Sociality in the phylogenetically basal allodapine bee genus *Macrogalea* (Apidae: Xylocopinae): implications for social evolution in the tribe Allodapini

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Recent phylogenetic studies based on DNA sequence data indicate that the tropical African bee genus *Macrogalea* is the sister group to the remaining extant allodapine fauna, whereas previously it was thought to be a distal group. This leads to some fundamental changes in our understanding of social evolution in the allodapines. Earlier studies suggested that *Macrogalea* showed only weak forms of social behaviour and these were not well characterized. However, large samples of *Macrogalea zanzibarica* presented here show that this species exhibits marked social behaviour. Nearly half of nests collected contained two or more adult females, with up to 10 females per nest. Brood are reared progressively and brood ages within colonies are staggered, giving rise to colonies with very mixed age structures and therefore frequent opportunities for alloparental care. Ovarian dissections indicate non-simple forms of reproductive partitioning within colonies and most multi-female colonies show evidence that more than one female has contributed to egg production, though reproductive episodes among colony members are frequently asynchronous. Some females show signs of much higher wing wear than their nestmates, but always show signs of previous reproduction. Reproductive division of labour appears to be temporally marked, ovarian differentiation among nestmates is linked to relative body size, but permanent worker-like or queen-like castes appear to be absent. This is similar to the communal, continuously brooded and multivoltine behaviour of some tropical halictine species and may be due to the aseasonal nature of brood development in tropical regions. Patterns of per capita brood production indicate large benefits to multi-female nest occupancy, and sex allocation is strongly female biased. These findings strongly suggest that the capacity for complex social interactions and alloparental care is an ancestral trait for all of the extant allodapine lineages. Therefore comparisons among extant allodapines are unlikely to throw light on the initial origin of social behaviour, though they may uncover origins of true caste behaviour and reversals to solitary nesting. Sex ratios in *Macrogalea* and most other allodapine genera, spanning a broad phylogenetic and ecological range, suggest that one or more allodapine traits have provided persistent selection for female-biased sex allocation. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2002, 76, 211–224.

ADDITIONAL KEYWORDS: alloparental care – sex allocation – per capita brood production.

INTRODUCTION

The allodapine bees (Apidae, Xylocopinae) have been thought to provide extensive opportunities for investigating the evolutionary origin and elaboration of insect social behaviour. Sociality varies widely within and between species and genera, providing an opportunity for comparative approaches to social evolution at multiple hierarchical levels (Schwarz, Bull & Hogendoorn, 1998). Allodapines are almost entirely restricted to sub-Saharan Africa, south-east and southern Asia, and Australia. A single genus, *Exoneuridia*, occurs in the Palearctic and is restricted to southern Turkish highlands, Iran and the Arabian Peninsula (Terzo, 1999). Early behavioural studies (Brauns, 1926; Skaife, 1953) demonstrated at least temporary episodes of sociality, involving matrilineal cooperation, in several African species. Work by Michener (1962, 1965, 1971a, 1977a, 1985) revealed...
the wealth of material available for studies of social evolution and suggests that the allodapines could be used to elucide early steps into sociality in bees. The existence of morphological castes in the Australian allodapine *Exoneurella tridentata* (Houston) shows that social behaviour in at least one allodapine species encompasses highly eusocial organization that in bees had previously only been identified in corbiculates and some halictines bees (Houston, 1977; Hurst, 2002). Research by Schwarz and coworkers since the late 1980’s on Australian *Exoneura* species indicates that social behaviour in allodapines is very complex (review in Schwarz *et al.*, 1998), involving nest co-founding with active recognition of previous nestmates, high degrees of reproductive skew and relatively large colony sizes. In contrast, members of the related clade *Brevineura* possess extended opportunities for sib-rearing and derive apparently much larger benefits from group living, yet multi-female colonies are less frequent and much smaller in size (Tierney, Schwarz & Adams, 1997; Tierney *et al.*, 2000). The development of complex social behaviour among the Australian allodapines appears to be influenced by phylogenetic history and environmental constraints to independent nesting rather than the existence of matrifilial relationships.

Reports of sociality in Australian exoneurine allodapines (encompassing the genera *Exoneura*, *Exoneurella*, *Brevineura* and the parasitic genus *Inquilinea*) suggest that sociality is more complex than that of the more abundant and diverse African fauna (*Allodape*, *Allodapula*, *Dalloapula*, *Compsonelissa*, *Halterapis*, *Macrogalea*, plus the parasitic *Eucondylops* and *Effrectapis*). This may be because Australian studies were able to obtain larger sample sizes covering more of the life cycle, utilized large-scale field experimentation, and employed more recent methodologies in analysis. Nonetheless, Michener’s (1971a, 1975) prodigious volumes on the biology and taxonomy of the African allodapines provide a rich framework for current research.

