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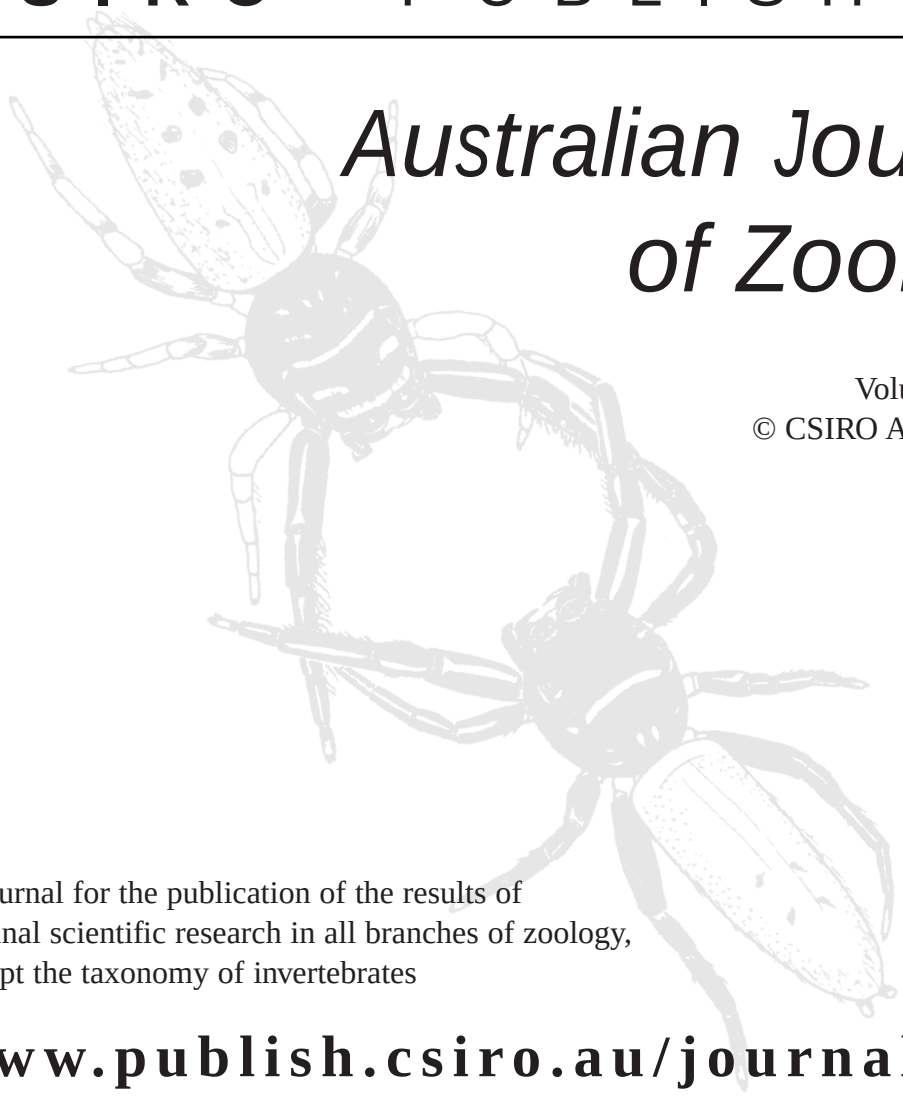

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Social Behaviour in an Australian Allodapine Bee *Exoneura* (*Brevineura*) *xanthoclypeata* (Hymenoptera : Apidae)

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Abstract

The allodapine bees are well suited for comparative studies of social evolution because of the wide variation in social behaviour within and between genera. There are three main clades in the endemic Australian genus *Exoneura*. Two groups (*Exoneura sensu stricto* and *Exoneurella*) have received extensive study. In this paper we provide the first detailed study of social behaviour in the third group, *Brevineura*, based on a heathland population of *Exoneura* (*B.) xanthoclypeata* Rayment. This species has two seasonal pulses of egg-laying and brood rearing occurs throughout most of the year, including winter. This extended period of egg-laying and brood development differs from the two other Australian *Exoneura* subgenera and provides extensive opportunities for eusocial-like sib-rearing. Dissection data indicate that reproductive differentiation among adult nestmates is well developed and dependent on body size, with smaller females being mostly or entirely non-reproductive. *Per capita* brood production is dramatically higher in multi-female nests than in single-female nests and relatedness between adult nestmates is moderately high ($r \approx 0{\cdot}5$). These two factors suggest that local fitness enhancement may be occurring and our limited sex allocation data suggest female-biased ratios. Because of the opportunities for sib-rearing in this species, local fitness enhancement has the potential to lower selective thresholds for eusociality.

Introduction

The allodapine bees have enormous potential for investigating the evolution of social behaviour because of the wide variation in social organisation within and between species and genera (Schwarz *et al*. 1997). Nevertheless, relatively few species have received detailed investigation. Five Australian species in two subgenera have been studied with sufficient depth to characterise levels of social behaviour. In the subgenus *Exoneura sensu stricto*, *E. bicolor* and *E. richardsoni* are characterised by high levels of social nesting involving semisocial, quasisocial and occasionally eusocial (*sensu* Batra 1966; Michener 1974; Wcislo 1997) organisation and are unusual among non-apine bees in exhibiting well-developed cofounding of new colonies by close genetic relatives (Schwarz 1994; Schwarz *et al*. 1996; Cronin and Schwarz 1997). In the subgenus *Exoneurella*, *E. lawsoni* and *E. setosa* are largely solitary (Michener 1962; Neville *et al*. 1997) whereas *E. tridentata* has very large colony sizes and appears to be highly eusocial (Houston 1977; Schwarz *et al*. 1994). No detailed studies have been made on species in the third subgenus, *Brevineura*. Michener (1977) suggested that *Brevineura* is a sister clade to *Exoneura* s.s., with *Exoneurella* being basal. Knowledge of sociality in *Brevineura* is therefore important for inferring patterns of social evolution in the *Exoneura* group.

