Reproductive hierarchies in the African allodapine bee *Allodapula dichroa* (Apidae; Xylocopinae) and ancestral forms of sociality

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The social organization of allodapine bees has been described in detail for most genera, although there remains a notable gap for one major lineage, the genus *Allodapula*. Here, we provide the first detailed study of social organization in *Allodapula dichroa*. Colony sizes are small and the frequency of cooperative nesting is low compared with other allodapine taxa, but there is very clear evidence for reproductive differentiation among adult nestmates. Reproductively dominant females tend to be larger than their nestmates and have much higher levels of wing wear, suggesting that they perform most foraging activities. Multi-female colonies have: (1) lower rates of complete brood absence, suggesting a substantial benefit to cooperative nesting; and (2) larger numbers of brood, suggesting that the presence of a second adult female leads to a greater reproductive output. These data suggest a major phylogenetic split in the form of social organization within the allodapines. In the genus *Macrogalea* (sister clade to all other allodapines), body size does not preclude young females from laying eggs, and there appears to be, at most, weak reproductive queues. However, in most other allodapines, reproductive hierarchies are prominent and younger and/or smaller females queue for reproductive opportunities, adopt permanently subordinate roles, or disperse. Interestingly, the most common forms of reproductive hierarchies in allodapines do not involve subordinates undertaking foraging roles before reproduction, but instead involve the delaying of both reproduction and foraging. This has implications for the understanding of suggested developmental ground plans in the early stages of social evolution. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 520–530.

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INTRODUCTION

The allodapine bees (Apidae; Xylocopinae; Allodapini) are a useful group for studying social evolution. Social nesting is facultative and social organization varies widely, providing considerable material for comparative evolutionary studies (Michener, 1974). Recent molecular phylogenetic research has provided well-resolved and well-supported trees covering all non-parasitic genera (reviewed in Schwarz, Richards &

Danforth, 2007; Chenoweth *et al.*, 2008; Tierney *et al.*, 2008), so that behavioural studies can be informed by a knowledge of evolutionary descent. We can now begin to infer ancestral traits for the tribe and identify phylogenetic locations of key evolutionary transitions.

Sociality is a plesiomorphic trait for the tribe, and varies from species which are largely subsocial (for example, Hogendoorn, Watiniasih & Schwarz, 2001) through to one species which is clearly highly eusocial (Hurst, 2001), but with the majority of species showing varying degrees of quasisociality, semisociality and 'primitive' eusociality (reviewed in Schwarz *et al.*, 2007; Tierney *et al.*, 2008). However, one clear

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pattern regarding sociality concerns the existence of size-based reproductive hierarchies, which have been found in the genera *Hasinamelissa*, *Halterapis*, *Braunsapis*, *Exoneura*, *Brevineura*, and *Exoneurella*. Studies of the genus *Macrogalea*, which comprises the sister group to all other allodapines, indicate that, in multi-female colonies, females become reproductive shortly after reaching adult eclosion (Tierney *et al.*, 2002; Schwarz, Bull & Cooper, 2003; Smith & Schwarz, 2006; Smith, Schwarz & Chapman, 2006; Thompson & Schwarz, 2006), and that, if size-based hierarchies exist, these are either weak or only involve older females who would be entering a second or later phase of egg laying (Thompson & Schwarz, 2006). However, one major allodapine lineage, the African genus *Allodapula*, has not been studied in sufficient depth to determine whether size-based hier-

archies exist, and the only study to date suggested that multi-female colonies were rare for this genus (Michener, 1971).

Figure 1 summarizes the relationships among the non-parasitic allodapine genera, with tree branches denoting the known existence of reproductive hierarchies. If size-based hierarchies exist in *Allodapula*, this would coincide with the most basal divergence within allodapines, namely the divergence between the *Macrogalea* clade and the lineage leading to all other genera. A lack of such hierarchies in *Allodapula* would suggest a more complex pattern of origins and losses.

