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Original Article

Non–nest mate discrimination and clonal colony structure in the parthenogenetic ant *Cerapachys biroi*

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Understanding the interplay between cooperation and conflict in social groups is a major goal of biology. One important factor is genetic relatedness, and animal societies are usually composed of related but genetically different individuals, setting the stage for conflicts over reproductive allocation. Recently, however, it has been found that several ant species reproduce predominantly asexually. Although this can potentially give rise to clonal societies, in the few well-studied cases, colonies are often chimeric assemblies of different genotypes, due to worker drifting or colony fusion. In the ant *Cerapachys biroi*, queens are absent and all individuals reproduce via thelytokous parthenogenesis, making this species an ideal study system of asexual reproduction and its consequences for social dynamics. Here, we show that colonies in our study population on Okinawa, Japan, recognize and effectively discriminate against foreign workers, especially those from unrelated asexual lineages. In accord with this finding, colonies never contained more than a single asexual lineage and average pairwise genetic relatedness within colonies was extremely high ($r = 0.99$). This implies that the scope for social conflict in *C. biroi* is limited, with unusually high potential for cooperation and altruism. *Key words:* aggression, asexuality, chimera, cooperation, Formicidae, thelytoky. *[Behav Ecol]*

INTRODUCTION

Major transitions in evolution occur when formerly independent units join to form integrated entities of higher complexity (Maynard Smith and Szathmáry 1995; Bourke 2011). These steps can also be conceptualized as transitions in the level of "organismality" (e.g., Queller and Strassmann 2009). Examples include the transition from unicellular to multicellular organisms, and the transition from solitary insects to eusocial insect societies. However, although the cells in multicellular organisms are generally genetically identical, individuals in insect societies are usually not. This implies that the reproductive interests of individuals are not perfectly aligned, giving rise to social conflicts that have to be suppressed or resolved to maintain functionality at the colony level (Ratnieks et al. 2006). Recently, however, it has become apparent that several ant species reproduce predominantly via thelytokous parthenogenesis, where females produce female offspring without fertilization. This can result in female offspring being genetically identical (or at least extremely similar) to their mother (Schilder et al. 1999; Hasegawa et al. 2001; Pearcy et al. 2004, 2006, 2011; Fournier et al. 2005; Hartmann et al. 2005; Foucaud et al. 2006, 2007, 2010; Ohkawara et al. 2006; Dobata et al. 2009, 2011; Kellner and Heinze 2011a; Rabeling et al. 2011; Wenseleers and Van Oystaeyen 2011; Kronauer et al. 2012; Rabeling and Kronauer 2013).

Some of these idiosyncratic reproductive systems could potentially result in colonies composed entirely of clonally identical individuals, especially in cases where colonies are founded by a single asexual individual. Such colonies should largely be void of internal social conflict. Concomitantly, in such colonies, conflicts are still possible under 2 situations. First, mutations in a subset of individuals can give rise to genetic mosaics. If these mutants evade reproductive control, they can manifest themselves as "social cancers" and compete over contribution to the offspring generation (Strassmann and Queller 2004). In species where new colonies are founded by single queens, the impact of such "social cancers" is curtailed by the recurrent genetic bottlenecks during the colony founding stage. This is analogous to the fact that cancerous somatic mutations are not passed on to the offspring in multicellular animals, where individuals develop from a single cell and the separation between germline and soma occurs at an early stage during embryonic development (Buss 1987; Michod 1996). On the other hand, in social insects with dependent colony founding by budding or fissioning, "social cancers" have the potential to be transmitted vertically from generation to generation. This is analogous to some forms of asexual reproduction in plants where germline and soma are not clearly separated (D'Amato 1997; Folse and Roughgarden 2010; Clarke 2012). Second, fusions of individuals from genetically distinct clonal lineages can give rise to chimeric colonies. Chimeras are the rare exception among multicellular animals, probably because they often lead to destructive

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internal conflicts and rarely constitute a selective advantage at the organism level (Foster et al. 2002; Strassmann and Queller 2004). However, in some ascidians, slime moulds, and cooperative bacteria, for example, chimeric associations form readily, possibly because of selective advantages associated with larger group size (e.g., Stoner et al. 1999; Foster et al. 2002; Kraemer and Velicer 2011). Chimeric associations are also regularly found in colonies of some parthenogenetic ant species (Hasegawa et al. 2001; Kellner et al. 2010), where they can involve "transmissible social cancers," that is, genetic "cheater" lineages that spread horizontally between colonies (Dobata et al. 2009). Thelytokous social insects are, therefore, potentially powerful study systems to investigate the dynamics of social conflict and cooperation (Wenseleers and Van Oystaeyen 2011; Rabeling and Kronauer 2013).

