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Origin of male-biased sex allocation in orphaned colonies of the termite, *Coptotermes lacteus*

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Abstract In eusocial insects, sex allocation often constitutes a ground for intracolony conflicts. This occurrence provides ideal opportunities to test kin-selection theory. A vast literature on this topic is available for social Hymenoptera, but the same field remains almost untouched in termites. A preeminent case is that of some species of *Coptotermes*, where the sex-allocation ratio in nymphs shifts from near equity to all-male when the primary reproductives are replaced by neotenics. To shed light on the developmental origin of this shift, we compared the sex ratio of the various castes and instars in primary- and neotenic-headed mature colonies of *Coptotermes lacteus*. The male-biased sex allocation in the latter type of colony results from two concurrent events: first, the sex ratio of the youngest instars (larvae) is male-biased by a 3:1 ratio; and second, all female larvae become workers, while a large fraction of the male larvae proceed to the nymphal and alate stages. Colony-founding experiments showed that inbreeding by itself cannot account for the male bias at hatching. We suggest that both genetic factors, due to the reproductive behaviour of neotenics, and environmental factors (colony condition and resource availability) may influence this process. Their exact nature and respective impact have not yet been clarified.

Keywords Isoptera · Rhinotermitidae · Subterranean termites · Sex ratio · Inbreeding

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Introduction

Theoretical and empirical studies of sex allocation in social Hymenoptera have burgeoned since Trivers and Hare (1976) drew attention to the possible involvement of sex allocation biasing in the onset of sociality, and to the likelihood of conflicts between queen and workers over sex allocation. Nowadays, considerable progress has been made towards the understanding of sex allocation processes and relevant conflicts in social Hymenoptera (Bourke and Franks 1995; Crozier and Pamilo 1996; Chapuisat and Keller 1999; Sundström and Boomsma 2001).

By contrast, only a handful of sex allocation studies are available for termites. The sex ratio of alates produced by individual colonies is distributed around 1:1 in *Nasutitermes corniger* and *Macrotermes michaelseni* (Thorne 1983; Darlington 1986). However, some laboratory-held colonies of *Reticulitermes lucifugus* produced a large excess of female alates (Herfs 1951), whereas extreme male biases were observed in two colonies of *N. dunensis* (McMahan et al. 1983). Sex ratios of unflown alates in colonies of the Formosan termite (*Coptotermes formosanus*) from the southern United States were unbiased to highly male-biased (45–96% male) (Jones et al. 1988), but recent counts of flying alates revealed a balanced sex ratio at the population level (Henderson 1996). A laboratory-kept colony of this species in China shifted from male-biased to all-male alate production (Crosland et al. 1994; Lenz and Roisin 1998).

The best-documented case is probably that of the Australian species, *C. lacteus*, in which extreme male biases were experimentally obtained (Lenz and Runko 1993). Colonies are normally headed by primary reproductives (queen and king), i.e. the pair of imagos that founded the colony after aerial dispersal and wing-shedding. In such colonies, the development of the young starts with two consecutive undifferentiated instars, respectively called larvae 1 and larvae 2 (Fig. 1). At the second moult, larvae 2 engage either in the apterous line, comprising several worker instars (at least eight) and

