

Does kin structure explain the occurrence of workers in a lower termite?

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Abstract Kinship plays a fundamental role in the origin of social life. It is also predicted to affect numerous details within animal societies, yet recent studies revealed equivocal results. We tested the influence of relatedness for the occurrence of workers in the termite *Cryptotermes secundus*. Here individuals are developmentally flexible to remain workers or to become dispersing sexuals that found new colonies. Furthermore, colony relatedness naturally increases with inbreeding and decreases when neighboring colonies fuse. Similar to recent studies on social Hymenoptera, our experimental change in relatedness gave equivocal results. Reducing relatedness within colonies did not have an effect, but individuals in inbred colonies were less likely to disperse and more likely to remain workers as predicted by kinship arguments. Several explanations for the interpretation of these equivocal results are provided.

Keywords Cooperation · Kinship · Relatedness · Social evolution · Termite

Introduction

It is mostly undisputed today that kinship is a major force in the evolution of social behavior in social insects (Bourke and Franks 1995; Crozier and Pamilo 1996; Foster et al. 2006) and, more generally, also in most other group-living animals (Griffin and West 2003). Besides, the genetic composition of animal societies is also thought to affect numerous details of social life, such as the allocation of resources, the partitioning of reproduction, and the occurrence and expression of conflicts within these societies. However, here the supporting evidence is equivocal and the cause for a failure to detect associations between relatedness and social behavior within colonies

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in some cases are highly debated (e.g., Keller 1997; Reeve 1998; Beekman and Ratnieks 2003; Boomsma et al. 2003). While a number of studies in social Hymenoptera support a strong influence of kin structure in some contexts, such as worker reproduction or sex allocation, others have failed to detect a clear association between social and genetic structure (e.g., Brown and Keller 2000; Hammond et al. 2003; for a review see Korb and Heinze 2004).

For termites, the second large group of social insects, similar studies addressing specifically the influence of relatedness on the social behavior within colonies are lacking and investigations so far concentrated on determining relatedness within colonies or populations (e.g., Atkinson and Adams 1997; Thompson and Hebert 1998; Husseneder et al. 1999; Bulmer et al. 2001; Shellman-Reeve 2001; Goodisman and Crozier 2002; Vargo 2003; DeHeer and Vargo 2004). A comparison between both groups of social insects, however, would be most revealing. In termites, within-colony conflicts that are based on relatedness asymmetries caused by haplodiploidy do not exist as termites are diploid (Korb 2005). Also genetical variations within colonies caused by primary polyandry or polygyny are exceptional because termite colonies generally originate by claustral colony foundation of one pair of reproductives (Nutting 1969). Instead variation in kin structure in termite colonies mainly arises due to fusion of colonies or inbreeding by neotenic replacement reproductives which develop from workers after the death of the queen or king. Although the general importance of colony fusion is not yet clear—but probably has been underestimated (DeHeer and Vargo 2004; see also below)—inbreeding is an influential evolutionary force for generating within-colony genetic variation as it is an ancestral trait common to all lower termites (Shellman-Reeve 1997; Thorne 1997; Roisin 2000). Thus, variation in relatedness was probably a prominent factor when sociality originated in termites, whereas in social Hymenoptera it seems to have evolved often after they became eusocial, at least in bees and ants (Crozier and Pamilo 1996).

Good model systems to investigate the influence of variation in kinship are the one-piece-life-type termites that nest in one piece of wood that serves at the same time as nest and food (Abe 1987). Such a lifestyle is associated with flexible development in which workers have the potential to explore all caste options (e.g., Shellman-Reeve 1997; Roisin 2000). (Note: Terminology in termites can be quite confusing—for a discussion see Thorne and Traniello 2003—for easy comparison with social Hymenoptera, here we use the term ‘worker’. It includes all individuals that stay in the nest and form the workforce. These individuals are sometimes also called ‘pseudergates’, ‘false workers’ or ‘helpers’; Roisin 2000.) They can remain as workers in the nest or they can switch toward becoming winged sexuals (alates) that found new colonies (primary reproductives). Development into a dispersing reproductive, thus, is a conditional strategy (sensu Gross 1996). Termites adjust their tactic to environmental conditions as has been shown experimentally for the drywood termite (Kalotermitidae) *Cryptotermes secundus* Hill (Korb and Katrantzis 2004; Korb and Lenz 2004; Korb and Schmidinger 2004) and which seems to apply to one-piece-life-type termites in general (Lenz 1994). For example, when food availability in the nest is reduced, an increased proportion of workers develop into alates that leave the nest to found new colonies. Hence, this flexible development offers unique opportunities to study the importance of variation in relatedness for developmental decision-making. Furthermore, by concentrating on the development of workers versus winged sexuals, a central evolutionary question can be addressed: the impact of relatedness for the occurrence of workers.