Here, we studied the social cohesion and genetic composition of colonies of the parthenogenetic ant *Cerapachys biroi*. This species is unusual in that queens and reproductive hierarchies are absent and all workers in a colony can reproduce via thelytokous parthenogenesis (Tsuji and Yamauchi 1995; Ravary and Jaisson 2004). A native of continental Asia, *C. biroi* has become introduced on tropical and subtropical islands around the world, probably as a consequence of human commerce (Kronauer et al. 2012; Wetterer et al. 2012). At least in the introduced range, the species reproduces almost entirely asexually, and genetically identical individuals are commonly found even across geographically distant populations (Kronauer et al. 2012). *C. biroi* is a specialized ant predator with army ant-like behavior, and the entirely subterranean colonies are usually composed of a few hundred individuals (Tsuji and Yamauchi 1995; Ravary and Jaisson 2002, 2004). New colonies are probably established by budding, that is, the genetic bottleneck of a single founding individual is absent (Tsuji and Dobata 2011). Using behavioral assays, we first asked whether *C. biroi* workers discriminate against foreign individuals, that is, whether colonies are able to distinguish self from nonself. Although non–nest mate discrimination is absent in many invasive species and can result in unicolonial population structures (e.g., Helanterä et al. 2009), recognition would probably be crucial to maintain genetic homogeneity at the colony level in a system like *C. biroi*. Second, we employed a population genetics approach to ask whether colonies in the field are monoclonal, chimeric, or mosaic. This information is important in delimiting the scope for cooperation and conflict in this species and will further our understanding of the ecology and evolution of thelytokous social insects.

MATERIAL AND METHODS

Samples

In April 2008, we collected 22 live colonies of *C. biroi* on Okinawa, where the species has been introduced, and transferred them to the laboratory (sample localities are given in Tables S1 and S2). In an effort to minimize effects of laboratory maintenance on colony recognition (e.g., via changes in cuticular hydrocarbon profiles), all behavioral assays were performed within 3 months after collection, and individuals that died in the laboratory over the first 3 months after collection were stored in 95% ethanol for genotyping.

Non–nest mate discrimination

Non–nest mate discrimination trials were performed between 19 pairs of colonies belonging either to the same or different asexual lineages (see below). For each original stock colony, we set up an experimental "host" colony composed of 18–22 unmarked workers in a standard petri dish with a moist plaster of Paris floor. From each stock colony, we also marked 10 workers with a dot of red paint (Humbrol Enamel Paint) on the abdomen or petiole and transferred them to a separate petri dish. For each pairwise colony comparison, a total of 20 individual behavioral trials were performed: 5 marked focal workers from colony A were introduced into colony B (1 at a time), 5 focal workers from colony A were introduced into colony A, 5 focal workers from colony B were introduced into colony A, and 5 focal workers from colony B were introduced into colony B. For each encounter between the focal individual and a resident individual from the host colony, we noted whether the interaction was "neutral" (the ants ignored each other or one or both ants performed slow antennation) or "discriminatory" (discriminatory interactions involved at least one of the following: 1) "antennal drumming," that is, rapid and prolonged antennation with parallel extended antennae [which is very distinctive from slow antennation], 2) biting of appendices [legs or antennae], and 3) attempting to sting, where one ant bends her gaster forward and extends the sting [actual stinging was never observed]). All "neutral" and "discriminatory" encounters of each focal worker were counted for 3 min in each trial. The host colony was switched after each trial to minimize prolonged arousal of host workers, and forceps were washed in acetone between trials to avoid transfer of pheromones or other odorants. Each marked worker was only used once in each pairwise colony comparison, but given the small size of field colonies, most workers had to be used more than once between different colony comparisons. All behavioral observations were conducted blindly with respect to the focal worker's colony of origin. Levels of discrimination against introduced ants in between-colony versus within-colony trials were compared using 1-sided Wilcoxon rank-sum (Mann–Whitney *U*) tests in S-PLUS.