The genus *Allodapula* consists of 16 species largely restricted to South Africa (Michener, 1975a, 2007). *Allodapula* is unique among allodapines in brood-rearing methods. Cohorts of larvae form a group
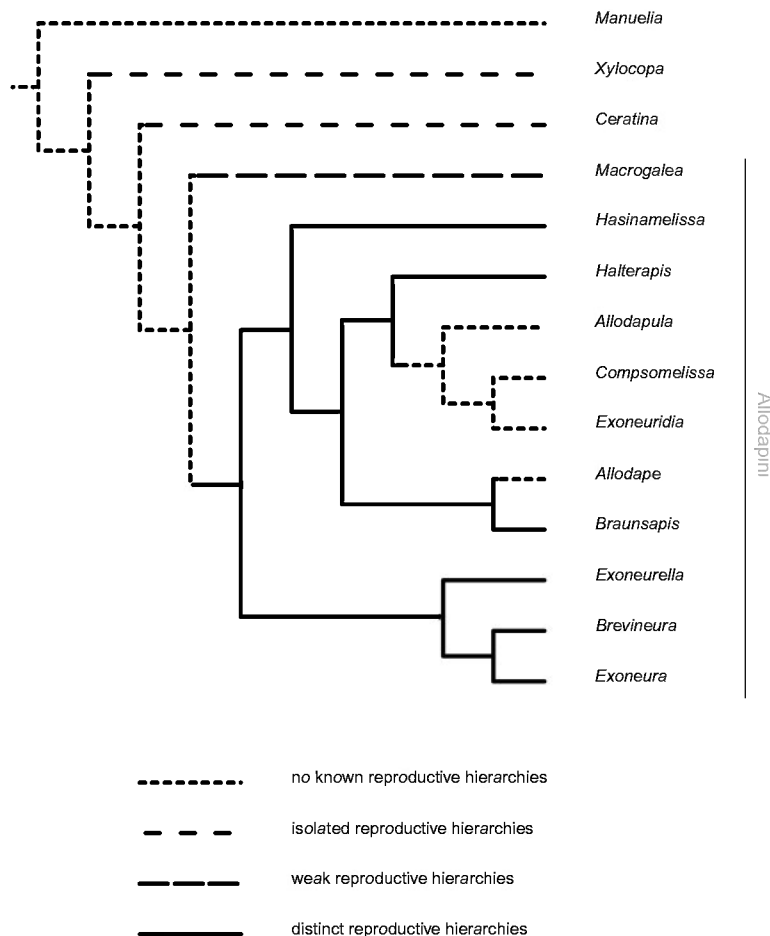

Figure 1. Reproductive hierarchies in the genera of Xylocopinae – the tribes Manuelini, Xylocopini and Ceratinini are monogeneric. Reproductive hierarchies based on age or body size are unknown in *Manuelia* (see Flores-Prado, Chiappa & Niemeyer, 2008), taxonomically sporadic in both *Xylocopa* and *Ceratina*, and present to varying degrees in Allodapini. Among the 11 non-parasitic genera of Allodapini, reproductive hierarchies are phylogenetically widespread, but are only weakly expressed in *Macrogalea*; they appear to have been lost in *Allodape* and, prior to this study, were unknown in *Allodapula*, *Compsomelissa* and *Exoneuridia*.

around a central common pollen mass, which is progressively provisioned by adults (Brauns, 1926; Skaife, 1953; Michener, 1971, 1975a, b). *Allodapula* species overwinter as adults and begin laying small cohorts of eggs in late winter (Michener, 1971; S. M. Tierney and M. P. Schwarz, unpubl. data). They exhibit a multivoltine life cycle with egg laying through spring and summer, resulting in nests that often contain immatures of all stages up until early autumn. July-laid eggs emerge as adults in late spring (late October–November), whereas eggs laid in spring and summer have much faster developmental times, 6–7 weeks (Skaife, 1953). Michener (1971) found only limited evidence for the existence of queen and worker-like roles, and multi-female nests never comprised more than 20% of sampled nests.

Here, we investigate colony development and reproductive hierarchies in *Allodapula dichroa* Strand during three stages of the main brood-rearing period. We then use these results to infer broader patterns in the evolution of sociality in allodapines.

MATERIAL AND METHODS

FIELD SITES AND SAMPLE DATES

A montane population of *A. dichroa* from the Skurweberg Mountains (Western Cape Province, South Africa) was sampled. Nests excavated in restioid reeds (Restionaceae) were collected from a westerly facing pass (~1800 m in altitude) leading into the Witzenburg valley, 25 km NNW of Ceres (33°22′S; 19°19′E). This mountain pass is dominated by open montane fynbos and is exposed to very cold winters with occasional light snow. Nests were sampled over three periods in 2000: 8–13 January (mid-summer); 25–28 September (early spring); 6–12 December (early summer).

COLLECTION, PRESERVATION AND DISSECTION METHODS

Intact colonies were collected at dawn, dusk or during periods of rain, when foraging does not occur. Nest occupants were preserved in Kahle's solution for later dissection. Larvae were divided into four categories: small (first and second instar), medium (third and early fourth instar), large (late fourth instar) and pre-pupae. Newly eclosed adults were identified by their pallid exoskeleton, flattened dorso-thoracic pubescence and pristine wing condition. We refer to such individuals as 'callows' and consider them as members of the immature brood (according to Michener, 1971). Colony efficiency is assessed in terms of per capita brood production (calculated as total brood/number of non-callow adult females; Tierney, Schwarz & Adams, 1997). Both pupae and

callow adults were used to estimate numerical sex ratios (r = proportion of males in the sexed brood). Dissections of adult females follow Schwarz (1986). Ovary size was measured as the summed lengths of the three largest terminal oocytes. Fertilization status was determined by investigating spermathecal content. Forewing length was used as an indication of body size, measured as the distance from the axillary sclerites to the apex of the submarginal cell. Nicks and tears in the distal wing margins were scored and used as a measure of flight activity.

STATISTICAL ANALYSES

Data were analysed using SPSS© version 11 for Mac OS X. Parametric tests were used when assumptions of homoscedasticity and/or normality were met, and regression analyses were only used when Q–Q plots indicated that residuals were evenly distributed over the ranges of the independent variables. When these could not be met using either raw data or transformed data, non-parametric tests were used.