We tested specifically the influence of changes in relatedness by inbreeding and by colony fusion on reproductive decision-making (i.e., staying as a worker or leaving as a winged sexual) in *C. secundus*. According to kinship arguments, we hypothesized that in abundant food colonies, lacking competition between individuals (Queller 1993; Frank 1998; West et al. 2002), increased within-colony relatedness results in individuals on average being more likely to stay in the colony, while the opposite should hold for fused colonies. Inbreeding as well as colony fusion are common phenomena in this species in which 16% of the natural colonies are headed by neotenic reproductives ($N = 237$) and at least 25% of all colonies ($N = 510$) consist of fused colonies originating when a dead tree is inhabited by several colonies that grow and extend their nests until neighboring colonies come into contact. During the fusion event, often both reproductives of one colony are killed by the reproductives of the other colony, so that the remaining workers are unrelated to the new offspring of the colony. (Note: Workers do not intervene and show no signs of aggression against alien individuals; Roux 2004.) In addition, we examined in inbred laboratory colonies the mechanisms that lead to staying versus leaving by analyzing development and behavior of focal individuals. We have no indications for inbreeding depression; the cycles of inbreeding seem to be few.

In order to interpret our results, we additionally present data that show the importance of direct fitness benefits for staying at the nest.

Methods

Collection

Colonies were collected from dead *Cerriops tagal* trees in a mangrove area near Palmerston-Channel Island in Darwin Harbor, Northern Territory, Australia (12°30'S 131°00'E; for more details see Korb and Lenz 2004). All colonies used were complete monogamous unfused colonies with a natural composition of instars and castes. Colony sizes ranged from 20 to 220 individuals reflecting the natural size range. Colonies were set up in standardized *Pinus radiata* wood blocks adjusted to colony size providing abundant food conditions (one termite: 10 cm³ wood; see Korb and Lenz 2004). The use of *P. radiata* wood does not affect behavior, development, growth or caste composition of the colonies (Korb and Lenz 2004; Korb and Katrantzis 2004).

Dispersal under decreased relatedness conditions—fused colonies

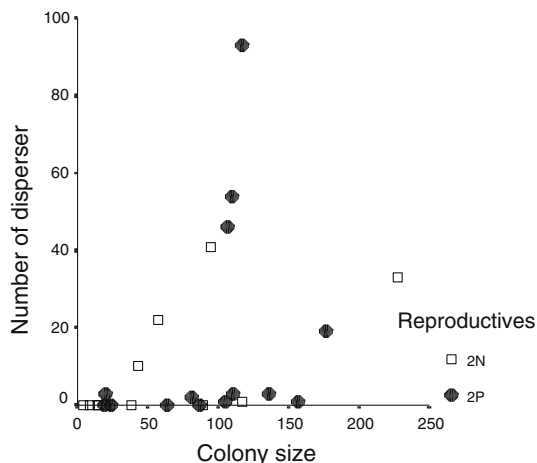
To study the influence of decreased relatedness on the development of dispersing sexuals 25 control colonies (predicted average relatedness among offspring in monogamous colonies—0.5) and 13 mixed colonies (fused colonies; predicted average relatedness among offspring when half of the individuals are exchanged—0.25, range 0–0.5) were set up in July 1999. Both types of colonies consisted of 60–80 workers and two primary reproductives; in control colonies the workers were the offspring of the reproductives, whereas in fused colonies half the workers were exchanged by workers from another colony. A mixing of individuals is possible; no aggression occurs among workers or soldiers, during fusion events aggression is limited to fights among reproductives (see above). Although no fights

occurred when exchanging workers, workers have the information that colony fusion took place as they can recognize kin from non-kin after mixing individuals (Korb 2006). The colonies were then placed in the field at the site where they had been collected (for more details Korb and Lenz 2004) and prior to the swarming period of the next year, in July 2000, they were sampled to determine the number of dispersing individuals.