Allodapines are unique among bees in progressively rearing their larvae in a non-partitioned communal nest. *Halterapis* is the only genus to display mass provisioning, and its sister genus *Compsonelissa* displays partial mass provisioning. Mass provisioning is present in all non-allodapine Xylocopinae and because of this it was initially considered that *Halterapis* and *Compsonelissa* represented intermediate steps between mass provisioners and the remaining allodapines (Michener, 1977a). Recent phylogenetic analyses of some key allodapine genera, using one nuclear and two mitochondrial gene fragment sequences (Schwarz, Bull & Cooper in press) conflict with previous morphology-based analysis (Michener, 1977a; Reyes, 1998) by placing *Macrogalea* as the sister group to all other extant allodapines. This suggests that the kind of provisioning displayed by *Halterapis* and *Compsonelissa* is not plesiomorphic, but represents a new character state. Because the order of oviposition and provisioning in *Halterapis* is the reverse of other mass provisioning Xylocopinae, mass provisioning in *Halterapis* is not a simple reversion to an ancestral state. Given that the sequence-based analyses and re-consideration of morphological and behavioural data indicate that *Macrogalea* forms the sister group to all other extant allodapine groups, it is now important to characterize sociality in *Macrogalea* because of the insights this may give to origins and elaboration of sociality within the tribe Alldapidini.

*Macrogalea* is distributed in east and south-west tropical Africa and Madagascar. There are presently five recognized species, one of which is parasitic (Michener, 1971b, 1977b, 1977c). *Macrogalea candida* (Smith) is the most widespread species, occurring in Sudan, Ethiopia, Kenya, Uganda, Tanzania, Angola and Namibia, but is not known from Democratic Republic of Congo westward to Senegal. *Macrogalea mombasae* (Cockerell) is a parasitic species collected from nests of *M. candida* and has only been collected in Kenya and Tanzania. *Macrogalea elliotti* (Saussure) and *M. infernalis* Michener are located in Madagascar, whilst *M. zanzibarica* Michener is recorded from the Tanzanian island of Unguja (Zanzibar).

Very little has been previously recorded about the biology and sociality of *Macrogalea*. Both adults and immatures are morphologically unique among allodapines. Adults are large and robust with extensive pubescence, prepupae are unusually curled, and mature larvae are covered with short dense hairs which are often hooked dorsally and thought to aid in maintaining position against the nest wall (Michener, 1971b, 1976). Michener’s (1971a) description of *M. candida* is the only account of the biology in this genus and details the contents of 23 nests, the majority of which contained solitary females. Whilst some multi-female nests implied the possible presence of sociality, reproductive skew and caste-like behaviour could not be investigated. Sample sizes precluded analysis of the extent or nature of social nesting, leading to the general assumption that sociality in this genus was relatively undeveloped. Here we conduct a more detailed examination of social behaviour in *Macrogalea* using the previously unstudied species *M. zanzibarica*, and reveal a significantly more developed expression of sociality than previously anticipated. Our findings strongly indicate that sociality is a plesiomorphic trait for extant allodapines and this has major consequences for understanding social evolution in this tribe.
MATERIAL AND METHODS

*Macrogalea zanzibarica* was investigated from two localities, adjacent to the villages Jambiani and Matemwe, on the eastern coast of Unguja (Zanzibar Island) situated 35 km off the coast of Dar es Salaam at latitude 6° S. Colonies were collected from herbaceous shrubs along coastal dunes from 24–27 December 2000. Nests were collected at dawn and dusk or during rain, when it was assumed all nest mates were in residence. The entire contents of nests were preserved in Kahle’s solution.

Reproductive condition of females was assessed by dissection of adults according to the methods of Schwarz (1986). Ovary size was measured as the sum of the three largest terminal oocyte lengths. The presence of degenerating nurse cells (yellow bodies) in the lateral oviducts indicate recent egg laying history (Michener, 1971a) and was also noted. Insemination status was gauged by the opacity of the spermatheca, and if this was ambiguous *in situ* it was ruptured on a slide mount, whereby the resilient and spheroid sperm mass became apparent if present. Wing length was used to estimate relative body size and was measured as the distance from the axillary sclerites to the apex of the marginal cell. Wing wear was measured as the number of nicks and tears on the distal margins of the forewings.