Studies on montane populations of *E. bicolor* and *E. richardsoni* have shown that high levels of social nesting are associated with three principal factors: (i) substantial increases in *per capita* brood production with increasing colony size; (ii) re-utilisation of natal nests by succeeding generations; and (iii) highly aggregated and annually renewed nesting sites, a

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factor that enables most colonies to be cofounded by groups of closely related females. These factors suggest that cooperative nesting is associated with benefits accruing from cooperation among kin, at least at the colony level.

The existence of colony-level benefits to social nesting, combined with the maintenance of high levels of relatedness among nestmates, has consequences for the evolution of eusociality. In colonies with small clutch sizes, mean daughter fitness can become a function of sex allocation, leading to selection for female-biased ratios. This phenomenon has been termed local resource enhancement (LRE, Clarke 1978; Charnov 1982; Schwarz 1988) and, more recently, as local fitness enhancement (LFE, Schwarz 1994). Schwarz (1988, 1994) has argued that in haplodiploids, LFE-mediated sex ratio bias may increase relatedness between potential altruists and beneficiaries and that this may lower selective thresholds for sib-rearing. In haplodiploids, sisters are more closely related to each other ($r = 0{\cdot}75$) than they are to their own offspring ($r = 0{\cdot}5$). However, males are related to their sisters by only $r = 0{\cdot}25$ so that when sex ratios are unbiased, there is no net genetic gain to be realised by rearing siblings instead of direct offspring. However, if sex ratios are biased towards females, then the average relatedness of siblings to a female will increase above $r = 0{\cdot}5$ and therefore favour sib-rearing as a strategy. Because of the potentially facilitating effect of female-biased ratios on eusociality, it is important to investigate whether colony-level interactions leading to LFE are more widespread than is currently indicated.

In this paper we investigate social behaviour in a heathland population of the Australian allodapine bee *Exoneura* (*Brevineura) xanthoclypeata* Rayment. We describe the life cycle and social organisation of this species and compare this with other well-studied *Exoneura* species. We also consider intra-colony relatedness and *per capita* brood production as a function of colony size and suggest that LFE may also be expected in this species. Lastly, we discuss the implications of our results for understanding patterns of social evolution in the *Exoneura* group.

Materials and Methods

Study Site

Our study was based on nests collected from Cobboboonee State Forest and the eastern margin of the adjacent Lower Glenelg National Park, approximately 25 km north-west of Portland, south-west Victoria, Australia (38°50′S; 141°25′E). This area has been previously used to study sociality in a heathland population of *E. bicolor* (Schwarz 1994; Silberbauer and Schwarz 1995; Bull and Schwarz 1996). Vegetation in these areas is largely open heath forest, with an overstorey dominated by *Eucalyptus ovata*, interspersed with open heathy meadows and *Melaleuca* swamps. In this habitat, *E. xanthoclypeata* nests in the dead flower scapes of the grasstree *Xanthorrhoea minor* and in dead flower canes of the sedge *Gahnia sieberana*. These substrates contain pithy interiors that can be excavated to create a narrow burrow. *X. minor* is widely dispersed throughout most of the study site and *G. sieberana* occurs commonly around the edges of swamps and along low-lying roadsides.

Nest Collection Methods

Entire colonies of *E. xanthoclypeata* were collected in early morning, early evening or during rainy or cold weather when all nest occupants were assumed to be present. Nesting substrates were searched for the nest entrances of *E. xanthoclypeata*: in *X. minor* these comprise small round holes at the tops of broken scapes, and in *G. sieberana* these are elliptical exit holes created by stem-boring insects along the length of the cane. Entrances were blocked with cotton wool in the field and intact nesting substrates were detached from the plants and placed on ice until processed in the laboratory. Adults and brood were either preserved in Kahle's solution or frozen at –60°C for later electrophoresis. Nests were collected between 13 January 1994 and 16 January 1995 (Table 1).

Dissection Methods

Female adults from the February, May, June, October, December and January samples were dissected according to the techniques described by Schwarz (1986). Variables measured for adult females included

the length of the three largest terminal oocytes (summed to give a measure of ovary size), insemination status, and wing length (the distance from the axillary sclerites to the apex of the marginal cell), used as a measure of body size. Insemination status was determined by examining the spermathecae *in situ* or as temporary slide mounts in 70% ethanol. Sperm presence is indicated by spermathecal opacity; absence is indicated when the spermathecae are transparent. If *in situ* examination was ambiguous, spermathecae were detached and examined as slide mounts (Schwarz 1986).

Per Capita *Brood Production*

Two ways in which benefits of sociality in insects have been assessed are effects on colony survival and effects on *per capita* brood production (referred to hence as PCBP) and previously termed 'reproductivity per female' (RPF) by Michener (1964*a*), Schwarz (1988, 1994) and Wenzel and Pickering (1991). We measure PCBP as the number of brood being reared in a nest divided by the number of adult females and prefer this term to RPF which could be interpreted as being a measure of egg production only. Egg production may not indicate the reproductive output of a nest because of the possible effects of oophagy.