Molecular protocols

We initially extracted DNA from 1 individual per colony ($n = 22$ total) using the QIAGEN DNeasy Blood & Tissue kit. Each individual was genotyped at 30 microsatellite loci and sequenced for 2 mitochondrial gene fragments (658 bp of *cytochrome oxidase I* and 575 bp of *cytochrome oxidase II*) as has been described in Kronauer et al. (2012). An earlier analysis (Kronauer et al. 2012) revealed that these 22 individuals clustered into only 2 distinct multilocus (asexual) lineages (MLLs, i.e., groups of multilocus genotypes [MLGs] that are derived from a single sexual recombination event) (MLLs 1 and 6 in Kronauer et al. 2012). This analysis also showed that the Okinawa population seems to reproduce exclusively asexually (Kronauer et al. 2012). We then extracted DNA from an additional 203 individuals (for a total of 225 individuals; see Table 1 for details) by heating homogenized individual ants in 100 µL of 5% Chelex 100 (Bio-Rad) to 96 °C for 15 min. After brief centrifugation, the supernatant was used as template in all subsequent polymerase chain reactions. Ants from colonies where the previously genotyped individual belonged to MLL1 were genotyped for 11 loci that showed some level of heterozygosity within that asexual lineage (microsatellite loci ED32S, EGR4W, D71AW, D4XW2, ETJ3E, D8M16, ETWBP, ED6BM, B8PND, ESA52, and D8EP1). Ants from colonies where the previously genotyped individual belonged to MLL6 were genotyped for 12 loci that showed some level of heterozygosity within that asexual lineage (microsatellite loci ED32S, EFAFC, EGR4W, D8ZOW, ETJ3E, D8M16, D9Y4L, ETWBP, ED6BM, E27C5, ESA52, and D8EP1). Eight of these 15 different loci were assayed in both lineages (microsatellite loci ED32S, EGR4W, ETJ3E, D8M16,

Table 1
Non–nest mate discrimination in *Cerapachys biroi*

	MLL1				MLL6			
Colony no.	6	8	13	17	3B	9	12	14
6	—							
8	2/1 ns	—						
13	3/11 ns	2/9 ns	—					
17		1/7 ns	0/2 ns	—				
3B		1/20**	1/35**	0/35**	—			
9		0/57**	2/45**	1/170***	6/51**	—		
12		1/42***	2/38 ns	5/42***	1/23 ns	0/0 ns	—	
14					4/26 ns		0/8*	—

Discriminative behaviors were recorded in pairwise colony comparisons. The figure before the slash gives the total number of incidences of discriminatory behavior observed in within-colony trials, whereas the figure after the slash gives the number for between-colony trials. Wilcoxon rank-sum tests were used to test whether these behaviors were preferentially directed toward non–nest mates (significance levels are ns [not significant], *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). The upper left and lower right quadrants give pairwise comparisons within asexual lineages, and the lower left quadrant gives pairwise comparisons between asexual lineages. Not all possible pairwise comparisons were tested.

ETWBP, ED6BM, ESA52, and D8EP1). Details for genetic markers and genotyping protocols are given in Kronauer et al. (2012). The final microsatellite data matrix is deposited in the Dryad repository.

Population genetic analyses

We used the program GenClone 2.0 (Arnaud-Haond and Belkhir 2007) to compute and plot genetic pairwise distances ("allele distances," i.e., the number of allele differences) between all MLGs based on the 8 microsatellite loci shared across all samples. Under sexual reproduction, the distribution of genetic distances should be unimodal, whereas a bimodal distribution with a second peak at very small genetic distances is expected under asexual reproduction, indicating MLGs derived from the same sexual reproductive event (Arnaud-Haond et al. 2007). A distinct peak at small genetic distances was apparent in our data set (1–5 alleles across 8 diploid loci; Figure S1), indicating samples that belonged to the same MLL and only differed due to mutations, partial loss of heterozygosity as a result of recombination during asexual reproduction, or genotyping errors. We, therefore, grouped all individuals with 5 or less allelic differences into the same MLL (Arnaud-Haond et al. 2007; Kronauer et al. 2012). This resulted in only 2 MLLs that perfectly corresponded to MLL1 and MLL6, which have been previously reported from Okinawa (Kronauer et al. 2012).