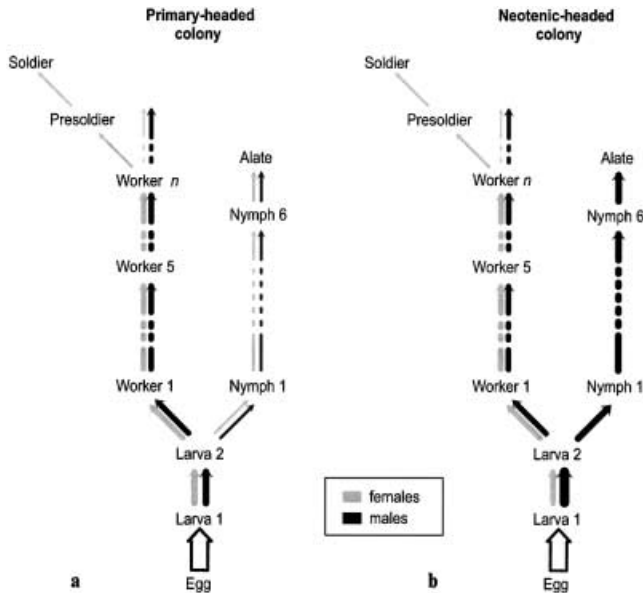


Fig. 1a, b Caste developmental pathways in mature colonies of *Coptotermes lacteus*. Differentiation of neotenic from late nymphal instars not represented. The thickness of arrows indicates (without proportionality) relative numbers of individuals engaging in the various pathways. **a** Primary-headed colonies (modified from Roisin and Lenz 1999). **b** Neotenic-headed colonies

soldiers, or in the nymphal line, comprising six wing-budded instars (called nymphs), followed by the winged dispersive morph (alate). Workers appear to be permanently sterile. The sex ratio is near equity in all castes except soldiers, which all derive from old female workers (probably of instars 8–10; Roisin and Lenz 1999; Fig. 1a). If the primary reproductives die or are removed, male and female neotenic replacement reproductives differentiate from nymphs present in the colony and produce inbred offspring (Lenz and Barrett 1982; Lenz et al. 1988). A remarkable feature of *C. lacteus* is that the sex ratio of alates, which is near 1:1 in colonies headed by primary reproductives, shifts to almost 100% male in those headed by neotenic (Lenz and Runko 1993). In addition, instead of being seasonally raised, nymphs develop the whole year round in neotenic-headed colonies, but they complete their development to the alate stage in synchrony with primary-headed colonies. Such neotenic-headed colonies also occur naturally, although there have been only a few chance finds to date (Lenz and Barrett 1982; Lenz and Runko 1993). *C. acinaciformis*, and probably some populations of *C. formosanus*, react to orphaning in a similar fashion (Lenz and Roisin 1994, 1998).

A central difficulty for the interpretation of sex-allocation patterns in termites is the dearth of knowledge as to the sex-determination mechanisms themselves. The occurrence of one pair of sex-determining chromosomes (XX in females, XY in males) is thought to be the basal condition, but actual observations of heteromorphic sex chromosomes are few. Fusions or translocations involving sex chromosomes may substantially complicate this

pattern: rings or chains linking a substantial part of the genome to the Y chromosome at meiosis are known in many species (Syren and Luykx 1977; Fontana and Amorelli 1978; Vincke and Tilquin 1978; Fontana 1990, 1991; Luykx 1990; Martins and Mesa 1995; Reeve and Shellman-Reeve 1997; Martins 1999). Furthermore, the extent of genomic sex linkage may differ widely between congeneric species (e.g. in the European *R. lucifugus* complex: Fontana and Amorelli 1978) or even between conspecific populations (e.g. in *Kaloterme approximatus* and *Incisitermes schwarzi*: Syren and Luykx 1981; Luykx 1987). Thus far, the only available account of a *Coptotermes* karyotype, concerning a Hawaiian population of *C. formosanus*, reveals a haploid chromosome number of 21 in either sex but does not provide decisive information as to the actual mode of sex determination (Wang and Grace 1999).

To identify possible targets for further investigation of the proximate mechanisms and ultimate causes of the sex-allocation shift in neotenic-headed colonies of *C. lacteus*, a basic issue is to determine at which stage the sex ratio becomes biased. For instance, a bias discovered at the egg-hatching stage would suggest a direct effect of inbreeding on sex determination (or on sex-specific survival of the embryos), or a pre-determination in the egg by the queen (e.g. through the hormonal content of the egg) or by the king (e.g. through an excess of sperm containing the Y chromosome). Alternatively, the selective elimination of one sex in the course of larval development would suggest that further investigations should rather be focused on worker behaviour.