Relatedness Estimates

Allozyme electrophoresis was used to assess genetic relatedness between 49 adult females from 20 nests (ten 2-female; four 3-female; two 4-female; two 5-female) collected on the 11 October sample. Bees used for electrophoresis were frozen live in individual Eppendorf tubes and stored at –60°C until assay. Electrophoresis was carried out on cellulose acetate gels (Cellogel™) according to the techniques of Richardson *et al*. (1986). The following enzymes were assayed for the presence of polymorphism: aconitase hydratase (ACON, EC 4.2.1.3), acid phosphatase (ACP, EC 3.1.3.2), aminoacylase (ACYC, EC 3.5.1.14), alcohol dehydrogenase (ADH, EC 1.1.1.1), adenosine kinase (ADK, EC 2.7.1.20), fructose-bisphosphate aldolase (ALD, EC 4.1.2.13), arginine kinase (ARGK, EC 2.7.3.3), diaphorase (DIA, EC 1.6.99.), enolase (ENOL, EC 4.2.1.11), esterase (EST, EC 3.1.1.), fumarate hydratase (FUM, EC 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), guanine deaminase (GDA, EC 3.5.4.3), glutamate dehydrogenase (GDH, EC 1.4.1.3), lactoylglutathione lyase (GLO, EC 4.4.1.5), aspartate aminotransferase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glycerol-3-phosphate dehyrogenase (αGPD, EC 1.1.1.8), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), alanine aminotransferase (GPT, EC 2.6.1.2), glutathione reductase (GSR, EC 1.6.4.2), 3-hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), hexosaminidase (HEX, EC 3.2.1.30), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), cytosol aminopeptidase (LAP, EC 3.4.11.1), L-lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37), 'malic' enzyme (ME, EC 1.1.1.40), nucleoside-diphosphate kinase (NDPK, EC 2.7.4.6), dipeptidase (PEP-A, EC 3.4.13.), tripeptide aminopeptidase (PEP-B, EC 3.4.11.), dipeptidase (PEP-C, EC 3.4.13.), proline dipeptidase (PEP-D, EC 3.4.13.), phosphoglycerate mutase (PGAM, EC 5.4.2.1), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 5.4.2.2), pyruvate kinase (PK, EC 2.7.1.40), L-iditol dehydrogenase (SORDH, EC 1.1.1.14), and triose-phosphate isomerase (TPI, EC 5.3.1.1). The nomenclature for referring to loci and allozyme follows Adams *et al*. (1987). Relatedness was estimated from polymorphic loci by Queller and Goodnight's Relate 4.2 program (Queller and Goodnight 1989). This program is derived from Grafen's (1985) relatedness coefficient, which uses identity by descent rather than as a genetic regression.

Results

Life Cycle of E. xanthoclypeata

The phenology of colony development was investigated by examining the numbers of eggs, larvae (categorised as small, medium and large), pre-pupae, pupae and adults in nests collected in 12 samples taken between 9 February 1994 and 14 January 1995. Colony composition is summarised in Fig. 1 and Table 1. Seven major collection trips were selected as representative samples over an annual period (denoted by ●, Table 1). These trips were chosen on the basis of their spread throughout the year and sample size, remaining samples (denoted by ○, Table 1) are not graphed in order to reduce clutter.

Table 1. Nest collections and number of adult females per nest

Nests were sampled on 12 collection trips over an annual period. Seasons are abbreviated as follows: summer (Su), autumn (Au), winter (Wi) and spring (Sp). Trip status identifies the seven major representative samples discussed, which are denoted by ●; the remaining minor samples are denoted by ○. Females per nest shows the number of nests collected containing 1–7 adult females. Multi-female nests represents the percentage of nests containing at least 2 adult females for each collection trip

Collection trip	Trip status	Nests collected	Females per nest 1	2	3	4	5	6	7	Multi-female nests (%)
9.ii.1994 –Su	●	12	6	3	2	–	–	–	1	50·0
16.iii.1994 – Au	○	8	7	1	–	–	–	–	–	12·5
30.iii.1994 – Au	●	13	8	5	–	–	–	–	–	38·5
20.iv.1994 – Au	○	8	5	1	2	–	–	–	–	37·5
11.v.1994 –Au	●	15	10	3	1	–	–	–	–	33·3
11.vi.1994 –Wi	●	16	7	2	3	3	1	–	–	56·3
21.vii.1994 – Wi	○	26	20	2	1	2	–	1	–	23·1
24.viii.1994 –Wi	○	13	10	2	1	–	–	–	–	23·1
11.x.1994 –Sp	●	61	31	14	9	2	4	1	–	49·2
7.xii.1994 – Su	●	34	29	2	2	1	–	–	–	14·7
27.xii.1994 – Su	○	6	4	2	–	–	–	–	–	33·3
14.i.1995 – Su	●	25	12	6	3	4	–	–	–	52·0

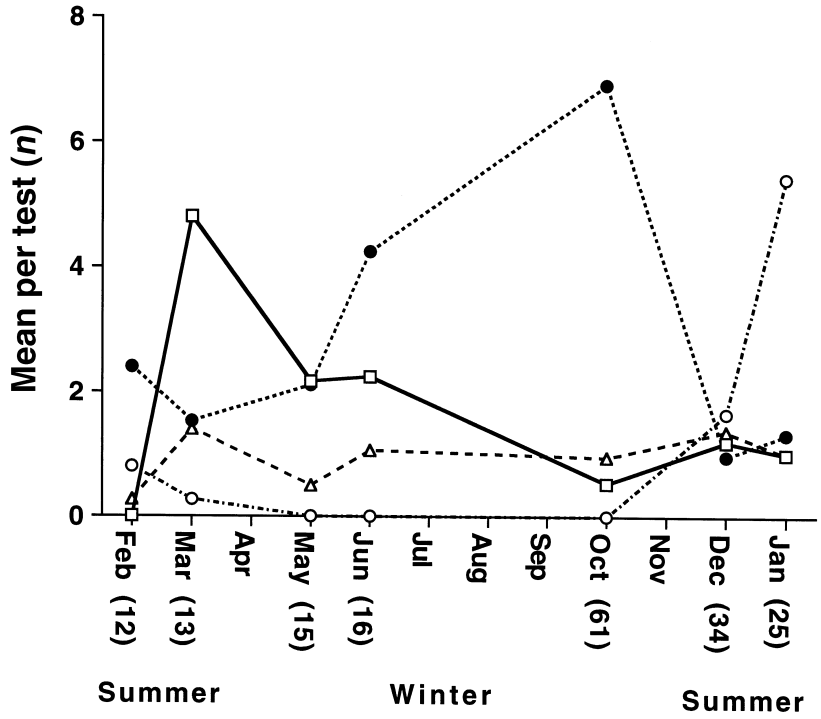

Fig. 1. Phenology of brood development, summarising data from the seven major collections periods between 9 February 1994 and 4 January 1995. Brood instars have been grouped into the following four categories: eggs (□); small and medium larvae – first- to early fourth-instar larvae (●); large larvae – mid-to-late fourth-instar larvae (△); and pre-pupae and pupae (○). Numbers in parentheses represent collection sample sizes.