RESULTS

COLONY PHENOLOGY

A total of 120 colonies were sampled from September ($N = 21$), December ($N = 56$) and January ($N = 43$) collections. The mean numbers of eggs, larvae, pre-pupae + pupae and callow females per nest are summarized in Figure 2. Multi-female colonies comprised 14%, 29% and 33% of all nests for each collection, respectively, and the proportions of solitary
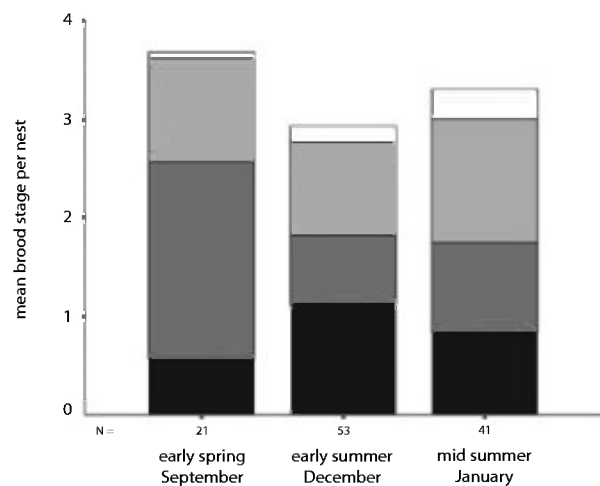
Figure 2. Histogram of brood proportions. Colours define mean numbers of eggs (black), larvae (dark grey), pre-pupae and pupae (light grey) and callow females (white) collected within nests of *Allodapula dichroa* for the three sample dates.

versus multi-female colonies did not differ significantly across samples ($\chi^2_2 = 2.423$, $P = 0.298$). The maximum number of adult (non-callow) females over these samples was three, and only five colonies (~4% of the total) had this many females. Figure 2 suggests that egg production in our samples was greatest in early summer (December), which probably represents secondary brood cohorts, keeping in mind that seasonal egg laying in this genus begins as early as July (Skaife, 1953). In line with this assumption, the number of larvae was greatest in spring (September), but with a second increase by mid-summer (January), and the number of callows increased as the season progressed. However, Kruskal–Wallis tests showed that only total larvae number varied significantly over these samples ($\chi^2_2 = 10.026$, $P = 0.007$) and that the other stages did not ($P > 0.213$ for all other comparisons).

Our samples indicate that colony size was small across the sampled seasons, and that the proportion of multi-female nests did not vary substantially. Brood rearing was well underway by our spring sample (presence of pupae and pre-pupae), and would have continued after our mid-summer sample (which contained eggs). Our data show that egg laying, larval rearing and maturation of brood into callow adults occurred throughout all the sampled periods. This means that newly eclosing adult females are faced with opportunities for both direct reproduction (as oviposition was occurring in all samples) and rearing of brood that may already be present in their natal nests. Given this, we need to explore what kinds of reproductive hierarchies and alloparental care might exist.

REPRODUCTIVE HIERARCHIES

For this study, neither ethological studies on observation nests nor genotyping were feasible; thus, reproductive skew and its determinates were inferred from colony census and dissection data. We began by looking at ovarian development, body size and wing wear from adult females. Nests containing three adult females were excluded from analyses because of the small sample sizes.

Firstly, we addressed the reproductive differentiation among nestmates. For each two-female colony, we classed females as having either the larger or smaller ovary size in their nest. A graph of these data (Fig. 3) suggested bimodality and therefore ovarian differentiation. Testing for such differentiation in multi-voltine species is not straightforward, as nestmates will virtually never have exactly the same ovary size as each other, even if females do not fall into worker-like or queen-like roles. We explored the existence of reproductive differentiation
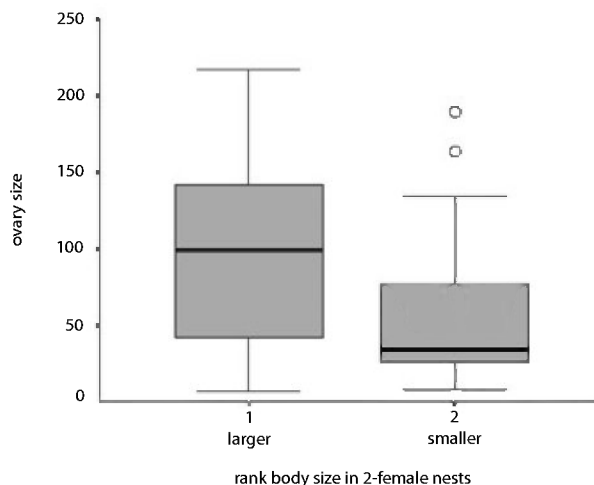

Figure 3. Boxplot of ovary size as a function of body size (wing length), ranked within colonies for two-female nests ($N = 28$); circles indicate outliers.

by asking whether ovary sizes of nestmates were random with respect to each other, given the distribution of ovary sizes in the sample as a whole. To do this, we calculated the mean difference in ovary size between nestmates from the 28 two-female colonies. We then used a re-sampling procedure to randomly draw 28 pairs of ovary sizes from a pool of all females in two-female colonies (not taking colony membership into account), and then calculated the mean difference among these pairs. This procedure was then repeated 1000 times, giving a null distribution that could be used to determine whether the observed differences in ovary size between nestmates were a result of stochastic variation alone. Only six of the 1000 simulated mean ovary size differences were greater than the observed mean difference, indicating that ovarian differentiation is indeed marked in multi-female nests.