Dispersal under increased relatedness conditions—neotenic colonies

To study the influence of increased relatedness on the dispersal of individuals 14 colonies with two primary reproductives (primary colonies; expected average relatedness among offspring: 0.5) and 13 colonies with two neotenic replacement reproductives (inbred neotenic colonies; expected average relatedness among offspring: 0.75) were set up in July 2000. Genetic analysis using microsatellite markers (Fuchs et al. 2003) confirmed that field colonies with two primary reproductives have an average relatedness not significantly different from 0.5 (mean: $r = 0.45 \pm 0.215$ S.D.). In inbred neotenic colonies relatedness is increased, indicative of one generation inbreeding among full-siblings (mean: $r = 0.68 \pm 0.186$ S.D.). Furthermore, the dark brown color of the neotenic reproductives showed that they were active reproductives since an extended period of at least 1 year. The colonies were placed in the field at the site where they had been collected and prior to the swarming period of the next year, in July 2001, they were sampled to determine the number of dispersing individuals. As it was not possible to collect enough similar sized colonies in July 2000, colony size varied between 20 and 220 individuals. To account for this, we included colony size as a co-variable in our statistical ANCOVA analysis (see below). Furthermore, colony size did not differ between colonies with primary and neotenic reproductives (t -test for equal variances: $t = 0.89$, $N_1 = 14$, $N_2 = 13$, n.s.; Fig. 1), so that this factor does not flaw our comparison. However, the differences in the production of dispersing sexuals between this experiment and the former experiment, in which colony size was fixed to 60–80 individuals, might be caused by colony size differences.

Fig. 1 Number of dispersing sexuals in reproducing colonies with primary (2P) and neotenic (2N) reproductives in relation to colony size



Development and behavior under increased relatedness conditions

For the observation of development and behavior of single individuals colonies were set up as described above, but kept in climate chambers in Regensburg (Germany) at 28°C and 70% relative humidity with a 12-h day/night cycle. These conditions are appropriate for *C. secundus* and do not affect growth or development of colonies (for more details see Korb and Schmidinger 2004; Korb and Katrantzis 2004). We established 14 colonies, seven each with either a pair of primary reproductives (primary colonies) or neotenic replacement reproductives (inbred neotenic colonies). As above the neotenic reproductives were established since a long period as indicated by their dark brown color.

From each colony we made focal observations on at least 15 workers which had the potential to develop into dispersing sexuals for the next nuptial flight (Korb and Schmidinger 2004). At the start of the experiment each worker was classified to instar under a dissecting microscope according to wing bud development and thorax shape (for more details see Korb and Katrantzis 2004). All individuals were marked with a unique color code, consisting of two small dots of enamel paint (Revell, Germany). Individuals were checked throughout the duration of the experiment to ensure they retained their identifying markings and to assess their state of molting readiness. Termites that are about to molt have a whitish, opaque appearance; when this happened they were separated until they had molted, this treatment did not affect development (Korb and Schmidinger 2004). After the molt, wing bud development and thorax shape were re-determined and the molts were classified as progressive, stationary, or regressive according to an increase, no change or decrease of wing bud development and size. Progressive molts are indicative of a development into dispersing sexuals via five nymphal instars (i.e., individuals with wing buds), while regressive molts characterize individuals that remain as workers in the colony (Korb and Katrantzis 2004). Newly molted termites were marked again with their previous color code and repatriated into their colony which was set up in an observation chamber of adjusted size that had been drilled in the wood blocks and which was covered with a sealed glass plate (see Korb and Schmidinger 2004).