The number of each major developmental stage for the seven main samples in our study are summarised in Fig. 1. Data obtained from the other minor samples are consistent with the trends outlined below. A major pulse of egg production commences in autumn and brood grow during late autumn and winter (Fig. 1), as indicated by the development of small and medium larvae. Larvae begin to pupate in spring and summer, and eclose as adults in summer and autumn. Egg production occurs during spring to early summer (Fig. 1), with a subsequent second pulse of small and medium larvae occurring from December to January (early to mid-summer). Pupae and callow adults were present in at least some nests throughout summer and autumn, and this coincides with the presence of eggs and feeding larvae. The extended period of brood production and the ability to rear brood throughout the entire year means that opportunities for sib-rearing by older, newly eclosed females may be frequent.

Numbers of adult females per nest for the sampling dates discussed above are summarised in Table 1. Solitary adult female nests were the most frequent nests collected on all sampling dates and the most common multi-female colony size was only two adult females per nest. Levels of cooperative nesting tend to be highest during early winter (June), mid-spring (October), mid-summer (January), and late summer (February). Levels of multi-female nests in late autumn and winter suggest that most or all recently eclosed females remain in their natal nests until spring or later. High levels of multi-female nest occupancy over winter and spring are followed by comparatively high proportions of solitary nesting in the 7 December sample. This could imply a dispersal period in late spring and early summer or a reduction of colony size due to adult mortality. Whilst trap nests set during our study were not occupied, trap nesting by Schwarz *et al*. (unpublished observations) indicate that all new nests of *E. xanthoclypeata* are solitarily founded.

The decrease in mean numbers of pre-pupal and pupal stages in February indicates adult eclosion of the first pulse of brood around mid-to-late summer (Fig. 1). Thus, pre-reproductive assemblages probably account for the increased proportion of multi-female nest in the January and February samples.

Opportunities for Eusociality

Nests of *E. xanthoclypeata* can contain small and medium larvae throughout the entire year, and eggs were found for every sample but February (Fig. 1). With seemingly continuous brood rearing in this species, we would expect frequent generational overlap with opportunities for newly eclosed callow females to assist in rearing their younger siblings. When collecting nests it is impossible identify the relationship between female nest mates (i.e. mother–daughter or sister–sister) without the aid of many highly polymorphic genetic markers, or unless some nestmates are newly eclosed callows. A situation where callows females reside with young feeding instars means that there is a definite opportunity for daughters to help rear their younger siblings, nests that contain newly eclosed callow females in the presence of larval instars therefore display an overlap of generations with an opportunity for cooperative brood care. The period where such sib-rearing opportunities can be mostly easily detected occurs from mid-summer to mid-autumn because of the frequency of both eggs and mature brood at this time. Colony compositions for nests that unambiguously indicate generational overlap are presented in Table 2, with the exception of the 7 December sample. Some fully pigmented adults in Table 2 may nevertheless be recently emerged, since the duration of callow coloration could be brief.

In the 9 February sample, 42% of the total nests collected contained callow females coexisting with feeding larvae or eggs. Whilst it is possible that some fully pigmented adults in this sample may be recently emerged it is unlikely that all eggs and larvae could have been produced by such females given the range of larval sizes. The early autumn (30 March) sample showed the greatest proportion of recognisably potential eusocial nests, making up 69% of all nests. This sample also displays the most eggs (Fig. 1) and the most callow

females of any collection date. In many of these nests it is very unlikely that the large number of eggs and larvae could be reared to pupation solely by the one or two fully pigmented females present at the time. In such cases, sib-rearing would be unavoidable unless many existing brood are destroyed or left to die. However, the number of small and medium larvae increase during winter and peak in spring (October) (Fig. 1), suggesting that brood existing in autumn nests are indeed reared over later time intervals.

Samples taken during winter and spring do not show any co-existence of callow adults and feeding brood, and no callow females were present in the 7 December sample. However, the presence of high proportions of large larvae, pre-pupae, and pupae together with early-instar brood (eggs and small larvae) in the December sample suggests that further development of these brood would have resulted in many opportunities for generational overlap and sib-rearing as colonies moved into summer. This is further demonstrated in the 20 January 1995 sample (Table 2), where nests contain newly eclosed callow females in the presence of dependent small larvae and eggs.

Social Organisation in E. xanthoclypeata

We investigated the possible existence of behavioural castes and reproductive differentiation among adult nest mates by looking for relationships among measurements of

Table 2. Opportunities for sib-rearing

Colony compositions for nests that exhibit an unambiguous overlap of generations. This situation presents the opportunity for sib-rearing and the potential for eusociality to occur. Percentages indicate the proportion of nests where sib-rearing could occur, relative to the total nests collected for that sample

Nest collection date	Adult females	Eggs	Small larvae	Medium larvae	Large larvae	Pre-pupae	Pupae	Callow females	All nests collected (%)
9.ii.1994	1	–	2	1	–	–	–	1	
9.ii.1994	1	–	2	–	–	–	–	2	
9.ii.1994	2	–	2	–	–	1		1	41·7
9.ii.1994	2	–	2	–	2	–	–	2	
9.ii.1994	3	–	2	1	2	–	1	1	
30.iii.1994	1	1	3	1	–	–	–	2	
30.iii.1994	1	2	2	–	1	–	–	2	
30.iii.1994	2	5	–	–	1	–	–	3	
30.iii.1994	2	6	–	–	2	–	–	1	
30.iii.1994	1	7	–	–	2	–	–	2	69·2
30.iii.1994	1	8	4	3	1	–	1	3	
30.iii.1994	2	8	1	2	5	–	2	2	
30.iii.1994	2	10	–	–	3	–	–	1	
30.iii.1994	2	16	2	3	5	–	1	3	
20.iv.1994	3	3	–	1	2	–	–	1	12·5
7.xii.1994	2	–	7	2	4	3	–	–	
7.xii.1994	2	5	–	5	5	4	–	–	
7.xii.1994	3	6	–	4	6	15	–	–	14·7
7.xii.1994	3	7	2	–	10	9	5	–	
7.xii.1994	4	9	2	–	14	9	4	–	
20.i.1995	3	3	1	–	1	–	9	1	
20.i.1995	2	6	–	–	2	1	12	2	8·0

