M. & Sundström, L. 2003: Worker nepotism among polygynous ants. Nature **421**, 910.

Hartmann, A., Wantia, J., Torres, J. A. & Heinze, J. 2003: Worker policing without genetic conflicts in a clonal ant. Proc. Natl Acad. Sci. USA **100**, 12836—12840.

Hartmann, A., Wantia, J. & Heinze, J. 2005: Facultative sexual reproduction in the parthenogenetic ant *Platythyrea punctata*. Insect. Soc. **52**, 155—162.

Heinze, J. & Hölldobler, B. 1995: Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant, *Platythyrea punctata*. Naturwissenschaften **82**, 40—41.

Heinze, J., Foitzik, S., Hippert, A. & Hölldobler, B. 1996: Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. Ethology **102**, 510—522.

Holzer, B., Kümmerli, R., Keller, L. & Chapuisat, M. 2006: Sham nepotism as a result of intrinsic differences in brood viability in ants. Proc. R. Soc. Lond. B Biol. **273**, 2049—2052.

Johns, P. M., Howard, K. J., Breisch, N. L., Rivera, A. & Thorne, B. L. 2009: Nonrelatives inherit colony resources in a primitive termite. Proc. Natl Acad. Sci. USA **106**, 17452—17456.

Keller, L. 1997: Indiscriminate altruism: unduly nice parents and siblings. Tree **12**, 99—103.

Kellner, K., Barth, B. & Heinze, J. 2010: Colony fusion causes within-colony variation in a parthenogenetic ant. Behav. Ecol. Sociobiol. **64**, 737—746.

Korb, J. 2006: Limited food induces nepotism in drywood termites. Biol. Lett. **2**, 364—366.

Kronauer, D. J. C., Schöning, C., D'Ettorre, P. & Boomsma, J. J. 2010: Colony fusion and worker reproduction after queen loss in army ants. Proc. Biol. Sci. **277**, 755—763.

Liersch, S. & Schmid-Hempel, P. 1998: Genetic variation within social insect colonies reduces parasite load. Proc. R. Soc. Lond. B Biol. **265**, 221—225.

Nakagawa, S. & Cuthill, I. C. 2007: Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. **82**, 591—605.

Nehring, V., Evison, S. E. F., Santorelli, L. A., d'Ettorre, P. & Hughes, W. O. H. 2010: Kin-informative recognition cues in ants. Proc. Biol. Sci. doi: 10.1098/rspb.2010.2295.

Prezoto, F. & Santos-Prezoto, H. H. 2005: Occurrence of nest fusion in the swarm-founding wasp *Polybia paulista* (Hymenoptera: Vespidae). Sociobiology **45**, 99—103.

Ratnieks, F. W. L., Foster, K. R. & Wenseleers, T. 2006: Conflict resolution in insect societies. Annu. Rev. Entomol. **51**, 581—608.

Reber, A., Castella, G., Christe, P. & Chapuisat, M. 2008: Experimentally increased group diversity improves disease resistance in an ant species. Biol. Lett. **11**, 682—689.

Schilder, K., Heinze, J., Roy, G. & Hölldobler, B. 1999: Microsatellites reveal clonal structure of populations of the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera: Formicidae). Mol. Ecol. **8**, 1497—1507.

Statsoft 2003: Statistica (data analyses software system), Version 6.0. Tulsa, Oklahoma.

Sundström, L., Chapuisat, M. & Keller, L. 1996: Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science **274**, 993—995.

Trivers, R. L. & Hare, H. 1976: Haplodiploidy and the evolution of social insects. Science **191**, 249—263.

Tsuji, K. 1988: Inter-colonial incompatibility and aggressive interactions in *Pristomyrmex pungens* (Hymenoptera: Formicidae). J. Ethol. **6**, 77—81.

Tsuji, K. 1990: Kin recognition in *Pristomyrmex pungens* (Hymenoptera: Formicidae): asymmetrical change in acceptance and rejection due to odour transfer. Anim. Behav. **40**, 306—312.

Tsuji, K. & Ito, Y. 1986: Territoriality in a queenless ant *Pristomyrmex pungens* (Hymenoptera: Formicidae). Appl. Entomol. Zool. **21**, 377—381.

Wenseleers, T. 2007: Nepotism absent in insect societies-or is it? Mol. Ecol. **16**, 3063—3065.

Zinck, L., Chaline, N. & Jaisson, P. 2009: Absence of nepotism in worker-queen care in polygynous colonies of the ant *Ectatomma tuberculatum*. J. Insect Behav. **22**, 196—204.

van Zweden, J. S., Brask, J. B., Christensen, J. H., Boomsma, J. J., Linksvayer, T. A. & d'Ettorre, P. 2010: Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. J. Evol. Biol. **23**, 1498—1508.