The second issue we addressed was whether ovary size scaled with body size independently of any social interactions. We examined single-female nests using analysis of covariance (ANCOVA) with sample date as the treatment and body size (measured as wing length) as the covariate; this indicated no interaction between covariate and treatment ($F_{2,36} = 2.534$, $P = 0.093$); when the interaction was removed, there was no indication of a body size effect ($F_{1,38} = 0.01$, $P = 0.922$). Given this lack of evidence for a body size scaling effect in single-female nests, we asked whether relative body size might influence ovary size in multi-female colonies.

In two-female nests, we ranked individuals according to body size and ovary size, and then carried out independence tests between these two ranks for all

samples combined. This indicated a significant dependence ($\chi^2_2 = 9.053$, $P = 0.011$), with females with the lowest ranked ovary sizes tending to have the lowest ranked body size. We then investigated absolute ovary size as a function of ranked wing length. Analysis of variance (ANOVA) showed no sample effect on ovary sizes of first- or second-ranked females from two-female nests ($P > 0.4$ for both tests), and we then combined the three collection periods ($N = 28$). A t-test indicated that ovary sizes were significantly different between first and second body size-ranked females in two-female nests ($t_{54} = 3.071$, $P = 0.003$). Ovary sizes are summarized as a boxplot in Figure 3, which shows that, although there is a strong difference between the two groups in median ovary size, some second-ranked females nevertheless have large ovaries. The mean ovary size of females from solitary nests (97.38 ± 45.96 SD) was not significantly different from that of the first-ranked physically 'larger' female (101.25 ± 60.06 SD) in two-female nests ($t_{68} = -0.305$, $P = 0.761$). However, the ovary size of solitary females was significantly greater than that of second-ranked 'smaller' females (56.86 ± 47.39 SD) in two-female nests ($t_{68} = 3.570$, $P = 0.001$).

We also examined wing wear as a function of body size and ovary size; for these comparisons, we used non-parametric tests because the distribution of wing wear is zero-truncated. We tested whether wing wear differed between females with first- or second-ranked body size among samples using Kruskal–Wallis tests, which indicated no seasonal differences ($P > 0.39$ for both tests). We then pooled the data across samples and compared wing wear between first and second body size-ranked females using a Mann–Whitney U-test, which indicated a significant difference ($P = 0.037$); the data are summarized in Figure 4. When wing wear was similarly compared between first and second ovary size-ranked females, we found a highly significant difference (Mann–Whitney U-test, $P < 0.001$), and these data are summarized as a boxplot in Figure 5. This graph forms a strong contrast with Figure 4, and the two figures suggest that wing wear is more strongly affected by ovarian rank than by body size rank.

Taken together, the analyses of multi-female nests indicate that: (1) there is a strong tendency for females with greater relative body size to have the largest ovaries in their nest, with some exceptions; (2) the physically largest female in the nest tends to have higher levels of wing wear, but again there are exceptions to this; and (3) females with the largest ovaries in their nest tend to have much greater levels of wing wear, and the majority of second-ranked females have very little or no wing wear. These patterns are similar to many other non-*Macrogalea* allodapines, which suggests the existence of size-
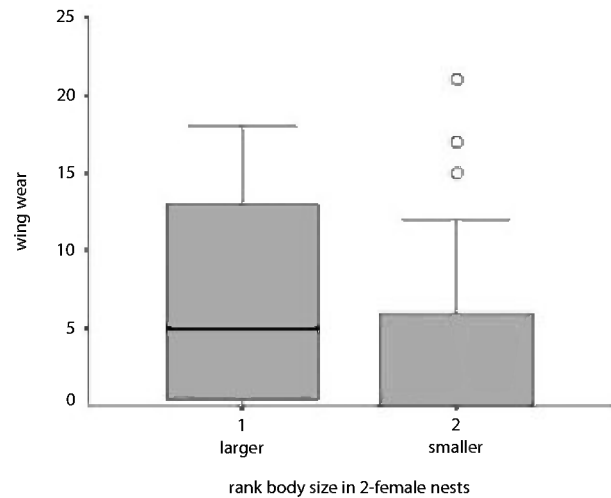