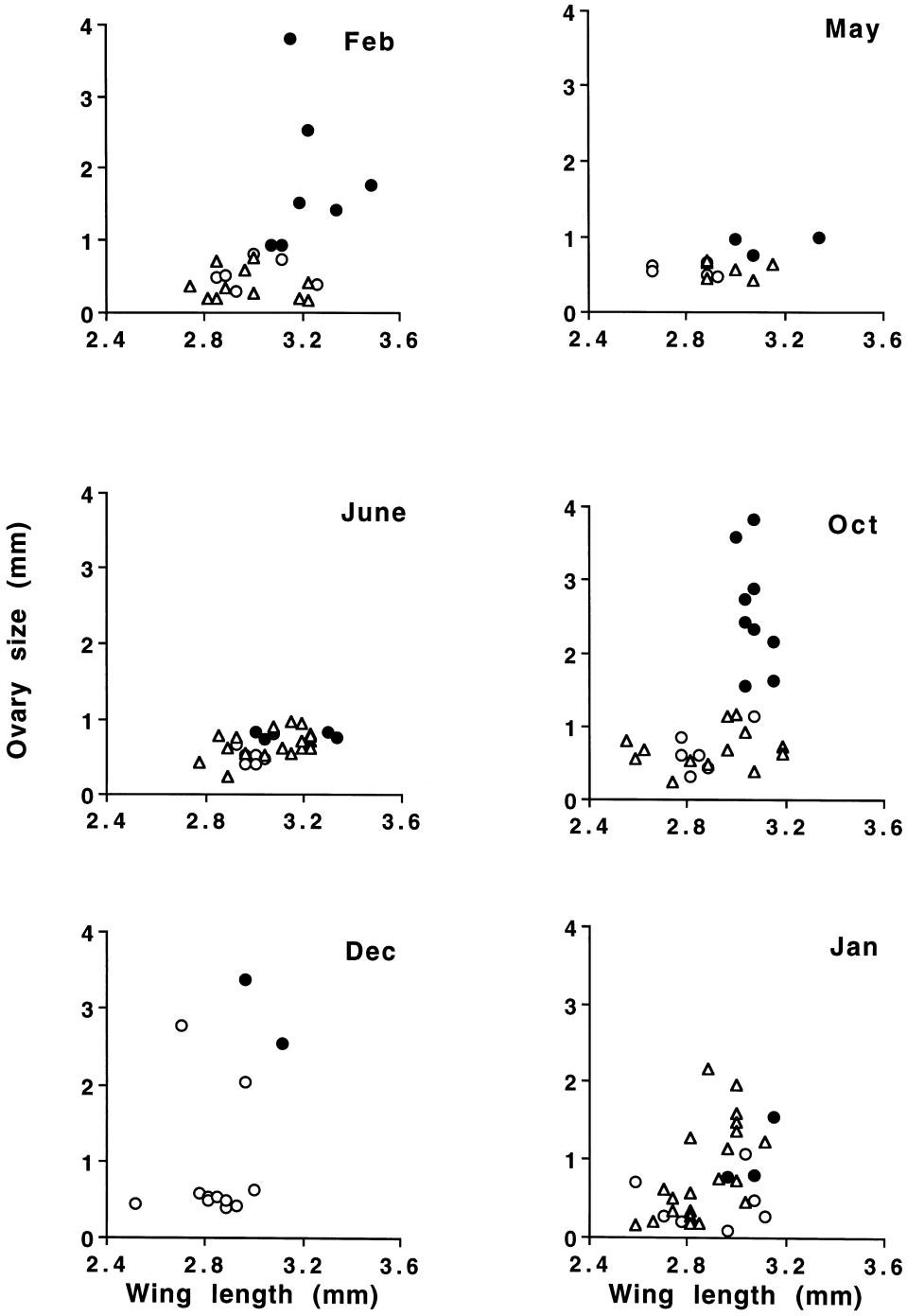

Fig. 2. Relationships between ovarian development, body size (as determined by wing length) and insemination status for the February, May, June, October, December and January samples. Insemination status was categorised as inseminated (●), uninseminated (○), and spermatheca not found (△).

body size, ovary size and insemination status in the sampling periods examined above (Fig. 2). Insemination status is frequently very difficult to determine in these very small bees, and this accounts for the high number of individual cases where insemination status was unknown. Spermathecae devoid of sperm are difficult to detect during dissection and it is likely that, where spermathecae could not be located, females were uninseminated.

The February sample indicates bimodality of ovary size involving small- and large-ovaried females (Fig. 2). We assessed whether ovary size was associated with insemination status by a 2-way ANCOVA, with nests and insemination status as crossed factors and wing length (used as a measure of body size) as a covariate. Because not all nests contained both inseminated and uninseminated females, we used experimental decomposition of sums of squares (Norusis/SPSS Inc., 1993). This ANCOVA showed no nest effects ($F_{6,12} = 2{\cdot}028$, $P = 0{\cdot}257$), a significant effect of insemination status ($F_{1,12} = 14{\cdot}540$, $P = 0{\cdot}019$) and a marginally non-significant covariate effect ($F_{1,12} = 5{\cdot}913$, $P = 0{\cdot}072$). The relationship between ovary size and insemination status is also apparent from data graphed in Fig. 2.