Larvae were categorized as small (1st and 2nd instars), medium (3rd and early 4th instar) or large (4th instar). Brood rearing efficiency associated with social nesting was assessed as the total number of immatures present per adult female and is referred to here as per capita brood production (PCBP – Tierney et al., 1997). We use the terms ‘brood’ and ‘immatures’ as synonyms referring to a collection of any of the following ontogenetic phases found in a nest: eggs, larvae, prepupae, pupae, callow adults. PCBP provides a ‘snap-shot’ of colony production and does not indicate lifetime colony output. However, it does provide a useful gauge of colony efficiency as a function of the number of female nestmates at the time of collection.

Numerical and investment sex ratios were investigated in 46 nests of *M. zanzibarica* that contained pupae. Ratios were calculated as the number of male pupae divided by the total number of pupae per nest. Relative levels of investment in the two sexes were assessed using dry pupal weights, where pupae were dried at 50°C for 3 days before weighing.

RESULTS

COLONY SIZE AND COMPOSITION

Nests of *M. zanzibarica* were up to 30 cm long with narrowed entrances but without evidence of the constructed entrance collars found in other allodapine genera such as *Exoneura*, *Braunsapis* and *Allodapula* (Michener, 1971a). Eggs are weakly attached to the nest lumen and become loose in the nest prior to larval eclosion, multifemale nests rarely possessed attached eggs and this may be a result of increased nest traffic. Brood are aligned within the nest by age as in other allodapines with the youngest stages at the terminal end, unlike some nests of *M. candida* which show arrangement independent of age (Michener, 1971a).

Figure 1 plots total brood against nest length for *M. zanzibarica*, with each colony labelled according to the number of adult females. Nest length is related to the number of brood, with larger nests tending to contain more adult females, and the scattergram does not suggest that colony size is constrained by available nesting substrate.

Colony sizes are summarized in Figure 2. There was a mean of 2.01 females per nest (range 1–10, \( N = 79 \)) and 44.3% of nests contained more than one adult female. There were up to 22 immatures in a nest with a mean of 5.67 per nest. The composition of brood is discussed in more detail below. Adult males were only found in five nests and all of these also contained adult females.

We classified brood into five categories, i.e. eggs, small, medium and large larvae, and pupae + prepupae. Ninety-one percent of nests contained immatures and the mix of brood developmental stages in the nests are summarized in Figure 3, which also indicates the number of adult females in each nest. All develop-
Mental stages were common in our sample of nests, suggesting that brood production is continuous, which is not surprising given the coastal tropical habitat of our study sites. Interestingly, 84% of single female nests possessed brood at some level of development, and this is a larger percentage compared to most African allodapines previously studied (Michener, 1971a).

All nests containing only one developmental stage (N = 17 nests), or two consecutive stages (N = 9), were single female nests, whereas multi-female colonies tended to contain three or more developmental stages. Of the 14 colonies containing all developmental stages, all were multi-female. The narrow spread of brood ages in single female nests suggests that they have been recently founded and the resident adult is rearing a single cohort of brood. In contrast, the presence of most or all brood stages in multi-female colonies suggests that once the oldest brood have become adults, and if they remain in their natal nest, brood production proceeds as a more or less continuous process, leading to a wide spread of immature stages. This is important for understanding social behaviour, as it means that opportunities to rear siblings arise commonly in this species because some brood are becoming adults while others are at egg or feeding larval stages. For example, 39 of the 79 colonies contained eggs or small larvae simultaneously with pupae and it is likely that these pupae would have become adults while dependant feeding stage immatures were still present in the nest, giving rise to opportunities for newly eclosed females to help rear immature nestmates.

**Per capita brood production**

Per capita brood production (PCBP) was calculated for each colony size (number of adult females) and the resulting data are summarized in Figure 4. The relationship between PCBP and colony size was assessed using a two-way ANOVA with PCBP as the dependent variable, and with site (Jambiani or Matemwe) and colony size (number of adult females per nest) as crossed factors. PCBP was log transformed to produce homogeneity of variances. The results show no interaction between site and females per nest (F₁,₆₆ 1.304, P = 0.278). A subsequent ANOVA with interaction effects removed showed no site effect (F₁,₆₆ = 0.152, P = 0.697) but a significant effect of the number of females (F₇,₆₆ = 3.820, P = 0.002). Mean PCBP values for each colony size category where then compared using multiple unplanned post hoc tests. Both Tukey and Least Significant Difference tests indicated that PCBP for single female nests was significantly lower than for 2-female colonies (P < 0.001), and no other pair of colony sizes showed a significant difference in PCBP. The lack of differences for the larger colony sizes is probably linked to small sample sizes but may also suggest lower PCBP values than for two-female nests.