Figure 4. Boxplot of wing wear as a function of body size (wing length), ranked within colonies for two-female nests ($N = 28$); circles indicate outliers.
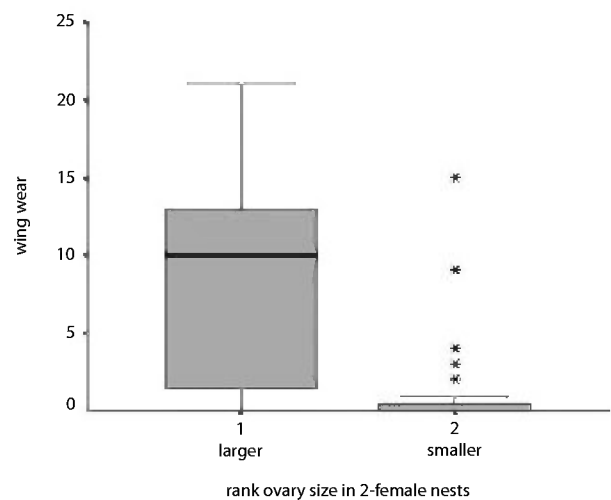

Figure 5. Boxplot of wing wear as a function of ovary size ranked within colonies for 2-female nests ($N = 28$); asterisks indicate extremes.

based reproductive hierarchies within colonies, in which reproductively more active females appear to do most or all of the foraging in their colony. It is also possible that females with small ovary sizes may simply be recently eclosed, and hence have little or no wing wear. Michener (1971) regarded fully pigmented females with undeveloped ovaries and no wing wear as recently emerged individuals. This latter scenario would require that recently eclosed females were smaller than their nestmates, or that larger recently eclosed females had very quickly dispersed, or had been evicted, from the natal nest, leaving only the smaller ones behind.

INTERPRETATION OF WING WEAR

The above arguments are based on the assumption that wing wear is an indicator of foraging activity, and not simply caused by age (for example, Mueller & Wolf-Mueller, 1993). Unfortunately, it was not feasible to independently examine the wing wear of foragers versus non-foragers. Instead, we inferred this using single-female nests to determine whether the amount of wing wear was related to the total number of brood within the nest at the time of collection, under the assumption that larger brood sizes require greater foraging effort. We first conducted an ANCOVA with sample date as a fixed factor and total brood number as a covariate; this showed no interaction between the covariate and sample date ($F_{2,32} = 1.644$, $P = 0.209$), no effect of sample date ($F_{2,32} = 0.207$, $P = 0.814$), but a highly significant effect of brood size on the amount of wing wear in solitary females ($F_{1,32} = 13.922$, $P = 0.001$). We then regressed wing wear for solitary nesting females on the total number of brood, pooling all samples; this showed a significant positive effect ($F_{1,36} = 16.824$, $P < 0.001$, $\beta = 0.564$), implying that wing wear may well be an indicator of an individual's foraging effort. Foraging effort is also likely to be correlated with the age of brood (older brood will have required more foraging trips). To examine this, we developed a non-parametric weighted measure of required foraging effort based on both the number and developmental stage of brood (i.e. eggs, small, medium and large larvae, pre-pupae, pupae and callows). This was calculated as (number of eggs × 1) + (number of small larvae × 2) + . . . (number of callows × 7). We then calculated a Spearman's non-parametric correlation between this parameter and wing wear; this indicated a highly significant association ($\rho = 0.603$, $P < 0.001$), again suggesting that wing wear is correlated with previous foraging activity.

In our study, of the 45 females from two-female nests where insemination status could be determined, only two were uninseminated, and neither of these females had any wing wear and both had the smallest ovaries in their nest. Michener (1971: 264, 268) noted that, in multi-female nests of *Allodapula acutigera* Cockerell and *A. dichroa*, auxiliary females were usually inseminated, and emphasized this as a major difference between *Allodapula* and the sympatric southern African species of *Allodape* and *Braunsapis*.

BENEFITS OF GROUP LIVING

Dissection data suggest a size-based reproductive hierarchy in which reproductively dominant females appear to do the foraging. This raises the question of whether reproductively subordinate females actually contribute to brood rearing at all (for example, via

defence, foraging, maintenance), or may instead be simply waiting for an opportunity to inherit the nest after death of the dominant female.

Two benefits of cooperative nesting have been identified in allodapines (Schwarz *et al.*, 2007): (1) prevention of total brood failure; and (2) increases in per capita brood production. We examined the first possibility using a Fisher exact test to determine whether the presence or absence of brood varied with colonies containing one or more adult females. We found a highly significant effect ($P = 0.007$), with 24 of the 87 single-female nests lacking brood, but only one of the 28 multi-female nests lacking brood. It is possible that single-female nests lacked brood because they had only been initiated recently, in which case nest length should be shorter. However, a *t*-test indicated no difference in nest length between single-female nests with and without brood ($t = 0.092$, $P = 0.927$), suggesting that the time of nest initiation could not explain the absence of brood.

We then compared per capita brood production between single- and two-female nests, using only nests that contained at least one brood instar. ANOVA indicated no interaction between sample date and colony size ($F_{2,84} = 0.543$, $P = 0.583$), no effect of sample date ($F_{2,84} = 3.084$, $P = 0.245$) and no effect of the number of adult females ($F_{2,84} = 0.015$, $P = 0.908$). Consequently, our data suggest that the benefits of cooperative nesting involve the prevention of total brood failure (either failure to produce brood or failure to prevent brood loss), but no benefits for increased rearing efficiency once brood absence is removed as a factor. Lastly, we determined whether cooperative nesting affected the total number of brood being reared, even though there was no apparent increase in rearing efficiency. To do this, we used ANOVA in which the total brood number was the dependent variable, and sample date and colony size (single- or two-female colonies) were crossed factors. This indicated no interaction between factors ($F_{2,84} = 1.051$, $P = 0.354$), no effect of sample date ($F_{2,84} = 1.267$, $P = 0.441$), but a significant effect of cooperative nesting ($F_{1,84} = 19.774$, $P = 0.024$), with mean brood numbers substantially larger in two-female nests than single-female nests (Fig. 6). These findings suggest that, although cooperative nesting does not increase rearing efficiency, it does impact positively on the total number of brood being produced. We now ask why this may be the case.