In this paper, we examine the developmental pathways in neotenic-headed colonies of *C. lacteus* and provide a first test of the most straightforward mechanical explanation for the observed pattern. To determine the origin of the male bias among the alates produced by neotenic-headed colonies, the following hypotheses were put forward: (1) broods are male-biased from the earliest instars; (2) young female nymphs or female larvae developing into nymphs are selectively eliminated; (3) all female larvae moult to workers, whereas a fraction of the male larvae moult to nymphs. Since a bias was found among early larval instars, a subsequent question was whether inbreeding, which is the most obvious correlate of neotenic reproduction, by itself triggers a mechanism biasing the sex ratio towards males at hatching. An answer to this question was sought by establishing inbred and outbred founding pairs to compare the sex composition of their respective brood. In the same series of experiments, all-female pairs were set up to detect possible effects of parthenogenesis.

Methods

Sex ratio in field colonies

For sex-ratio comparisons, we used 11 colonies of *C. lacteus* (Froggatt): 4 from Armidale (30°31'S, 151°39'E) and 7 from Termeil (35°28'S, 150°21'E), in southeastern Australia. All were sam-

Table 1 Sex ratio of the various castes of *Coptotermes lacteus* in primary- and neotenic-headed colonies. Numbers indicate percentage of males in sample; sample size in parentheses. For a synthesis and statistical interpretation of these data, see Fig. 2

Colony no.	Time since orphaning	Larvae 1	Larvae 2	Workers 1–5	Soldiers	Nymphs+alates
Primary-headed colonies						
CM26	–	53 (60)	38 (40)	43 (101)	0 (30)	–
CM40	–	56 (50)	58 (53)	44 (104)	0 (30)	51 (100)
CM42	–	40 (52)	62 (52)	47 (100)	0 (30)	51 (100)
CM78	–	54 (50)	48 (52)	52 (101)	0 (50)	44 (59)
CM80	–	56 (77)	44 (25)	45 (100)	0 (50)	40 (5)
T2	–	53 (40)	48 (62)	50 (102)	0 (52)	56 (100)
Mean		52.0	49.7	46.9	0	48.4
Neotenic-headed colonies						
CM3	20 months	80 (54)	78 (54)	42 (106)	0 (30)	100 (76)
CM23	20 months	89 (9)	72 (18)	34 (103)	0 (31)	100 (100)
CM17	4 years	62 (50)	62 (52)	41 (108)	0 (30)	100 (100)
CM66	4 years	72 (103)	81 (53)	54 (103)	0 (31)	100 (100)
CM82	5 years	85 (60)	66 (90)	53 (100)	0 (31)	100 (100)
Mean		77.6	71.8	44.8	0	100

pled in September 1993, except colonies T2 (February 1997) and CM82 (August 1998). Five of these colonies had been orphaned in the field 20 months to 5 years beforehand by excavation and removal of the royal cell, as described in Lenz and Runko (1993), and were headed by neotenic replacement reproductives at the time they were excavated again (Table 1). The remaining six colonies were headed by primary reproductives. Samples from all castes were collected after mound dissection and the search for reproductives. Specimens were fixed in FAA (formol: alcohol: acetic acid). Individuals were sorted by caste under a dissecting microscope. In late nymphs and alates, the sex was determined by checking the size and shape of the abdominal sternites. In other individuals, the sex was determined after dissection and hematoxylin staining (Roisin and Lenz 1999). Among workers, we focused on individuals with fewer than 14 antennal segments, and thus not older than instar 5. The reason for doing so was to evaluate worker sex ratio, before soldier differentiation (which involves only old female workers in primary-headed colonies; Roisin and Lenz 1999), could cast confusion.