Schwarz (1994) argued that prevention of total brood loss in some allodapines was an important source of benefits from cooperative nesting and Zammit, Schwarz & Hogendoorn (unpubl.) experimentally confirmed that ant predation incurs very high rates of colony and brood failure in single female nests. We examined whether brood failure in *M. zanzibarica* might contribute to the low per capita brood numbers in single female nests. Seven nests in our sample did not contain any brood and all of these were single female nests. The proportion of colonies with and without brood was compared between single and multi-female nests using a two-tailed Fisher Exact test and this showed significant dependence (P = 0.016). Because it is possible that single female nests without brood may have been only very recently initiated nests, we compared nest length of single female nests with and without brood. This indicated no significant difference (means of 48.24 and 48.57, respectively, t₄₂ = 0.02, P = 0.984). Given that lack of brood was restricted to single female nests, we examined whether this may have lead to the significant ANOVA result on PCBP above. We performed another ANOVA on PCBP omitting nests that lacked brood from the analysis, but obtained very similar results and a
Figure 3. Range of immature stages within nests of *Macrogalea zanzibarica*. Each row represents an individual nest (*N* = 72 nests with brood). Larvae are classified into three stages (small; medium; large), prepupal and pupal stages are grouped in the same category. The detached far right column indicates solitary (single open box) or multi-female colonies with 2–3 (single filled box), or ≥4 (double filled box) females per nest.
highly significant effect of adult females per nest \( (F_{7,59} = 3.867, P = 0.002) \).

The above analyses indicate benefits to cooperative nesting, but measures of PCBP for multi-female colonies *M. zanzibarica* with asynchronous brood development may underestimate actual rearing efficiency, because of the possible presence of recently eclosed adult females which were indistinguishable from old females. In our PCBP measures, recently eclosed females may have been counted as adults contributing to colony maintenance, but might more appropriately be counted as brood. Such misappropriation would falsely and simultaneously inflate the number of adults and discount the number of brood, leading to much lower PCBP values. However, it is difficult to confidently identify such nests when life cycles are continuous (not regulated by season) and callow females are only discernible for a brief period after eclosion.

**DISSECTION DATA**

Eighty females from 21 multi-female colonies were dissected and ovary size, presence of yellow bodies, wing wear, and wing length were recorded. Ovary size is plotted against wing wear for all females from multi-female colonies in Figure 5 and the presence or absence of yellow bodies is indicated. This figure shows that females both with and without wing wear showed highly varying degrees of ovarian development. This range in both wing wear and ovary size is concordant with brood development data suggesting that multi-female colonies potentially contain adults of very different ages. Of females from multi-female colonies, 52.5% possessed yellow bodies, indicating previous reproductive activity. Ovary size and wing wear also varied markedly for females with yellow bodies, suggesting that current reproductive status is not consistently linked with previous reproductive activity. The range in female age makes it difficult to use these parameters to examine whether females adopt queen-like or worker-like roles. However, queen-like and worker-like roles in many bee species are often linked to body size, and we examined whether body size was associated with ovarian condition and wing wear. Several approaches were used to examine these possible relationships.

Firstly, we tested whether current reproductive differentiation among nestmates was associated with relative body size. To account for scaling effects, an ‘ovarian index’ (sum of oocyte lengths/wing length) was used to indicate ovarian development adjusted for body size. Within each nest females were ranked according to ovarian index, then wing length was compared between the highest and lowest ranked individuals. This was performed using a paired-samples...
t-test, to account for possible variation in ovarian development and body size among colonies. Females with tied ranks were excluded. Females with the greatest ovarian index were significantly larger in body size than nestmates with the least ovarian development ($t_{19} = 2.868, P = 0.01$). We then ranked individuals within colonies by wing length and compared ovarian indices between the largest and smallest nestmates using a paired $t$-test. This suggested that the largest females tended to have greater ovarian indices than their smallest nestmates ($t_{13} = 2.215, P = 0.045$).

We also examined whether wing length was associated with previous reproductive activity, assessed by the presence of yellow bodies. Wing length was grouped into four categories (4.80–5.05 mm; 5.06–5.30 mm; 5.31–5.55 mm; 5.56–5.80 mm) and the proportion of females in each category that had yellow bodies was measured. Proportions are summarized in Figure 6 where error bars are standard deviations based on a binomial distribution. This figure suggests a strong relationship between body size and previous reproductive activity. A chi-square analysis showed that presence of yellow bodies is not independent of body size ($\chi^2_3 = 16.407, P < 0.001$).