We then conducted separate analyses in GenClone 2.0 for each MLL, using the full set of genetic markers. First, we grouped all individual multilocus microsatellite genotypes into recurrent MLGs. For each colony, we computed the number of observed MLGs (G_o) and MLLs (L_o) and estimated the effective number of MLGs (G_e) and MLLs (L_e) using the sample-size corrected estimator of Nielsen et al. (2003) (their equation 16). We calculated a nonsampling error for each colony, assuming that a putative second MLL in fact accounted for 25% of the workers in that colony, as $F_L = 0.75^n$, where n is the sample size. We then computed pairwise genetic distances between all MLGs within a given MLL in GenClone 2.0 as described above. Based on this distance matrix, we calculated average pairwise relatedness within colonies as $r = (A - D)/A$, where A is the maximum possible allele distance given the markers (i.e., in this case, this equals the number of microsatellite loci times 2, because all loci were polymorphic) and D is the average observed allele distance between individuals in a given colony. Because colonies never contained more than 1 MLL (see below), calculations of r always involved individuals of a single MLL that had been genotyped for an identical set of microsatellite loci. We also calculated average pairwise allele distance D between individuals from different colonies for each asexual lineage separately. Given that *C. biroi* reproduces asexually and the studied populations show very low clonal diversity, we did not perform standard calculations of pairwise regression relatedness (e.g., Queller and Goodnight 1989).

RESULTS

Non–nest mate discrimination

When all pairwise colony comparisons were combined, significant levels of non–nest mate discrimination were found both across comparisons within and between different asexual lineages (both $P < 0.001$). When analyzed separately, significant levels of discrimination against non–nest mates were observed in 10 out of 19 pairwise colony comparisons (Table 1). Eight out of 9 pairwise comparisons between colonies of different MLLs and 2 out of 10 pairwise comparisons between colonies of the same MLL were statistically significant (Table 1; 2-tail Fisher's exact test $P = 0.005$). This indicates that colonies of *C. biroi* can indeed recognize and discriminate against non–nest mates, and that they are more likely to discriminate against non–nest mates from a different asexual lineage than non–nest mates from the same asexual lineage.

Population genetic analyses

The 225 workers in our data set belonged to 2 distinct asexual lineages that have been described from Okinawa previously (MLL1 and MLL6 in Kronauer et al. 2012), and no signs of sexual reproduction were detected. Fourteen colonies contained workers with MLL1 genotypes and 8 colonies contained workers with MLL6 genotypes. Colonies with a mix of MLL1 and MLL6 workers were not found (Table 2). The average probability that we failed to detect a second MLL that occurred at moderate frequency (0.25) due to insufficient sample sizes was small ($F_L = 0.06$) (Table 2). Although in 9 colonies all genotyped workers were genetically identical across all microsatellite loci, we detected small differences between some workers in the remaining 13 colonies (Table 2 and Tables S1 and S2). On average, we detected 1.9 MLGs per colony and the average effective number of MLGs per colony (G_e) was 1.5 (Table 2). Average pairwise relatedness within

Table 2
Genetic composition of *Cerapachys biroi* colonies on Okinawa

Colony no.	n	G_o	G_e	L_o	L_e	F_L	r
1	10	3	3.3	1	1	0.06	0.96
2	11	3	1.9	1	1	0.04	0.97
3a	11	1	1	1	1	0.04	1
3b	10	1	1	1	1	0.06	1
4	9	2	1.3	1	1	0.08	0.99
5	11	2	1.2	1	1	0.04	0.99
6	11	2	1.2	1	1	0.04	0.99
7	10	2	1.2	1	1	0.06	0.99
8	10	1	1	1	1	0.06	1
9	11	2	1.5	1	1	0.04	0.99
10	11	1	1	1	1	0.04	1
11	11	4	2.4	1	1	0.04	0.97
12	10	1	1	1	1	0.06	1
13	10	2	1.2	1	1	0.06	0.99
14	10	1	1	1	1	0.06	1
15	11	1	1	1	1	0.04	1
16	11	1	1	1	1	0.04	1
17	10	2	1.2	1	1	0.06	0.97
18	10	1	1	1	1	0.06	1
19	10	4	4.2	1	1	0.06	0.93
20	7	2	1.4	1	1	0.13	0.99
21	10	3	1.6	1	1	0.06	0.98
Mean ± SE	10.2±0.2	1.9±0.2	1.5±0.2	1±0	1±0	0.06±0	0.99±0