Establishment of inbred and outbred pairs from alates

To test for a possible immediate effect of inbreeding on the sex of the young, inbred and outbred pairs were established from alates taken from three mounds (A, B, C) of *C. lacteus* in the Canberra region in mid-September 1999. This period lies within the flying season of this species in this part of southeastern Australia (Hill 1942). The mounds were located in plantation forest of *Pinus radiata*. Mounds were distant from each other by several kilometres. Part of the outer casing of the mounds was removed until sections of the inner carton, holding large numbers of alates, could be removed. Many alates flew as the mounds were opened, an indication that the alates were mature and ready to leave the nest once environmental conditions were suitable. The sections of carton were stored in plastic containers and taken to the laboratory. Within 1–3 days from the date of field collection, alates were selected at random out of containers, sexed and de-winged by gently bending the wings backwards with soft forceps until they snapped at the sutures. Pairs were placed into 70-ml plastic vials with screw-top lids which were filled with 16 g of moist, powdered carton material from a *C. lacteus* mound (see Lenz et al. 1987 for details on this material as a food source and a matrix for holding moisture) and one block of *Eucalyptus regnans*, measuring 3×1×1 cm. Pairs were stored in a climatized room at 27°C and 80% r.h.

We established 180 inbred pairs, with male and female from the same colony (A, B or C: 60 pairs from each colony), and 300 outbred pairs, with male and female from different colonies (50

repeats of each possible combination of male and female from colonies A, B and C). In addition, 20 all-female pairs were also set up (5 of each of the following combinations: A/A, A/B, B/B, A/C). Pairs were kept until early January 2000 when maintenance conditions became accidentally too wet and most incipient colonies showed signs of decline. All surviving colonies were then opened and the individuals fixed in FAA for sex determination.

Statistical procedures

For a given caste, sex-ratio differences among colonies or between colony types were assessed by the method detailed by Fleiss (1981) for comparing proportions from independent samples. This chi-square-based method separates the respective effects of two factors (in the present case, colony identity and colony type). Differences between successive instars were assessed by analysing the logarithms of the “odds ratios” (Fleiss 1981), which is a method suited for splitting the effects of two factors (colony identity and caste) in dependent samples. A weighted, single-factor ANOVA on arcsine transformed data (Campbell 1974) was run to test sex-ratio differences between outbred and inbred foundations. This method is better suited than chi-square-based methods when cell values are small. Confidence intervals on sex ratios were established as explained by Fleiss (1981); the total numbers of males and females for each caste and colony type served as a basis for such estimates. This procedure is valid as long as sex-ratio values do not differ significantly among colonies of the same type.

Results

Sex ratios in mature colonies

Soldiers were all female in all colonies. Average sex ratios and associated confidence limits were calculated for all non-soldier castes in both colony types (Fig. 2; for statistical procedure, see Fleiss 1981). In primary-headed colonies, all larvae, workers and nymphs showed a sex ratio near 1:1. In neotenic-headed colonies, larvae were male-biased; worker sex ratio was close to equity (with a slight female bias), and nymphs and alates were exclusively male (Table 1). Worker-like individuals derived from nymphs after regression of their wing buds were found in 2 colonies (CM23, CM17) and were all male

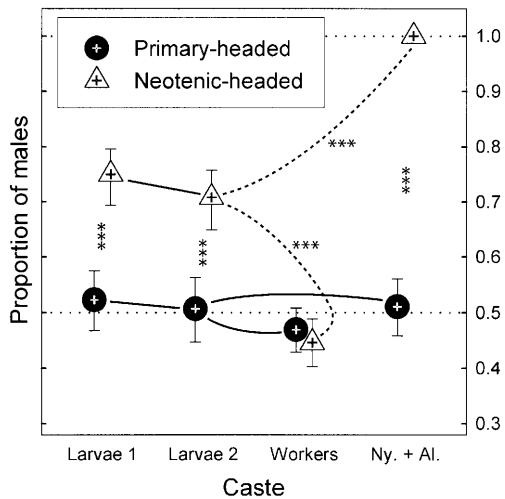


Fig. 2 Statistical interpretation of data from Table 1. Symbols, with 95% confidence limits, stand for average proportion of males for each caste in either colony type. Vertically set asterisks indicate significant differences ($P < 0.001$) between colony types. Solid lines link consecutive instars that display non-significantly different sex ratios. Horizontal asterisks next to dashed lines indicate sex-ratio differences ($P < 0.001$) between consecutive instars (Ny. + Al. nymphs and alates combined)