Whilst we can not directly attribute wing wear to foraging (e.g. Cartar, 1992) or guarding (e.g. Hogendoorn & Schwarz, 1998) activity, wing wear has been widely used as an indicator of age (e.g. Michener et al., 1955; Hogendoorn, Watiniasih & Schwarz, 2001). We explored the relationship between wing wear and body size, using the same wing length categories as above. There was no indication that presence or absence of wing wear was associated with body size ($\chi^2_3 = 2.850, P = 0.415$). Lastly, we examined whether wing wear was associated with presence of yellow bodies. Wing wear was grouped into four categories: no wear; 1–9 nicks; 10–18 nicks; and ≥19 nicks, in order to ensure adequate sample sizes for chi-square analysis and at the same time represent the range of wear exhibited in our sample. The proportion of individuals that possess yellow bodies (± binomial standard deviation) was plotted for each wing nick category (Fig. 7). Chi-square analysis indicates that presence of yellow bodies was not independent of degree of wing wear, although the significance was only marginal ($\chi^2_3 = 8.127, P = 0.043$). This suggests that individuals with higher levels of wing wear in multi-female nests are more likely to have engaged in reproductive activity, which is not unexpected if presence of wing wear is correlated with age.

Our analyses therefore suggest that both current reproductive status and reproductive history is associated with body size, and possibly with wing wear, but
the patterns in our data are not as clearly defined as they are for many temperate Australian allodapines (review in Schwarz et al., 1998; Tierney et al., 2000). This is potentially related to the tropical habitat of _M. zanzibarica_ which involves continual brood rearing behaviour, with asynchronous brood development in multi-female colonies. Continuous and asynchronous brood development should lead to staggered recruitment of new individuals into colonies combined with staggered death or dispersal of older nestmates and this has the potential to produce colonies with very wide ranging adult and brood ages.

To provide an indication of the range of adult compositions that occur in multi-female colonies of _M. zanzibarica_, we briefly present and discuss the composition of some colonies that span the range of colony variation in our sample. Table 1 summarizes ten multi-female colonies of varying size and composition.

**Table 1.** Selected nests of _M. zanzibarica_ that illustrate the range of reproductive activity among colonies of varying size. Individuals within each nest are ranked according to wing length in descending order. The range of brood within each nest is denoted as eggs (E); larvae (L); prepupae & pupae (P). History of egg laying is assessed using the presence (+) or absence (−) of yellow bodies. Wing wear was categorized into four categories: no wear (no asterisk); 1–9 nicks (*); 10–18 nicks (**); ≥19 nicks (***) . Insemination status is represented positive (+); negative (−); or ambiguous (?). Callow females denoted by (C)

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For each colony, individuals are ordered according to wing length, with ovarian size, insemination status, wing wear and presence of yellow bodies indicated. All colonies listed contained more than one inseminated female. Larger colonies contained two or more actively reproductive females and an even greater mean number had previously laid eggs (presence of yellow bodies). Most nests have one female with extensive wing wear, who is inseminated, has previously laid eggs, but whose reproductive activity varied at the time of collection. Nest 21 is probably a relatively new nest (nest length = 72 mm) containing an older female who has reproduced in the past and is currently the principal egg layer (inseminated, large ovaries with yellow bodies and high level of wing wear), who is accompanied by another female (probably one of her daughters) who has not been reproductive (uninseminated, small ovaries, no yellow bodies, and low level of wing wear) but may be helping to rear her younger nestmates (probably siblings) in a small colony that contains a wide range of brood. A similar scenario occurs in the larger and presumably older nest 68 (nest length = 243 mm) which has one old, well-worn, reproductive female with three younger females (probably daughters, one still callow), two of which are uninseminated and one of which has low wing wear. Nest 10 contains an actively reproductive old female who has laid in the past, an unworn female who has equally developed oocytes, and two other females with smaller ovaries, one of which has laid in the past. Nests 1, 42 and 65 each contain an old inseminated female with younger adults (including a callow in nest 42) some of which are taking on reproductive roles, and some of which have also reproduced in the past. Nests 72 & 76 represent situations where perhaps the foundress has died and two females of similar age (low degree of wear) are both reproductive.

**SEX ALLOCATION**

The numerical sex ratio of brood produced by all nests in this sample (r = total male pupae/total pupae) was r = 0.12 which differed little from the value calculated as a mean of individual colony ratios r = 0.13 (N = 46 colonies). Analysis of pupal dry weights indicated no significant difference between male (1.33 mg ± 0.09 SE) and female (1.57 mg ± 0.04 SE) pupae (t_{105} = -1.774, P = 0.079). Consequently, numerical ratios are likely to indicate investment ratios. We calculated numerical sex ratios for each nest with pupae and these are summarized in Figure 8 as a function of the number of adult females in each colony. Sex ratios were female biased for colony sizes of <3 females, and in larger and presumably older nests (>4 females) ratios appear to be less biased. Mean sex ratios for each colony size, with colonies of >4 females aggregated, were compared using a Kruskal–Wallis test which showed a significant effect of colony size (χ^2 = 9.974, P = 0.016). However nonparametric post hoc multiple comparisons (Siegel & Castellan, 1988) did not identify significant differences between nest sizes, suggesting the relationship with colony size is not strong, at least based on these small sample sizes.