The marked individuals were observed during two 15-min periods with 2 months in between to monitor whether behavioral changes occurred during the course of the experiments (for more details see Korb and Schmidinger 2004). Two months were chosen because this period roughly corresponds to the mean interval between two consecutive molts (results from previous experiments: 64.5 ± 3.2 days, $N = 134$). Observations were initiated 2 months after the annual nuptial flights, and the total observation time for the experiment covered 6.5 months. During each observation, we used focal sampling to record all behaviors performed by an individual (active) and those in which it was involved as a partner (passive). In total, we observed 315 and 176 different individuals during the first and second observation, respectively. Differences in sample sizes for different behaviors are caused by missing values. The following behaviors were analyzed:

- antennation: contact between individuals with the antennae;
- allogrooming: one individual grooms another by moving the mouth parts over the others body;
- proctodeal trophallaxis: exchange of substances between individuals via the anus; anus–mouth contact.

If possible, for each observation the number of days until the next molt of the focal individual was recorded to check whether this molting variable confounds the individual's behavior. There were significant correlations between the time until the next molt and the frequency of proctodeal feeding. During the first observation period individuals approaching their next molt did more active feeding (i.e., provide proctodeal food; Pearson correlation: $R = -0.155$, $F_{1,172} = 4.29$, $P < 0.05$), while the frequency of passive feeding (i.e., being fed) increased with the number of days until the next molt during both observation periods (Pearson correlation—first observation, $R = 0.261$, $F_{1,172} = 12.49$, $P = 0.001$; second observation, $R = 0.318$, $F_{1,59} = 6.66$, $P < 0.05$). All other behaviors did not correlate with the time until the next molt ($P > 0.10$). Therefore, for further analysis the data for proctodeal feeding were corrected for time to the next molt by using the residuals of these correlations.

Quantifying reproductive options for staying and leaving the colony

Cryptotermes secundus workers can gain direct reproduction by leaving the colony as winged sexual to found a new colony or by staying and becoming a neotenic replacement reproductive when the same-sex reproductive of the colony dies. To determine the probability for successful colony foundation and for colony inheritance we used data from a comprehensive field study that was performed near Darwin (Northern Territory, Australia) between 1999 and 2003 in which we collected more than 500 complete colonies from seven different areas by dissecting over 1,400 dead mangrove trees (Korb and Lenz 2004). The probability for successful colony foundation was determined for two areas where all dead trees (about 200) were removed to collect complete colonies ($N_1 = 28$, $N_2 = 29$) before the annual nuptial flight. It was calculated as twice the number of 1-year-old colonies (each colony is headed by a king and a queen) divided by the number of dispersing alates. The number of dispersing alates used in this estimate should technically be the number from the previous year, however, as the number of dispersers do not change between years (Korb and Lenz 2004), it is valid to use the number of alates from the same year. One-year-old colonies are easy to identify by their colony composition (about 20 workers and 1 soldier) and the presence of a single colony chamber as continuous monitoring of field colonies during the course of a year has shown (J. Korb, personal observation). Furthermore, this model rests on the assumption that the immigration of alates into an area matches their emigration. From our long-term data, we have no indication that this is not the case. The probability to inherit a colony was determined as the number of new neotenic replacement reproductives (indicated by their color) in relation to the number of workers that could potentially inherit a colony for all seven areas ($N = 276$).

Statistics

All variables were checked for normal distribution by visual inspections and one-sample Kolomogorov–Smirnov tests. Data that differed from normal distribution were analyzed with non-parametric statistics. If the same data set was subjected to multiple testing, the step-up false discovery rate (FDR) approach was used to correct P -values (Benjamini and Hochberg 1995), a method suggested to overcome

problems of Bonferroni correction procedures (Garcia 2004). All statistical analyses were conducted using SPSS 10.0 (SPSS Inc.). All tests were two-tailed. The means and standard errors of the results are provided.