(17 and 51 individuals, respectively). No significant sex-ratio differences were found among colonies of the same type among first-instar individuals ($\chi^2_{\text{primary}} = 3.950$, $df = 5$, $P > 0.5$ and $\chi^2_{\text{neotenic}} = 7.850$, $df = 4$, $0.1 > P > 0.05$), second-instar individuals ($\chi^2_{\text{primary}} = 7.572$, $df = 5$, $0.25 > P > 0.1$ and $\chi^2_{\text{neotenic}} = 6.382$, $df = 4$, $0.25 > P > 0.1$) and nymphs plus alates ($\chi^2_{\text{primary}} = 3.555$, $df = 4$, $0.5 > P > 0.25$ and $\chi^2_{\text{neotenic}} = 0$, $df = 4$, $P > 0.999$), but there was a significant excess of males in neotenic-headed colonies compared to primary-headed ones (larvae 1: $\chi^2_{\text{diff}} = 33.109$, $df = 1$, $P < 0.001$; larvae 2: $\chi^2_{\text{diff}} = 23.212$, $df = 1$, $P < 0.001$; nymphs plus alates: $\chi^2_{\text{diff}} = 295.36$, $df = 1$, $P < 0.001$). The sex ratio of workers was similar across all colonies ($\chi^2_{\text{total}} = 15.865$, $df = 10$, $0.25 > P > 0.1$). Statistical testing of sex-ratio differences between successive instars within each colony type (see Fleiss 1981) gave congruent results. In neotenic-headed colonies, the sex ratio was similar for larvae 1 and larvae 2 ($\chi^2_{\text{total}} = 8.625$, $df = 5$, $0.25 > P > 0.1$), but there was a significant excess of males among larvae 2 compared to workers ($\chi^2_{\text{assoc}} = 37.62$, $df = 1$, $P < 0.001$). By contrast, there was a significant excess of females among larvae 2 compared to nymphs plus alates ($\chi^2_{\text{assoc}} = 43.89$, $df = 1$, $P < 0.001$). Not surprisingly, no significant difference was found between the sex ratio of successive instars in primary-headed colonies (larvae 2 vs larvae 1, $\chi^2_{\text{total}} = 8.444$, $df = 6$, $0.25 > P > 0.1$; workers vs larvae 2, $\chi^2_{\text{total}} = 6.249$, $df = 6$, $0.5 > P > 0.25$; nymphs plus alates vs larvae 2, $\chi^2_{\text{total}} = 3.320$, $df = 5$, $0.75 > P > 0.5$).

Incipient colonies

Of 180 inbred and 300 outbred primary pairs set up with male-female pairs of de-alated imagos, 24 and 28

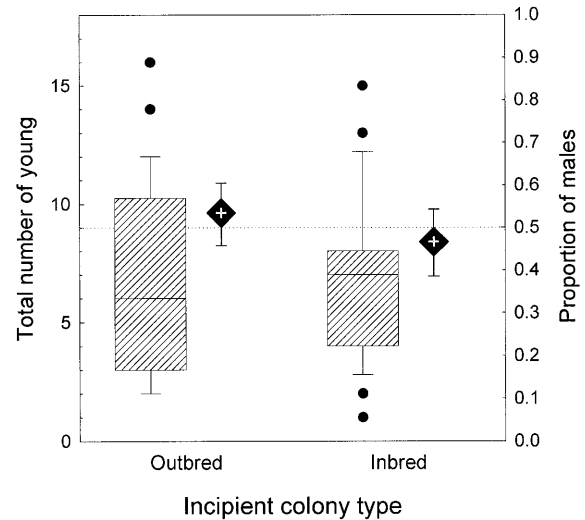


Fig. 3 Comparison between outbred and inbred pairs with at least one young. Hatched bars; total number of young (median and quartiles); 10–90 percentiles shown as error bars, outliers as dots. Diamonds; average proportion of males in young (all instars); error bars indicate 95% confidence limits. Differences between types of incipient colonies are not significant