Brood phenology data (Fig. 1) indicate that whilst February nests did not contain any eggs, nests collected in March contained, on average, more eggs per nest than other samples. This suggests that ovary size data from the February sample of adult females indicate reproductive differentiation shortly prior to the main period of egg production. That is, there is strong reproductive differentiation in multi-female colonies during the major period of egg production and this differentiation is associated with insemination status.

Dissection data from the autumn and winter samples of May and June indicate that ovary sizes of all female nestmates were small. Our May collection contained only 8 females from multi-female nests and 2-way ANCOVA showed no effects of nest ($F_{4,7} = 2{\cdot}593$, $P = 0{\cdot}432$), insemination status ($F_{1,7} = 0{\cdot}013$, $P = 0{\cdot}928$) or wing length ($F_{1,7} = 29{\cdot}894$, $P = 0{\cdot}115$). Analysis of the June sample with experimental decomposition of sums of squares indicated no nest effect ($F_{5,10} = 1{\cdot}165$, $P = 0{\cdot}480$) but a significant effect of insemination status ($F_{1,10} = 19{\cdot}303$, $P = 0{\cdot}022$) and wing length ($F_{1,10} = 13{\cdot}960$, $P = 0{\cdot}033$), with inseminated females having larger ovaries and ovary size varying positively with wing length.

Our October dissection data are similar to that from February, in that there is a marked group of inseminated females with very large ovaries. These inseminated females have significantly larger body size than uninseminated females, again indicating reproductive differentiation based on body size. ANCOVA with experimental decomposition of sums of squares for October showed no nest effects ($F_{7,14} = 1{\cdot}106$, $P = 0{\cdot}472$), a significant effect of insemination status ($F_{1,14} = 7{\cdot}821$, $P = 0{\cdot}038$) and a highly significant effect of wing length ($F_{1,14} = 19{\cdot}111$, $P = 0{\cdot}007$).

As only two females were detected as inseminated in the December sample and recording of insemination status was not possible in the remaining females, no analysis could be performed on these data (although it is graphically summarised in Fig. 2). However, both inseminated females detected in our sample displayed large ovary size and body size, suggesting a similar, but perhaps less marked, trend to that of February and October.

The January sample shows an absence of females with very large ovaries, although some females had moderately large ovaries. This result again is consistent with brood phenology data (Fig. 1), indicating a near absence of eggs in late summer. There may be a positive relationship between ovary size and wing length, but only three of the females in our sample could be positively identified as inseminated. A 2-way ANCOVA displayed no significant effect of nest ($F_{6,9} = 0{\cdot}756$, $P = 0{\cdot}706$), insemination status ($F_{1,9} = 0{\cdot}899$, $P = 0{\cdot}517$) or wing length ($F_{1,9} = 1{\cdot}430$, $P = 0{\cdot}443$) on ovarian development.

Further evidence for two pulses of egg-laying is also apparent from the above data. Ovarian development and subsequent egg production is most prominent during two periods (Fig. 2): (i) late summer/early autumn (February) and (ii) mid-spring (October) to early summer (December).

Per Capita *Brood Production*

Mean (± s.e.) PCBP values were calculated for each colony size (measured as the number of adult females per nest) in each sample in our study. Scattergrams of PCBP ± s.e. against the number of adult females per nest are presented in Fig. 3 for seven samples collected during February, March, May, June, October, December and January. Other samples are not graphed because sample sizes were smaller and because they did not show trends different from the main samples. In all samples other than February, PCBP in two-female nests was greater than that in single-female nests, and in the May, June, October and December nests, *per capita* efficiency appeared to be greater in all multi-female colony sizes than that in single-female nests.

We statistically assessed the relationship between colony size and PCBP by ANCOVA with sampling date as a treatment and number of adult females as the covariate. PCBP was transformed as $\log_{10}$ (PCBP ± 1) to produce homogeneity of variances (Bartlett-Box F test and Cochrane's C test, $F = 1{\cdot}575$, $P = 0{\cdot}107$, and $C = 0{\cdot}147$, $P = 0{\cdot}362$, respectively). The ANCOVA showed a significant treatment effect ($F_{10,205} = 2{\cdot}39$, $P = 0{\cdot}011$), a significant covariate effect ($F_{1,205} = 26{\cdot}74$, $P < 0{\cdot}001$) and a significant interaction effect ($F_{10,205} = 2{\cdot}04$, $P = 0{\cdot}031$). Because of the interaction, and because the dependent variable was log transformed, it is difficult to quantitatively interpret the regression slope for the covariate. However, for most samples, PCBP increases with colony size (Fig. 3).

Two features of the PCBP analyses need to be pointed out here. First, although the number of nests with more than two females was comparatively small, examination of Fig. 3 suggests that increases of PCBP with colony size begin to saturate once colonies exceed more than two females per nest. Such saturation in studies of two other allodapine bees, *E. bicolor* and *E. richardsoni*, tends to occur at colony sizes that are larger than those for *E. xanthoclypeata* (Schwarz 1988, 1994; Schwarz *et al.* 1996). Second, in the June, October and December samples, PCBP in two-female nests was double or more that of one-female nests, with colonies containing more than two adult females also showing significantly larger *per capita* productivity than single-female nests. This increase in PCBP for two-female nests compared with single-female nests is much greater than increases for the other two allodapine species where this has been studied in detail (Schwarz 1988, 1994; Schwarz *et al.* 1996).

Intra-colony Relatedness

Seven of the presumptive loci assayed (*Gda*, *Idh*, *Me*, *Acyc*, *Est-3*, *Est-5* and *PepC*) showed banding variability consistent with Mendelian variation at single loci. Allele frequencies of these loci are summarised in Table 3. Of these loci, only three (*Gda*, *Idh* and *Me*) showed allele frequencies for the most common allele lower than 0·9 and these loci were regarded as being the most useful for relatedness estimation. Intra-colony relatedness between adult females was estimated by jackknifing individual colony estimates for all polymorphic loci and then for the three most heterozygous loci (Queller and Goodnight 1989) (Table 3). Two of the individual locus estimates (*Est-3* and *Est-5*) were clearly very different from estimates based on the other loci; both were esterases and both had most common allele frequencies greater than 0·95. The other individual-locus relatedness estimates ranged from 0·436 to 0·647.