To analyze dispersal under different relatedness conditions, we tested frequency tables of the occurrence (yes, no) of dispersing sexuals with χ^2 -contingency analysis; to show the effect of relatedness on the occurrence of dispersal sexuals, we calculated the ‘risk ratio’ (i.e., the statistical change in the probability that an event will happen) with 95% confidence intervals (Norusis 1993). The number of dispersing sexuals produced were analyzed (a) with a Student’s *t*-test for the comparison under decreased relatedness with a fixed colony size and (b) with a general linear model under increased relatedness using relatedness (primary versus neotenic reproductives) as between-subject factor and colony size as confounding covariate.

To analyze the development and behavior under increased relatedness conditions, we compared the frequency of molting types (regressive, stationary, and progressive) with χ^2 -contingency analysis and the behavior of individuals with Mann–Whitney *U*-tests between colonies with primary and neotenic reproductives, separately for both observation periods.

Results

Dispersal under decreased relatedness conditions

The likelihood of producing dispersing sexuals did not differ between control colonies and fused colonies (contingency analysis: $\chi^2_1 = 0.67$, n.s.). In control colonies 10 out of 25 colonies produced sexuals (40.0%), while the corresponding values for fused colonies were 7 out of 13 colonies (53.8%). The ‘risk ratio’ to have sexuals for colonies with primary reproductives was 0.7 times (c.l. 0.37–1.49) that for fused colonies. If colonies reproduced, also the number of dispersing sexuals produced did not differ between both types of colonies (control colonies: 7.2 ± 0.71 ; fused colonies: 6.0 ± 1.51 ; *t*-test for equal variances: $t = 0.79$, $N_1 = 10$, $N_2 = 7$, n.s.; effect size: 0.387).

Dispersal under increased relatedness conditions

Colonies with primary reproductives had significantly more often dispersing sexuals than inbred colonies with neotenic reproductives (contingency analysis: $\chi^2_1 = 4.49$, $P < 0.05$). In colonies with primary reproductives 11 out of 14 colonies produced sexuals (78.6%), while the corresponding values for neotenic colonies were 5 out of 13 colonies (38.5%). The ‘risk ratio’ to have sexuals for colonies with primary reproductives was 2.3 times (c.l. 1.03–5.24) that for colonies with neotenic reproductives. If colonies reproduced, the number of dispersing sexuals produced did not differ between colonies with primary and neotenic reproductives (ANCOVA: $r^2 = 0.046$; $F_{1,15} = 0.01$, n.s.; confounding variable, colony size: $F_{1,15} = 0.62$, n.s.; Fig. 1).

Development and behavior under increased relatedness conditions

There was a trend in the frequency of molting types to differ between colonies with primary versus inbred, neotenic reproductives [primary colonies: regressive, 22 (23.2%); stationary, 41 (43.2%); progressive, 32 (33.7%); neotenic colonies:

regressive, 44 (34.4%); stationary, 37 (28.9%); progressive, 47 (36.7%); contingency analysis: $\chi^2_2 = 5.63$, $P = 0.060$; Fig. 2]. Comparing regressive versus stationary molts only, colonies with primary reproductives had a significantly lower proportion of regressive molts than colonies with neotenic reproductives (contingency analysis: $\chi^2_1 = 5.37$, after step-up FDR: $P < 0.05$; Fig. 2).

The frequencies of allogrooming (active and passive) as well as antennation (active and passive) was significantly higher in colonies with neotenic than in those with primary reproductives during both observations, but the degree of proctodeal trophallaxis did not differ (Table 1).

Quantifying reproductive options for staying and leaving the colony

We found that the probabilities for successful colony foundation and nest inheritance had the same order of magnitude. The mortality rates during dispersal were extremely high with less than 1% (0.6 and 0.7%) of the alates successfully founding an own colony. In comparison, the probability to inherit a colony ranged between 0.3 and 0.5%, with an average of 0.4% ($N = 7$).