ALLOPARENTAL ASSESSMENT

Thompson & Schwarz (2006) found that, in a Malawian species of *Macrogalea*, the number of potential alloparents influenced the total brood number, in that the number of eggs was correlated with the number of

Table 1. Multiple regression analysis investigating the effect of the number of larvae, pre-pupae + pupae, callow females and adult females on the number of eggs in nests

Collection	Beta value and (significance)			
	Larvae	Pre-pupae + pupae	Callow females	Adult females
September	**−0.598 (0.008)**	−0.289 (0.095)	**0.773 (< 0.001)**	0.229 (0.231)
December	**−0.446 (0.004)**	**−0.371 (0.008)**	0.107 (0.431)	**0.380 (0.032)**
January	−0.270 (0.088)	0.114 (0.500)	**0.394 (0.011)**	0.114 (0.487)

Statistically significant variables within each sample analysis are shown in bold.
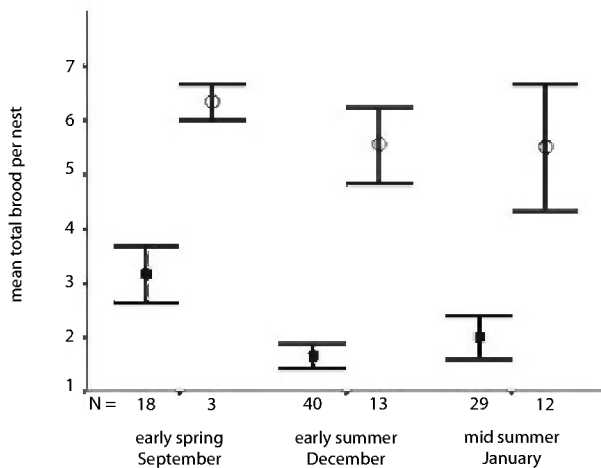
Figure 6. Mean total number of brood (± standard error) for single-female nests (filled squares) and two-female nests (open circles).

Table 2. Multiple regression analysis examining the effect of the number of adult females and callow females on the total number of larvae within nests

Collection	Beta value and (significance)	
	Callow females	Adult females
September	−0.142 (0.415)	**0.666 (0.001)**
December	−0.082 (0.554)	**0.451 (0.002)**
January	0.087 (0.586)	0.272 (0.093)

Statistically significant variables within each sample analysis are shown in bold.

potential future alloparents at the time that eggs were being laid. A similar situation was also found for an Australian bee, *Brevineura elongata* Rayment (Joyce & Schwarz, 2006), suggesting that reproductive females adjust oviposition rates in anticipation of future alloparental help. We examined this possibility in *A. dichroa* using multiple regression, where the dependent variable was the number of eggs, and the independent variables were the number of larvae, pre-pupae + pupae, callow females and adult females. These independent variables were included because: (1) older brood could affect egg production (for example, by placing demands on resources that could otherwise go into egg production); and (2) auxiliary females and callow females could represent actual or potential alloparental help for raising these eggs once they eclose into larvae. The results of these analyses are shown in Table 1 and indicate various significant effects. The only significant effect involving larvae and pre-pupae + pupae was negative, and could arise if a brood moves through its development as a cohort, so that as the number of older brood increases, the

relative number of younger brood decreases. In contrast, there were significant regression slopes for eggs regressed on callow females for the September and January data; for eggs regressed on adult females, there was a significant effect for the December sample only. These patterns are consistent with the prediction that reproductive females should increase egg production if actual alloparents (i.e. auxiliary adult females) are in place, or if they will soon come into effect, because of the presence of callow females.

If callow females and auxiliary adult females represent potential or actual alloparental help for rearing larvae, as the above analyses suggest, we might expect the number of brood being actively reared at any time (i.e. larvae) to be related more strongly to actual help (adults) than potential help (callows). However, we would not expect the number of larvae to be affected by the number of eggs or post-feeding brood (pre-pupae, pupae, callows). We therefore regressed the number of larvae on the number of adult and callow females in colonies for the three sample dates, and the results are summarized in Table 2. This shows that the number of larvae is positively related to the number of adult nestmates in September and December samples, but not to the number of callow females in any collection.