Inbreeding was investigated by calculating Wright's F_{it} statistic for all loci and for the three most heterozygous loci (*Gda*, *Idh* and *Me*) with means and standard errors jackknifed over colonies. Neither estimate indicated inbreeding (F_{it} ± s.e. = –0·053 ± 0·062 and –0·092 ± 0·078 respectively). The slightly negative values of these estimates may be due to the inclusion of more than one individual from each family group (Blows and Schwarz 1991).

Sex Allocation

We were only able to obtain sex allocation data from 15 nests (containing a total of 31 pupae and pre-pupae) collected in the January (n = 10 nests), February (n = 2) and March

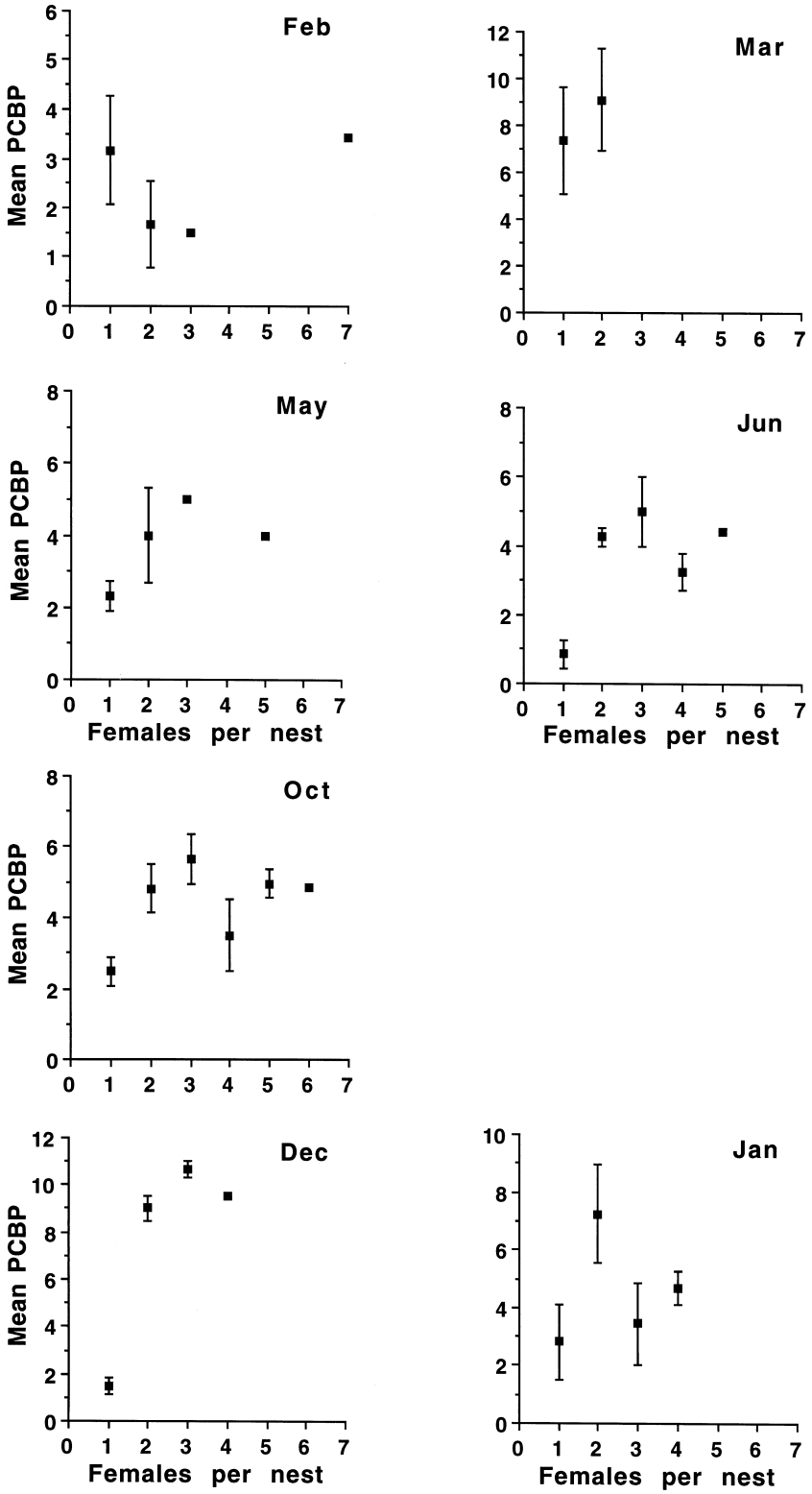

Fig. 3. Relationship between colony size and brood production for each colony (*per capita* brood production—PCBP) for the February, March, May, June, October, December and January samples.

Table 3. Allele frequencies and estimates of intra-colony relatedness between adult females
Standard errors were obtained by jackknifing over colonies. Two multi-locus estimates of relatedness were obtained, the first from all polymorphic loci and the second from all loci where the most common allele had a frequency of less than 0·9

Locus	Allele frequencies			Mean relatedness (r)	s.e. of r jackknifed over colonies	Colonies (n)	Individuals (N)
	a	b	c				
Gda	0·327	0·548	0·125	0·647	0·103	20	48
Idh	0·013	0·131	0·856	0·487	0·229	20	46
Me	0·235	0·765		0·436	0·153	20	48
Acyc	0·020	0·972	0·008	0·524	0·552	20	55
Est-3	0·981	0·019		–0·018	0·107	20	47
Est-5	0·013	0·025	0·962	–0·036	0·033	20	55
PepC	0·948	0·052		0·439	0·410		
	20	46					
All loci				0·491	0·061	20	55
Three most heterozygous loci (*Gda*, *Idh*, *Me*)				0·546	0·067	20	49

(n = 3) samples and, because of staggered brood development, we were not able to sex all brood within most of these nests. The small sample sizes and possibility of protogyny (females eclosing before males) or protandry (males eclosing before females) means that these data need to be treated very cautiously. Pooling data from all three samples, the mean pupal weight of males was 3·34 ± 0·26 (mean ± s.e.) and that of females was 6·08 ± 0·17. Only three of the pupae obtained were male. We calculated the sex ratio obtained for each nest and compared this with an unbiased numerical ratio (r = 0·5) by a one-sample t-test: the mean numerical ratio in our sample was significantly lower than 0·5 (t_{14} = –3·06, P = 0·009). Our wet weight and numerical data therefore suggest strongly female-biased investment ratios.