**DISCUSSION**

Our data show that social behaviour in *Macrogalea zanzibarica* is moderately well developed, with almost half of all nests containing more than one female. Brood development patterns and nest lengths suggest that nests are founded by solitary females, but the majority of older nests, where at least some brood have reached adulthood, contain more than one female. Examination of ovarian data indicate that multi-female colonies do not comprise only temporary pre-reproductive assemblages (Michener, 1985), which might be expected if daughters remain briefly in the natal nest before dispersing; rather there is strong evidence that more complex colony structures develop. Once brood have reached maturity, nests are lengthened and reused for subsequent egg laying and brood rearing. Whilst small, presumably newly founded colonies contain brood of a more-or-less uniform developmental cohort, nests which are being re-used contain brood of very wide ranging developmental
stages. This asynchronous and continual brood rearing in older nests means that there are very extensive opportunities for newly emerged females to help rear their younger, immature nestmates.

**Alloparental care and reproductive differentiation**

The nature of alloparental care in *M. zanzibarica* is difficult to characterize, given the range in ages of nest inhabitants. However, several traits can be discerned. Firstly, many if not most multi-female colonies exhibit signs that reproduction within nests involves more than one female. In some cases, an older female appears to continue reproduction and may be aided by some of her daughters. In other nests, older females may be joined by younger reproductive females, whilst continuing to reproduce, or reproduction may be taken on by younger females with the older female(s) engaging in tasks other than egg-laying. Because brood in allogynous nests are communally reared in an undivided burrow, the results of guarding and foraging activity spread to all immatures and cannot be restricted by adults to just one matrilineal group of immatures (Schwarz, 1988b).

The range in nestmate composition and variable reproductive roles for *M. zanzibarica* is similar to some other tropical bees, such as *Halictus ligatus* Say from Trinidad where over half of females collected from both nests and on flowers were inseminated and had enlarged ovarioles (Michener & Bennett, 1977). Whilst the largest individuals of *H. ligatus* were more reproductive than the smallest, some small bees had equally sized ovaries. Michener & Bennett (1977) also reported wide variation in wing wear and ovarian development among females and suggest that identifiable queen-like females did not appear to be inhibiting the reproductive activity of nestmates. Our data for *M. zanzibarica* also show some similarities to the life cycle of a subtropical allogynous *Braunsapis hewitti* (Cameron) from Taiwan (Maeta, Sakagami & Michener, 1992) which exhibits a multivoltine life cycle with staggered brood development. In *B. hewitti*, alloparental care consists of a serial progression of semisocial or eusocial monogynous colonies, with a slightly larger principal reproductive at any one time. Multi-female colonies and solitary nests with brood are both less common in *B. hewitti* than in *M. zanzibarica*.

Importantly, the existence of more than one female contributing to brood production within a nest suggests that intra-colony relatedness is unlikely to be high, at least in older colonies. Although some colonies with multiple reproductives appear to be comprised of mothers and daughters, there is evidence that some colonies contain two or more reproductives that are more likely to be sisters or less closely related. Studies of intra-colony relatedness using genetic markers are needed to assess the relationships between multiple reproductives, and between alloparents and the brood they help rear.

Although our data do not indicate the existence of effectively sterile worker-like individuals, they do indicate that body size is associated with reproductive status, with smaller females having a higher likelihood of not showing signs of previous or current reproductive activity. However, wing wear was positively but only weakly associated with previous reproductive activity. This association is difficult to interpret. It could mean that older females are more likely to have been reproductive at some time in the past or it could mean that reproductive females are more likely to engage in foraging or other activities that incur wing wear. In the Australian allogynous bee *Exoneura robusta*, reproductively dominant females tend to have higher wing wear than their subordinate nestmates and yet do not undertake the principal foraging roles (Hogendoorn & Schwarz, 1998) but instead undertake complex guarding roles (Bull et al., 1998). In another Australian allogynous, *Exoneurella tridentata*, morphologically distinct queens often have extremely worn wings, to the point that they are unable to fly (Houston, 1977), and appear to assume major guarding roles within their colonies and are very long lived (two or more years; Hurst, 2002). Behavioural observations of *M. zanzibarica* colonies are needed to resolve the nature of reproductive differentiation and alloparental care before measures of wing wear can be properly interpreted and behavioural roles inferred from them.