Table 2 Composition and sex ratio of brood in inbred and outbred incipient colonies of *Coptotermes lacteus*. Total numbers of individuals of each caste for all colonies with at least one live young. For a synthesis and statistical interpretation of these data, see Fig. 3

	Outbred (n=25)		Inbred (n=23)	
	Male	Female	Male	Female
Larvae 1	9	6	8	10
Larvae 2	40	42	30	38
Workers 1	43	29	31	29
Presoldiers	0	2	0	1
Soldiers	0	1	0	1
Total	92	80	69	79

respectively had retained both founders live after 3 months. Of these, 23 inbred and 25 outbred pairs had at least 1 live young. These proportions are not significantly different between the two types of incipient colonies ($\chi^2 = 2.000$, $df = 1$, $0.25 > P > 0.1$). The total number of young was not significantly different between inbred and outbred pairs (Table 2, Fig. 3) (Mann-Whitney U -test on all colonies with at least one live young: $N_1 = 25$, $N_2 = 23$, $U = 285.5$, $P > 0.95$). The two types of incipient colonies did not differ by their overall sex ratio, which was not significantly different from equity (single-factor ANOVA after angular transformation and weighting for sample size, see Campbell 1974: $F_{1,46} = 1.59$, $0.5 > P > 0.2$). Of the 20 all-female pairs, several laid eggs, but only in 1 did a single larva hatch. This larva died before it could be preserved.

Discussion

Our results are consistent with previous data on the sex ratio of all castes in primary-headed colonies of *C. lacteus* (Roisin and Lenz 1999) and confirm the male bias reported for alates and nymphs in neotenic-headed colonies (Lenz and Runko 1993). This bias persists at least 4–5 years after orphaning. Our most intriguing find is that this bias is generated in two steps (Fig. 1b). First, a significant excess of males appears among first-instar larvae, which supports hypothesis (1) formulated above (i.e. early male bias). Second, only male larvae engage in the nymphal pathway at the second moult. The fact that the proportion of males drops significantly between second-instar larvae and workers supports hypothesis (3) against (2), which means divergent destinies for male and female larvae rather than elimination of females. Moreover, assuming that all female larvae become workers, the fall in the male bias from 3:1 to an even ratio in workers implies either that the proportion of the male larvae selecting the nymphal pathway is as high as two-thirds, or that some elimination of males occurs at that time. In short, of four first-instar larvae hatching from the eggs, one would be female and become a worker, and three would be male, of which one would become a worker and two would proceed to the nymphal pathway or be eliminated. This relative amount of nymphs in comparison to workers is hard to corroborate by individual counts, first because nymphs may segregate from workers in the nest, and second because the normal duration and life expectancy of each instar are not known. Nevertheless, our data strongly suggest that the proportion of resources allocated to alates by neotenic-headed colonies is large, even though some male nymphs may be eliminated or revert towards a worker-like morphology at a later stage, giving rise to nymphs with regressed wing buds (Lenz and Runko 1993; colonies CM23 and CM17 herein). An opposite reaction after manipulation was observed in a colony whose primary queen escaped removal: this colony ceased to produce nymphs, probably to rebuild its worker population after damage (Lenz and Runko 1993).