Discussion

Brood Development and Life Cycle

Our results indicate that eggs and larvae are present throughout most of the year, with a major pulse of egg production occurring in autumn and a second weaker pulse occurring in spring. Interestingly, we found evidence that some brood provisioning occurs during winter. This extended and largely continuous pattern of brood rearing contrasts with brood production in species of *Exoneura* s. s. where egg-laying does not commence until winter and brood development is synchronised within nests. This also contrasts with most species of *Exoneurella*, which do not lay eggs or rear brood during the overwintering period. Extended brood rearing in *E. xanthoclypeata* may be related to the nesting patterns of this bee, in that most nests were in areas that received high levels of insolation, perhaps allowing adults to warm up enough for flight activity on warm winter days.

In montane populations of *E.* (*Exoneura* s.s.*) bicolor* and *E.* (*E.* s.s.*) richardsoni*, opportunities for recently emerged females to rear siblings are rare because of a univoltine life cycle and the existence of only one pulse of brood development. In heathland populations of these two species, some sib-rearing can occur because large colonies with advanced brood development produce a smaller second clutch of eggs in mid-summer. However, in *E. xanthoclypeata*, egg-laying and brood development occurs over most of the year and overlap of generations is common in summer and autumn, leading to opportunities for sib-rearing by previously immature brood. In *E.* (*Exoneurella) eremophila*, *E.* (*E.) setosa*

and *E.* (*E.) lawsoni* brood rearing does not occur over winter but at least some nests in each species produce two more-or-less distinct broods per year, creating opportunities for older daughters to help rear their younger siblings (Michener 1964*b*; Neville *et al.* 1997; Schwarz, personal observations). Because *Exoneurella* is a basal clade to *Brevineura* and *Exoneura* s.s. (Michener 1977), it is likely that winter brood rearing in *Brevineura* and univoltinism in *Exoneura* s.s. are both derived traits.

Reproductive Differentiation

Our dissection data indicate that reproductive differentiation among female nestmates in multi-female colonies is well defined for all periods except mid-summer (when many brood are reaching maturity but when egg-laying is absent) and mid-winter, when there is another hiatus in egg-laying. During times of egg-production, ovarian differentiation among nestmates is very well developed and our analyses indicate that during these times most multi-female nests contain both large- and small-ovaried females. This in itself need not reflect differentiation of nestmates into reproductive females and worker-like females, since such a pattern could arise if recently emerged daughters remain temporarily in nests before dispersal. However, the data indicate that large ovary size is also strongly linked to large body size and insemination status during all periods when egg-laying was occurring. The findings of a strong relationship between PCBP and the number of adult females per nest when ovary sizes are small further suggest that adult nestmates cooperate in brood rearing, and therefore that multi-female colonies comprise brood-rearing units.

Per Capita *Brood Production*

Our data show a very well-developed positive relationship between PCBP and colony size for all periods other than mid-summer, when most brood in nests are at, or reaching, maturity. The greatest increase in PCBP occurs when comparing single-female nests with two-female nests. Our data also indicate that in some samples PCBP may be larger in two-female and three-female nests than in four-female nests, but analyses are hampered by small sample sizes. The increase in PCBP here is much higher than that recorded for *E. bicolor* and *E. richardsoni*. PCBP has been analysed for only one species in *Exoneurella* and was not found to increase with colony size (Neville *et al.* 1997).

Intra-colony Relatedness

The estimates of intra-colony relatedness presented here are similar to relatedness estimates for two other Australian species, *E. bicolor* and *E. richardsoni*. These estimates are consistent with a situation where multi-female colonies comprise a mixture of mother–daughter or sister–sister assemblages, with both possibilities being consistent with our life-cycle findings. The dramatic increases in PCBP coinciding with the presence of a second female, when combined with moderately high relatedness, suggest that indirect fitness benefits for worker-like females may be high. Maintenance of kin-group integrity within colonies over time, and the existence of colony-level benefits to cooperative nesting, creates the possibility for local fitness enhancement (LFE) to occur. As with *E. bicolor* (Schwarz 1994), this could select for female-biased sex allocation, at least in smaller colonies, via LFE. Given the opportunities for sib-rearing to occur in *E. xanthoclypeata*, sex allocation patterns need to be more carefully studied in this species. While our data suggest that investment ratios may be female biased, our samples are too small to assert that LFE causes female-bias in this species.

In summary, previous studies on two species in *Exoneura s.s.* have shown that LFE is able to select for strongly female-biased sex allocation, but that opportunities for sib-rearing are limited by voltinism and brood development patterns. Our data on *E. xanthoclypeata* show

that opportunities for sib-rearing are common, and that PCBP data, combined with high intra-colony relatedness, suggest that LFE-mediated female-bias should occur in this species. Future studies on sex allocation in this and related species are required because they may furnish strong empirical evidence that LFE can lower selective thresholds for eusociality. Finally, our data provide the first detailed observations on sociality and life cycle in a *Brevineura* species, allowing variation in some traits in the Australian *Exoneura* group to be mapped onto phylogenies. This will be developed in future phylogenetic studies. However, it is worth noting that brood development and the ways in which opportunities for sib-rearing arise in *Exoneura* (*Brevineura*) *xanthoclypeata* differ markedly from species in *Exoneura* s.s. and *Exoneurella*, suggesting that it will be possible to identify the origins of some key social traits in the allodapine bees.

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