**Benefits of social nesting and sex allocation**

Measures of per capita brood production indicate that nest sharing by two females is accompanied by large benefits in brood rearing efficiency compared to single female nests. In several Australian allogynous, the increase in PCB of two female compared to single-female nests is largely due to prevention of brood loss, and single female nests frequently lack brood despite protracted opportunities for egg-laying (Schwarz, 1994; Bull & Schwarz, 1996; Tierney et al., 1997, 2000; Schwarz et al., 1998). Cane & Michener (1983) suggested that ants are a major threat to allogynous colonies, and experimental field manipulations by Zammit et al. (unpubl.) on the Australian bee *Exoneura nigrescens* Friese support previous speculation by clearly showing that single female nests suffer high rates of brood loss due to ant predation. Our data on *M. zanzibarica* indicate that all multi-female nests contained brood, whereas about 16% of single female nests lacked brood and this is unlikely to be due to
these nests being very young. If lack of brood results from enemies at the nest, our data suggest that brood defence may be an important benefit of cooperative nesting. However, analyses where broodless nests were excluded still indicated substantial increases in PCBP of two female nests compared to single female nests, suggesting that prevention of brood failure is not the only benefit of cooperation. One possible source of the higher PCBP values when brood loss is disregarded might be the future availability of potential alloparents once brood rearing has become continuous and asynchronous. If reproductive females have assurance that alloparents will become available, they may be able to provide larger numbers of brood than they could expect to rear to completion on their own. That is, females may adjust reproductive output to meet the anticipated number of future alloparents, rather than the currently available workforce, once staggered brood development is under way.

We found that sex allocation in *M. zanzibarica* was very strongly female biased. The sample-wide estimate of numerical allocation to brood is $r = 0.12$ (proportion of males), which is similar to *E. robusta* where local fitness enhancement has been proposed to lead to selection for very strongly female biased allocation ratios (Schwarz, 1988a, 1994). No other allodapines where sex allocation has been studied have more biased ratios than these two species. Female biased sex allocation in allodapine bees has been attributed to two non-mutually exclusive selective forces: (i) local fitness enhancement (LFE) (Schwarz, 1988a, 1994; Cronin & Schwarz, 1997) and (ii) ‘brood insurance via protogyny’ (BIP) (Bull & Schwarz, 2001). In the former model, allocation bias results from increases in mean daughter fitness due to benefits of cooperation between sisters, whereas in the latter model production of some ‘insurance daughters’ before further production of brood helps protect younger brood in the case of orphaning and, as a consequence, leads to a female bias in sex allocation. Selection for ‘insurance’ daughters to care for their younger siblings can arise via assured fitness returns (AFR) or ‘reproductive head-starts for workers’ (Queller, 1989; Gadagkar, 1990). This seems plausible for *M. zanzibarica* given that daughters who either (i) inherit a nest with brood, or (ii) choose to remain in the natal nest with their mother or sister, gain some indirect fitness advantages over solitary foundresses by being able to invest in already present, partially reared offspring and avoid the risks of dispersal. The potential gains from AFR should be higher in allodapines than for mass-provisioning bees, because larval instars are entirely dependent on adults for food and defence from predators throughout their development, so that orphaning in the absence of alloparents has higher fitness consequences than for mass provisioners where a mother’s parental investment is effectively secured each time a cell is completed.

**Implications for understanding social evolution in allodapines**

Until recently it was thought that the most basal allodapine clade was *Halterapis + Compsomelissa*, based on cladistic analyses of morphological and life-history data (Michener, 1977a; Reyes, 1998). The trait of mass provisioning brood in *Halterapis* was thought to be a retention of a plesiomorphic state, and levels of sociality in the genus were presumed to be mostly or entirely subsocial and this was also thought to be a plesiomorphy. The view that social behaviour within the tribe Allodapini had evolved *among* extant clades was based on these assumptions. Although *Halterapis* was believed to be subsocial, Michener (1971a) found an average colony size of 1.8 adult females and strongly female biased sex ratios. Multi-female nests mostly comprised an apparently old female together with one, or rarely more, relatively un worn and often callow females, suggesting that these younger females were daughters yet to disperse. However, this also allows the possibility of alloparental care in the case of orphaning, but opportunities for alloparental care would be very limited, given that eggs are mass provisioned. Michener (1971a) was able to sample only 10 nests of *Compsomelissa stigmoides* (Michener), and six of these contained two adult females. These colonies allow the possibility of social nesting, but could also represent mother–daughter associations where the daughter was soon to disperse. Further data on *Halterapis* and *Compsomelissa* are required to determine if those taxa do indeed exhibit alloparental care.