Whether this sex-allocation shift is adaptive per se is a puzzling question. Because neotenic-headed colonies are scarce in nature, their male-biased sex allocation is unlikely to influence population-level sex allocation in a significant manner. Among a population of colonies predominantly headed by primary reproductives, and which produce balanced alate sex ratios, any sex ratio should be, a priori, equally valuable for members of neotenic-headed colonies (Kolman 1960; Charnov 1982). Therefore, male bias may not be adaptive as such, but arise as a side effect of the prevailing sex-determination mechanism under inbreeding. However, if some factor specific to neotenic-headed colonies conferred a higher relative value to male alates than to female ones, such colonies should be expected to invest massively in males. As a result, male-biased allocation would become adaptive by itself.

Genetic correlates of reproduction by neotenic

Since inbreeding is the most distinctive feature of neotenic reproduction, a fundamental influence of this phenomenon on sex allocation can be hypothesised. Unfortunately, sex-determination mechanisms in *Coptotermes* are still poorly known (Wang and Grace 1999). Karyotypes of *Reticulitermes*, a genus closely related to *Coptotermes* (Kitade and Lo 1998), suggest a basic chromosomal pattern of sex determination with heteromorphic males and a variable extent of genomic linkage to the Y chromosome (Fontana and Amorelli 1978; Fontana 1990). If sex determination in *C. lacteus* conforms to such a pattern, inbreeding could have dual consequences. First, males could experience lower homozygosity than females because their Y-linked alleles can never become homozygous, contrary to the homologous alleles of females (Charlesworth and Wall 1999). If homozygosity effects were sufficient to generate a male bias among larvae in neotenic-headed colonies (either through a genetic determination of embryonic sex ratio or through differential mortality before or just after hatching), the same bias should appear in the offspring of inbred primary pairs. This was, however, not the case. The hypothesis of a purely mechanical effect of inbreeding on the sex of offspring can therefore be dismissed. Nevertheless, homozygosity might still influence the relative fitness of the sexes at a later stage (e.g. by affecting viability or fertility in adults).

A second effect of inbreeding is to modify Hamiltonian relatedness patterns within the colony. As soon as sex-linked genes are involved, the respective value of male and female alates to reproductives and workers of either sex may shift after reproductive replacement. For instance, in primary-headed colonies, sex linkage boosts relatedness between same-sex sibs and reduces relatedness between opposite-sex sibs (Luykx and Syren 1979; Crozier and Pamilo 1996), but this disparity is reduced by half when the progeny of replacement reproductives is considered (Roisin 2001). In addition, polygyny, polyandry and overlap of generations may substantially complicate relatedness patterns. Further research is required to determine whether males are more closely related than females to those colony members that control sex allocation, and therefore represent for them a more profitable investment.

Another possibility is that sex determination in *Coptotermes* does not follow the chromosomal XX/XY system. Some other termites, such as *Schedorhinotermes* and *Cornitermes*, possess all-female or all-male workers and soldiers (Renoux 1976; Noirot 1989; Roisin 1992). They must therefore produce unisexual young during the largest part of the year and shift to bisexual young when the nymphal brood is initiated. These facts demonstrate that termites can adjust sex ratios at hatching depending on environmental conditions, although the underlying mechanisms remain unknown.

A third process that could contribute to sex-ratio bias is parthenogenesis by neotenic females. While arrhen-

toky is essential for sex determination in Hymenoptera, parthenogenesis and its consequences are poorly documented in termites. Pairs of virgin females of *C. formosanus* failed to produce viable offspring (King and Spink 1974). Pairs of females of *R. virginicus* and *R. speratus* succeeded, but the sex of their brood was not determined (Howard et al. 1981; Matsuura and Nishida 2001). The discovery of a *Bifiditermes beelsoni* colony apparently composed entirely of females suggested the occurrence of thelytoky (Chhotani 1962). By contrast, in *Velocitermes* species, pairs of virgin females produced a much higher proportion of soldiers than male-female pairs (Stansly and Korman 1993). Since soldiers are male in *Velocitermes barrocoloradensis* and in related nasute genera (Roisin 1996), it is likely that these parthenogenetic broods were male-biased, but this requires confirmation. The fact that all-female pairs of *C. lacteus* largely failed to produce offspring suggests that the parthenogenetic potential of this species is low, although parthenogenesis by neotenic females cannot be totally ruled out. However, the fact that in the present case one young was produced may indicate that one of the females had been inseminated while still in the holding container, although only females with their wings intact were chosen for pairing.