Discussion

Our experiments revealed equivocal results for an influence of relatedness on the occurrence of workers versus dispersing sexuals in *C. secundus*. In contrast to predictions based on kinship, fused colonies with decreased relatedness did not produce more dispersing sexuals than monogamous control colonies. However as predicted by relatedness, inbred colonies with neotenic reproductives the likelihood that a colony produced dispersing sexuals was lower than in colonies with primary reproductives. This change was accompanied by increased allogrooming- and

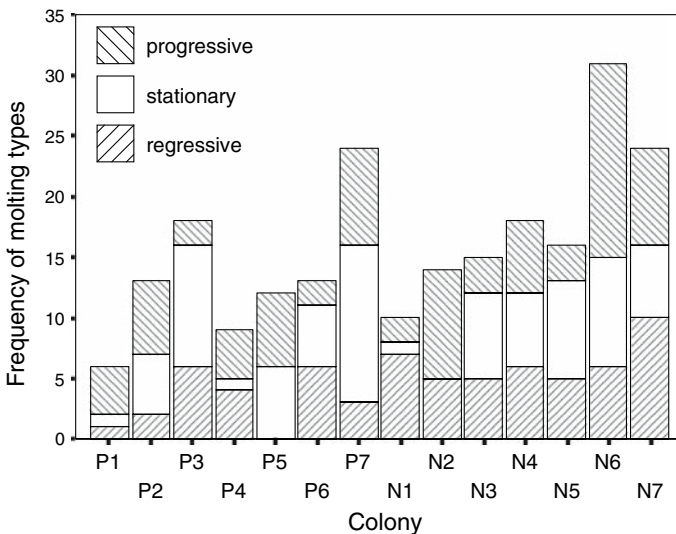


Fig. 2 Frequency of molting types (regressive, stationary, and progressive) in different colonies with primary (P1–P7) and neotenic reproductives (N1–N7)

Table 1 Comparison of behavior in primary and neotenic colonies during the first and second observation

		Primary	Neotenic	<i>U</i>	<i>N</i> ₁	<i>N</i> ₂	<i>P</i>
<i>First observation</i>							
Antennation	ac.	18.9 ± 0.92	27.2 ± 1.12	7,966.5	145	168	<0.001
	pa.	15.7 ± 0.68	26.9 ± 1.10	6,340	147	168	<0.001
proc tro—res	ac.	1.0 ± 0.11	1.0 ± 0.10	3,625	78	96	n.s.
	pa.	1.3 ± 0.11	1.4 ± 0.11	3,435	78	96	n.s.
Allogrooming	ac.	0.5 ± 0.11	1.3 ± 0.17	9,255.0	145	168	<0.001
	pa.	0.6 ± 0.10	1.4 ± 0.19	9,603.5	147	168	<0.001
<i>Second observation</i>							
Antennation	ac.	19.3 ± 1.34	23.7 ± 1.18	2,712.5	63	109	<0.05
	pa.	17.4 ± 0.95	22.0 ± 0.77	2,375.0	65	109	<0.001
proc tro—res	ac.	0.6 ± 0.12	1.0 ± 0.13	2,788.0	65	109	0.060
	pa.	1.1 ± 0.19	0.9 ± 0.10	375.0	36	25	n.s.
Allogrooming	ac.	1.2 ± 0.33	1.8 ± 0.25	2,649.0	63	109	<0.05
	pa.	0.7 ± 0.15	1.7 ± 0.32	2,824.0	65	109	<0.05

Shown are the mean (±s.e.m.) frequencies and results of Mann–Whitney *U*-tests comparing different behaviors between colonies with primary and neotenic reproductives during the first and second observation

ac. indicates active, behavior performed by the observed individuals

pa. indicates passive, observed individual was involved in behavior as a partner

proc tro. indicates proctodeal trophallaxis, res. indicates residuals after correction for molting time (for further information see Sect. 'Methods')

antennation-frequencies as well as higher proportions of regressive versus stationary molts (i.e., development to stay as worker in the colony) in inbred colonies.