The observed (G_o) and effective (G_e) number of MLGs, the observed (L_o) and effective (L_e) number of MLLs, the nonsampling error for a second MLL (F_L), assuming that this MLL in fact accounts for 25% of the workers in the colony, as well as the average pairwise relatedness between individuals (r) are given for each colony.

colonies was extremely high ($r = 0.99$) (Table 2). Looking at the 2 asexual lineages separately, the average allelic distance among workers within colonies was $D = 0.37$ (95% CI: 0.31–0.42) for MLL1 and $D = 0.18$ (95% CI: 0.13–0.22) for MLL6. The average allelic distance between individuals from different colonies was $D = 1.55$ (95% CI: 1.53–1.57) for MLL1 and $D = 0.19$ (95% CI: 0.17–0.21) for MLL6.

DISCUSSION

Previous research showed that the ant *C. biroi* reproduces almost exclusively asexually in the introduced range (Kronauer et al. 2012) and that all individuals in a colony have the potential to produce eggs via thelytokous parthenogenesis (Tsuji and Yamauchi 1995; Ravary and Jaisson 2004). Using a population genetic approach, we asked whether this unusual reproductive system gives rise to genetically uniform colonies or whether colonies are chimeras of different asexual lineages. Because this will largely depend on the extent to which workers drift between colonies and the propensity of colonies to fuse, we also conducted behavioral assays to study non–nest mate recognition and aggressive behavior toward foreign individuals. We found that relatedness within colonies was extremely high ($r = 0.99$) and that different asexual lineages never co-occurred in the same colony. In 9 colonies, all individuals had identical genotypes across all marker loci, whereas in the remaining 13 colonies, some individuals showed small differences in their MLGs (Table 2). In all cases, these differences were due to point mutations or recombination and loss of heterozygosity at specific loci during parthenogenetic reproduction rather than sexual recombination (see Kronauer et al. 2012 for details).

Colonies frequently discriminated against foreign individuals of a different asexual lineage (Table 1). Therefore, non–nest mate recognition seems to be an effective mechanism to prevent worker drifting and colony fusion between unrelated colonies of *C. biroi*. On the other hand, non–nest mate discrimination was significantly less pronounced between colonies of the same asexual lineage. This finding is somewhat analogous to the social amoeba *Dictyostelium discoideum*, where genetically more distantly related isolates are more likely to exclude each other during aggregation (Ostrowski et al. 2008). It is, therefore, unclear whether non–nest mate discrimination is sufficient to prevent drifting and fusion between clonally related colonies in the field. In combination with the small genetic diversity within asexual lineages, this makes it difficult to assess whether the small genetic differences between some workers in some colonies stem from worker drifting or colony fusion or whether they have mostly arisen independently and de novo within each colony. In other words, it is currently unclear whether these colonies are genetic chimeras or mosaics. Nevertheless, given that at least in MLL1, the average allelic distance between colony members was clearly smaller than that between non–nest mates (non-overlapping 95% CIs), we can conclude that even within asexual lineages, there is clear genetic structure at the colony level. That this test was not significant for MLL6 might not be surprising, given that the genetic diversity within that asexual lineage was very small and considerably less than in MLL1 (Tables S1 and S2). We did not perform ovary dissections to monitor the reproductive physiology of individuals used for the behavioral assays and therefore cannot evaluate the possibility that, at least in some cases, the reproductive physiology of the focal ant affected the behavior of its host colony. However, even if the reproductive physiology played a role in eliciting aggression, a systematic bias between comparisons within versus between asexual lineages seems unlikely.