A recent phylogenetic study of allodapines based on sequence data from one nuclear and two mitochondrial genes (Schwarz et al. in press) shows that *Macrogalea* is in fact the sister group to all other allodapines and that mass provisioning in *Halterapis* is therefore a derived character state. This means that the putative lack of sociality in *Halterapis* does not represent the plesiomorphic state for the Allodapini. Data reported here indicate that sociality is at least moderately well developed in *M. zanzibarica*, and that egg attachment and nest entrances without collars, also found in *Xylocopini* and *Ceratinini*, are likely ancestral traits for the Al lodapini. Based on a sample of 23 nests of *M. candida* from Kenya, Michener (1971a) suggested there was inconclusive evidence for worker-like individuals in some colonies. Only six colonies contained more than one adult female (range of 2–7), though all of these contained eggs or feeding stage immatures. Further data are needed to determine whether social behaviour in that species is similar to that of *M. zanzibarica*.
Although sociality is not well documented for the majority of African allodapine species, the existence of colonies with more than one adult cohabiting during active brood rearing, together with evidence for cooperative rearing involving alloparental care and size-based reproductive differentiation, is known for all genera except Halterapis and Compsomelissa. Recent studies (S. M. Tierney & M. P. Schwarz, unpublished) incorporating large sample sizes and more extensive sampling and analyses indicate that the extent of alloparental care in many African species is likely to be higher than indicated by Michener’s (1971a) samples. Well developed sociality has been reported from species for all non-parasitic Australian genera (Schwarz et al., 1998). Alloparental care is therefore known for all non-parasitic allodapine genera (excluding Halterapis and Compsomelissa – where it is possible but not demonstrated), and is now also recorded from the most basal clade, Macrogalea. Both morphological (Michener, 1977a; Reyes, 1998) and sequence data (Schwarz et al., in press) suggest that Compsomelissa and Halterapis form a holophyletic group, and sequence data indicate that this clade arose after the divergence between the Australian exoneurine group and the non-Macrogalea African genera. Consequently, parsimony arguments suggest that sociality involving alloparental care is a plesiomorphic state for the Allodapini and that species of Compsomelissa and Halterapis may represent a loss, or partial loss, of this. We now briefly discuss some implications of this conclusion.

Firstly, if sociality was present at the origin of the allodapines, within-tribe comparisons among the allodapines can not be used to infer factors contributing to the origin of sociality per se, although there may be useful insights into factors selecting for losses of sociality, alterations in socially important life-history strategies, and the development of true caste behaviour. Although differences in colony structure in allodapines have been thought to represent stages in an evolutionary pathway from subsocial to social organization (for example, Michener, 1974, 1985, 1990; Schwarz, 1988b), our data suggest that the de novo origin of sociality is not represented among extant taxa. Secondly, sociality in the extant allodapines has had a long evolutionary history, and the extent of this will depend on how old the tribe is. Engel (2001) has described a fossil tribe Boreallodapini, from Baltic amber approximately 45 Mya, which forms a sister group to the Allodapini, suggesting that the allodapines are likely to have an ancient origin. This means that social behaviour in extant species is likely to have resulted from a very long period of selection on long since established elements of sociality going back to the origin of the tribe, rather than representing early steps into sociality. Interestingly, social behaviour is recorded for at least several taxa in the tribe Cera
tinini (Hogendoorn & Velthuis, 1999), which is basal to Allodapini + Boreallodapini, various forms of sociality are also present in the majority of Xylocopini species that have received detailed study (Michener, 1985; Hogendoorn & Velthuis, 1999; Steen, 2000). It is therefore possible that some elements of social behaviour in the allodapines had an evolutionary origin prior to the origin of the tribe.

Inferring likely social traits of the ancestral allodapine lineage cannot be undertaken until further studies are completed on key African genera, particularly Alloolape and Allooladapula, where sociality is known but not well described. However, we do know that the existence of well defined alloparental care and caste-like behaviour does not seem to be restricted by voltinism, brood development patterns, or climatic conditions effected by latitude (Tierney et al., 2000). Alloolapine social behaviour is recorded from a wide range of genera in both tropical and temperate habitats, and for species that are univoltine, bivoltine and continuously brooded. Also, female biased sex allocation occurs in the majority of allodapine species where sex ratios have been reported (Michener, 1971a; Tierney et al., 1997; Schwarz et al., 1998; Bull & Schwarz, 2001). Two different selective models for allocation bias have been applied to temperate univoltine allodapine bees, LFE and BIP. The existence of strongly female biased sex allocation in Macrogalea, along with reports of female bias in other genera, suggests that selection for bias can occur in very different ecosystems and in phylogenetically and behaviourally very different clades. Increases in per capita brood production for multi-female colonies in Macrogalea zanzibarica exhibit similar trends to Australian temperate species, and this suggests that some alloolapine traits may enable such benefits to also arise across very different ecosystems.

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