The difference in response between the decreased- and increased-relatedness experiment can hardly be explained by statistical problems of power in the former. (Note: We did not calculate the power because recent discussions show the abuse of power analysis in post hoc testing; Hoenig and Heisey 2001; Colegrave and Ruxton 2003.) Sample sizes in both experiments were similar, the number of control colonies was even slightly larger in the decreased-relatedness experiment. The higher proportion of control colonies producing dispersing sexuals in the increased- compared to the decreased-relatedness experiment was caused by larger colony sizes in the former experiment. The proportions of dispersing sexuals were typical for colonies of these sizes as comparison with field colonies from several years shows (contingency analysis—increased-relatedness experiment, $\chi^2_1 = 1.83$, n.s.; decreased-relatedness experiment, $\chi^2_1 = 0.77$, n.s.; Korb and Lenz 2004). Differences between years in the likelihood of dispersal do not exist as our long-term field study has shown (Korb and Lenz 2004). Thus, our contrasting results are likely to have biological reasons and they are outlined in the following.

The lack of an influence of decreased relatedness on dispersal could be explained by a differential response of alien (i.e., exchanged workers from another colony) and offspring workers, namely, if non-related alien workers showed increased dispersal, which is compensated by decreased dispersal of offspring workers. However, currently performed studies suggest that this is not the case: alien and offspring workers leave the colony in equal proportions (J. Korb, unpublished data).

Mainly two explanations have been proposed for the failure to find an influence of relatedness on social behavior in colonies. Firstly, social Hymenoptera researchers have repeatedly evoked lack of sufficiently error-free information on relatedness, or

constraints in general, that prevent individuals from pursuing their own selfish interest (e.g., Keller 1997; Reeve 1998; Beekman and Ratnieks 2003; Boomsma et al. 2003). However, this is unlikely to apply for our study. *C. secundus* workers can recognize alien workers and in fused colonies they preferentially interact with related nestmates, but only under certain conditions, e.g., when the food availability is limited (Korb 2006). Secondly, individuals might be prevented by nestmates to pursue their own selfish interest if this poses a cost to the colony as a whole. So in social Hymenoptera a lack of nepotistic brood rearing or an influence on male parentage (Page et al. 1989; Ratnieks and Reeve 1992; Hammond et al. 2003) was proposed to be caused by colony level costs. However, in contrast to these Hymenopteran systems where few individuals would behave selfish at the cost of the majority of the colony, 'selfish' development into dispersing sexuals in *C. secundus* is in the interest of a huge fraction of the colony. In fact, all those individuals are expected to disperse that lost their reproductives in the process of colony fusion. In our experiment they accounted for half the colony size and in natural colonies they also constitute a large proportion of the fused colony because large, expanding colonies merge. Thus there is no clear-cut majority interest favoring impediment of dispersal suggesting that alien individuals are not forced to stay in the colony, but stay voluntarily in fused colonies. Why should individuals want to stay under fused colony conditions when they do have no related offspring they could raise? One possible explanation might be that individuals stay for other reasons than gaining indirect benefits by raising offspring. Our field data showed that the chances to inherit a colony as neotenic replacement reproductive and those to found successfully a new colony are in the same order of magnitude. This indicates that direct fitness benefits in the nest are an important factor influencing reproductive decision-making, as has also been proposed for termites (Myles 1988; Thorne et al. 2003). In fact, behavioral observations and experiments show that *C. secundus* workers are mainly staying in the nest for direct fitness benefits (Korb 2007). If the main benefit for remaining in the nest are direct fitness gains staying should not be affected by within-colony relatedness, but should depend on the probability to inherit the breeding position. This matches the pattern we observed, given that neotenic reproductives have on average a lower longevity than primary reproductives. Unfortunately, data on the longevity of both types of reproductives in *C. secundus* do not yet exist. Thus, although our data show that direct benefits are a potentially driving force for individuals to remain in the colony—explaining why unrelated individuals do not leave the colony—further experiments have to demonstrate whether the response to inbreeding is an effect of differential longevities between both types of reproductives or an effect of relatedness.

This study illustrates that equivocal results concerning the influence of relatedness on social life should solicit further research. Evoking constraints and a lack of information may be insufficient because alternative explanations may exist to account for the observed patterns of social behavior within colonies, regardless of whether they do or do not fit with expectations from kin structure. Such research is needed to clarify the ambivalent results on the effect of relatedness in shaping social societies.

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P20011508). The experiments performed comply with the current laws in Australia and Germany where the experiments were performed.

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