Lastly, if parental care is provided by younger adults after a dominant older female dies, we would

expect to see some colonies in which a younger female is now an alloparent for brood that she could not have produced herself. Given the relationship between wing wear and both the total number of brood and the oldest brood stage present (see above), we used wing wear to examine whether there were some colonies in which the sole female was unlikely to be the parent of all the brood present. We found many colonies that fitted this expectation. In two colonies, the sole adult had zero wing nicks and the oldest brood were recently emerged callows. In another nest, the sole female had one wing nick and was accompanied by three large larvae; in a further two nests, the sole adult female had two nicks and was also nesting with large larvae. Another solitary-nesting female had three wing nicks and was cohabiting with five pupae. In all of these cases, it is likely that the sole females were caring for brood that were not their own.

ANCESTRAL STATES AND CHARACTER STATE TRANSITIONS

Given the presence of reproductive hierarchies in *A. dichroa*, we can now infer likely ancestral traits for the allodapines more generally. When inferring origins and losses of reproductive hierarchies, it is important to determine whether or not such hierarchies are plesiomorphic for the sister clade. In the extant sister tribe to the allodapines (Ceratinini), knowledge of both sociality and phylogeny is poor (but see Discussion below), and therefore we explored both possibilities. We used MacClade (v4.08 OS X) to infer the most parsimonious reconstruction, assuming that gains and losses of hierarchies were equally likely, and treating *Compsomelissa* and *Exoneuridia* as having missing values. Studies by Michener (1971) indicate a lack of apparent hierarchies in those species of *Allodape* that have been studied, and in which large sample sizes were available. The resulting most parsimonious reconstruction was a tree containing two steps: a gain of hierarchies at the node uniting non-*Macrogalea* clades and a loss in the branch leading to *Allodape*. When the outgroup was assumed to have hierarchies, the most parsimonious

reconstruction was also a tree with two steps, namely a loss in *Macrogalea* and a loss in *Allodape*. If hierarchies are assumed to have been present in the sister clade to the allodapines, the most parsimonious model (involving two steps) involves two losses of social hierarchies – one in the lineage leading to *Macrogalea*, and another in the lineage leading to *Allodape*.

SEX ALLOCATION

Numerical sex ratios were calculated from pupae, combining all nests for each sampling period, and are presented in Table 3. Sex allocation was female biased in all sample dates, ranging from $r = 0.27$ to $r = 0.45$. These values are similar to those found in other *Allodapula* species (Michener, 1971; S. M. Tierney, unpubl. data), but are less female biased than most other allodapines (Michener, 1971; reviewed in Schwarz *et al.*, 2007).

DISCUSSION

Michener (1971) found very low levels (less than 3%) of multi-female nests in *A. dichroa*, whereas, for our samples, this varied from 14 to 33%. This discrepancy could be caused by temporal or habitat differences between study sites, and these possible effects are likely to have less influence for our sampling regime. Our results therefore indicate a higher level of social nesting than previously thought for the genus *Allodapula*. Benefits to cooperative nesting in *A. dichroa* derive from the avoidance of total brood failure, but there are no further benefits in terms of enhanced brood-rearing efficiency on a per capita basis. This is probably a result of the unusual feeding strategy of *Allodapula*, whereby larval cohorts congregate around a common food mass that is replenished by adults from above, placing a physical upper limit on brood number: a maximum of six large larvae at any one time in *A. dichroa* (Michener, 1971; this study). Thus, subsequent cohorts of larvae cannot be present until the former cease feeding because they occupy and block the inner circumference of the nest

Table 3. Numerical sex ratios calculated as an individual colony mean and at the population level

	Early spring	Early summer	Mid-summer	
	September	December	January	Combined
Colony r	0.27	0.48	0.30	0.38
Population r	0.36	0.45	0.41	0.42
	($N = 3$)	($N = 13$)	($N = 13$)	($N = 29$)

Ratios calculated by sample date as well as a combined total.

tunnel (Skaife, 1953). Nevertheless, colony sizes in *A. dichroa* are still small (three or less non-callow adult females) compared with most other allodapines, raising the question of whether sociality in *Allodapula* involves more than temporary matrifilial assemblages whilst newly emerged adults are waiting to disperse, which we now explore.

Our results show that, within colonies of *A. dichroa*, ovarian rank is positively associated with relative body size, although there were instances where smaller nestmates had larger ovaries. The relationship between ovarian rank and wing wear was much more marked, and nestmates with relatively smaller ovaries had either no or very little wing wear. We have no direct evidence that wing wear in reproductively dominant females is a result of foraging activity, although wing wear in solitary nesting females shows a strong correlation with both the total number of brood and age of the oldest brood in single-female nests, suggesting that it is a good measure of foraging activity. In observation nests of the sister species *Allodapula acutigera*, Skaife (1953: 13) noted that: 'A female that has emerged from the pupal stage in late January or during February will rest all through the autumn, only taking occasional flights from the nest for food, until about the middle of July, when she will commence to lay eggs'. Taken together, these results suggest that reproductive dominance is associated with relative body size and that dominant females do most of the foraging. This period of waiting is similar to that in many other allodapines (*Braunsapis*, *Exoneura*, *Brevineura*, *Exoneurella* [excluding *E. tridentata*], *Hasinamelissa* and *Halterapis*), but is very different from that in *Macrogalea*. We also found indirect evidence that females of *A. dichroa* will increase egg numbers in anticipation of future alloparental help, as well as evidence that some solitary females care for brood that are very unlikely to be their own.