Our study showed that colonies of *C. biroi* are genetically extremely homogeneous, even when compared with other thelytokous social insects. Several ant species are known to use thelytoky facultatively to produce new queens, whereas workers are produced sexually, thereby maintaining genetic diversity among the worker force (e.g., Pearcy et al. 2004,

2006, 2011; Fournier et al. 2005; Ohkawara et al. 2006; Foucaud et al. 2010). Furthermore, even in ants where all castes are predominantly parthenogenetically produced, this in itself does not necessarily imply genetic uniformity among the worker population. The first example is the ponerine ant *Platythyrea punctata*, where colonies are usually headed by a single reproductive female that reproduces strictly asexually (Heinze and Hölldobler 1995; Schilder et al. 1999; Kellner and Heinze 2011a). In this species, colony fusions occur and a significant proportion of colonies contain multiple genetic lineages (Kellner et al. 2010). This probably helps explain the frequent occurrence of worker policing behavior, a common manifestation of social conflict in insect societies (Hartmann et al. 2003). In fact, overall aggression levels were found to be higher in genetically heterogeneous colonies although direct kin nepotism was not observed (Kellner and Heinze 2011b). The second example is the myrmicine ant *Pristomyrmex punctatus*, where winged queens are absent and all individuals in a colony can reproduce thelytokously (reviewed in Tsuji and Dobata 2011). Despite the fact that individuals from different colonies recognize each other as foreign and behave highly aggressively toward each other (Tsuji 1990), colonies can be composed of different asexual lineages (including different mitochondrial haplotypes), opening the possibility of competition between different genotypes (Hasegawa et al. 2001; Dobata et al. 2009). Indeed, Dobata et al. (2009) discovered that certain parthenogenetic lineages are specialized in reproduction and do not forage or engage in other tasks related to colony maintenance. These socially parasitic "cheater" lineages are only viable in the presence of other genotypes and, over time, lead to the demise of the colonies they infect. To sustain their long-term survival, the socially parasitic lineages spread horizontally between host colonies and have therefore been equated to transmissible forms of cancer (Dobata and Tsuji 2009; Dobata et al. 2009, 2011; Tsuji and Dobata 2011). The lack of genetic diversity in colonies of *C. biroi* is particularly surprising because this species does not construct permanent nests and colonies emigrate frequently (Tsuji and Yamauchi 1995; Kronauer DJC, personal observation), thereby increasing the probability of colony fusions. Moreover, the fact that all individuals can reproduce thelytokously implies that any drifted worker could potentially contribute to a colony's reproductive output (Ravary and Jaisson 2004). Finally, colony founding by budding implies that the genetic bottleneck experienced during independent founding is absent or at least less severe in *C. biroi*, and genetic heterogeneity should therefore be passed on from mother to daughter colonies. In fact, the low levels of genetic diversity we found in some colonies of *C. biroi* might have arisen by this mechanism. Interestingly, this combination of traits is found in both *C. biroi* and *P. punctatus*, but is otherwise unique among social insects (Tsuji and Dobata 2011). The fact that genetic diversity is considerably higher in *P. punctatus* colonies (Hasegawa et al. 2001; Dobata et al. 2009; Tsuji and Dobata 2011) might be explained by the larger population size at both the founding and mature colony stages or, alternatively, by higher rates of drifting and colony fusions. However, as might be the case in *C. biroi*, the propensity of *P. punctatus* colonies to fuse depends on genetic similarity (Nishide et al. 2007), even though genotype-based kin recognition seems to be absent during this process (Nishide et al. 2012). Finally, the low overall genetic diversity in *C. biroi* on Okinawa (only 2 MLLs detected in this study) probably also contributes to the observed genetic homogeneity at the colony level in this particular population. The only other studied ant species that seems to show similar levels of genetic homogeneity within colonies is the parthenogenetic fungus-growing ant *Mycocepurus smithii*, where colonies contain multiple queens and workers are sterile (Rabeling et al. 2009, 2011).

Knowledge of the genetic composition of social groups is necessary to understand evolutionary dynamics and levels of cooperation and conflict (e.g., Lehmann and Keller 2006; West et al. 2007). The results from this study suggest that in *C. biroi*, the scope for social conflict is limited and the potential for cooperation and altruism is high. On the other hand, the lack of sexual recombination and the genetic uniformity of populations might limit the species' ability to adapt to novel situations, especially in the introduced range. Furthermore, even small genetic differences between individuals, such as those observed in *C. biroi* colonies, could give rise to reproductive competition and social conflict. It, therefore, remains to be tested to what extent the high genetic relatedness between individuals is reflected in the species' social behavior. Previous data on populations from India and China also indicated that colonies of *C. biroi* in the native range might be genetically more heterogeneous than in introduced populations (Kronauer et al. 2012). In order to achieve a complete understanding of the social environment under which the species' social behavior has evolved, it will therefore be important to study native populations of *C. biroi* in the future.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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