Colony condition and resource availability

Colonies headed by neotenics are in a delicate condition, which appears to be more than an immediate consequence of the physical damage caused by orphaning. Even colonies that apparently recover well from experimental manipulation tend to decline after a few years (Lenz and Runko 1993). Neotenic-headed colonies in Australian *Coptotermes* have seldom been encountered in nature despite their potentially everlasting reproductive resources. The intense production of alate dispersers by such colonies can be interpreted as a strategic choice of a secure short-term benefit against an uncertain long-term investment in colony reconstruction. Other termites have been shown to adopt this strategy under precarious conditions (Lenz 1994). However, this hypothesis explains neither why nor how, in *C. lacteus*, such colonies channel their reproductive investment almost exclusively into male alates.

Colony characteristics correlated to male bias in social Hymenoptera are of little comparative value. In several ant species, small colony size or limited resources were found to correlate with male-biased sex allocation, but most of the proposed explanations depend on the peculiar sexual composition of hymenopteran societies and are therefore not applicable to termites. For instance, several hypotheses depend on the assumption that investment shifts are easier between female castes (workers and gynes) than between males and females, so that resource-deficient colonies sacrifice gyne production to invest in workers but keep raising males (Bourke and Franks 1995; Crozier and Pamilo 1996; Chapuisat

and Keller 1999). Such an argument could not hold for *C. lacteus*, whose workers are of both sexes and lack sexual dimorphism.

However, other factors could potentially affect sex ratio and sex allocation in termites, in particular, in the more general framework of genetic conflicts within insect societies (Sundström and Boomsma 2001 and references therein). At the second moult, larvae of *C. lacteus* engage either in the worker developmental line or in the nymphal one. The signals governing this decision are not known, but the process is obviously altered in neotenic-headed colonies, as large numbers of males follow the nymphal line while all females are restricted to the worker line. This could be an expression of selfish behaviour by the individuals – i.e. they opt for a reproductive pathway in their own interest at the expense of the interests of the colony. Conversely, Lenz and Runko (1993) suggested that excluding females from the nymphal line could be a way for recently differentiated neotenic females to prevent competition from newly developing ones. In primary-headed colonies of several *Coptotermes* species, including *C. lacteus*, a small proportion of nymphs systematically develop into neotenics during each annual cycle, although such neotenics do not reproduce as long as the primary queen is active. The same phenomenon happens in neotenic-headed colonies but involves only males, since no female nymphs are present. This hypothesis may point in the right direction, although it cannot explain why *C. lacteus* developed the complicated process of male-biasing in the two steps discovered here, and why the male bias persists for at least 5 years after orphaning. In this context, it is interesting that the aforementioned laboratory-kept colony of *C. formosanus* in China with a 100% male bias of alates which persisted for 2 years, produced some female alates (about 0.4%) for 4 years before it died (Y.-H. Zong, personal communication).

The finding that the male bias in neotenic-headed colonies of *C. lacteus* is generated through a two-step process designates a double target for forthcoming studies of the factors governing sex allocation in this species: first, the process biasing the sex ratio before, or at the latest very soon after, hatching. We dismissed a straightforward, mechanical effect of inbreeding on sex ratio at hatching, but we could not test other hypotheses so far. Issues that badly need to be elucidated are the sex-determination mechanism itself, and the factors that could affect the survival rate of the embryos. A second target for future investigation lies at the developmental decision point, when larvae select either the nymphal or the apterous line of development. All these topics are poorly known at present, not only for *Coptotermes*, but for termites in general. These facts emphasise how much our present knowledge of termites lags behind that of social Hymenoptera on matters as basic as the mechanism of sex determination, the processes of caste differentiation, and the natural patterns of life history and sex allocation.

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