Macrogalea is the sister genus to all remaining taxa, but in no species is there evidence that recently emerged females forego reproduction or join a queue for dominance, and no reproductive castes inhibit newly emerged females from reproducing (Tierney *et al.*, 2002; Smith & Schwarz, 2006; Smith *et al.*, 2006; Thompson & Schwarz, 2006; Smith, 2007). *Macrogalea* differs noticeably from other allodapines in that its larvae lack appendages and elaborate setae (Michener, 1976; Schwarz *et al.*, 2003). We speculate that complex larval morphology, which allows the manipulation of food sources, may heighten competition for food among individual brood. If competition leads to a greater range in the sizes of adults, it could increase power asymmetries among emerging adults and increase the likelihood of the evolution of dominance hierarchies.

Most parsimonious reconstructions of Allodapini ancestral states for the presence or absence of hierarchies differ depending on the state assigned to the ceratinine outgroup. Although division of labour has been reported for some *Ceratina* species (Michener, 1990; Maeta & Sakagami, 1995; Hogendoorn & Velthuis, 1999), most subgenera have not been studied. In the subgenus *Ceratinidia*, reproductive skew is modulated by reciprocal oophagy (Maeta & Sakagami, 1995), something which has never been reported for allodapines, and, in *C. japonica* Cockerell and *C. okinawana* Matsumura & Uchida, some females will produce an undersized daughter who helps in nest defence and maintenance (Sakagami & Maeta, 1989; Sakagami *et al.*, 1993). Similarly, in the subgenus *Neoceratina*, multi-female nests of *C. australensis* Perkins and *C. dentipes* Friese typically consist of one reproductive female that is larger in size than the remaining non-reproductive, with evidence of division of labour and alloparental care (S. Rehan, pers. comm.). Unpublished data on other ceratinine subgenera suggest that reproductive hierarchies could well be an ancestral trait for this tribe (S. Rehan, pers. comm.), in which case reproductive hierarchies could represent the ancestral state for the [Ceratinini + Allodapini], with multiple losses in Ceratinini and two losses in Allodapini.

Finally, our results have implications for the understanding of the origins of caste specialization. A key emerging issue concerns the expression of developmental pathways involved in ovarian development and extra-nidal tasks – first coined as an 'ovarian ground plan' (West-Eberhard, 1996: 293; reviewed by Page & Amdam, 2007). Although females in solitary bee or wasp species will oscillate in the expression of reproduction and foraging behaviour, worker behaviour could potentially result from the de-activation of developmental pathways controlling the former, but not the latter, behaviour. This would result in reproductive specialization in some individuals and brood-care behaviours in others. Alternatively, it is possible that the earliest stages in social evolution involved dominance hierarchies with subordinates waiting until death or senescence of the dominant female before undertaking either reproductive or foraging behaviours. This would not require queen–worker differentiation at the earliest ontogenetic stages, but it could present a platform for future elaboration of alloparental care, and ultimately true worker behaviour. For example, there might be selection on them to undertake some alloparental work, such as guarding, before they assume dominance.

The life history of *Allodapula* resembles the circumstances under which West-Eberhard (1996) suggested social behaviour could have originated – namely, very small colonies of close relatives. Yet, *Allodapula*

and other allodapine genera (*Braunsapis, Brevineura, Halterapis, Hasinamelissa*), together with some xylocopine bees and a handful of polistine wasps (reviewed in Hogendoorn & Velthuis, 1999 and West-Eberhard, 1996, respectively), represent an exception to genetic developmental pathways; in the latter example, developmental pathways appear to have been lost. In all these taxa, because reproductively dominant females carry out all or most foraging work, there can be no ontogenetic de-activation of queen–worker behavioural regimes.

Interestingly, studies of *Macrogalea* do not suggest hierarchical barriers or queues for reproduction, nor do they indicate a social system in which worker-like behaviour occurs prior to reproduction (Schwarz *et al.*, 2007). Subordinate foraging prior to reproductive activity is clearly apomorphic in the allodapines and is only known in select species: *Exoneura robusta* Cockerell (Schwarz & O'Keefe, 1991), *Exoneurella tridentata* Houston (Hurst, 2001) and *Hasinamelissa minuta* (Brooks & Pauly) (Schwarz *et al.*, 2005). Owing to the fact that sociality is plesiomorphic for the tribe, we cautiously note that *Macrogalea* reproductive strategies potentially cast doubt on the concept of monogyny as the omnipresent ancestral state for social Hymenoptera (see Hughes *et al.*, 2008).

The above considerations suggest that, although social behaviour is ubiquitous in allodapines, they are clearly quite different from most other social insects. It is unlikely that social origins involved the differential expression of reproduction and foraging developmental pathways amongst dominant and subordinate nestmates. Rather, the plesiomorphic state is more likely to be: (1) a simultaneous delaying of reproduction and foraging by subordinates; or (2) immediate reproduction followed by foraging to provision larvae with food. These patterns are not concordant with arguments that the earliest stages of insect sociality involved developmental expression of foraging behaviour prior to reproductive activity.

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