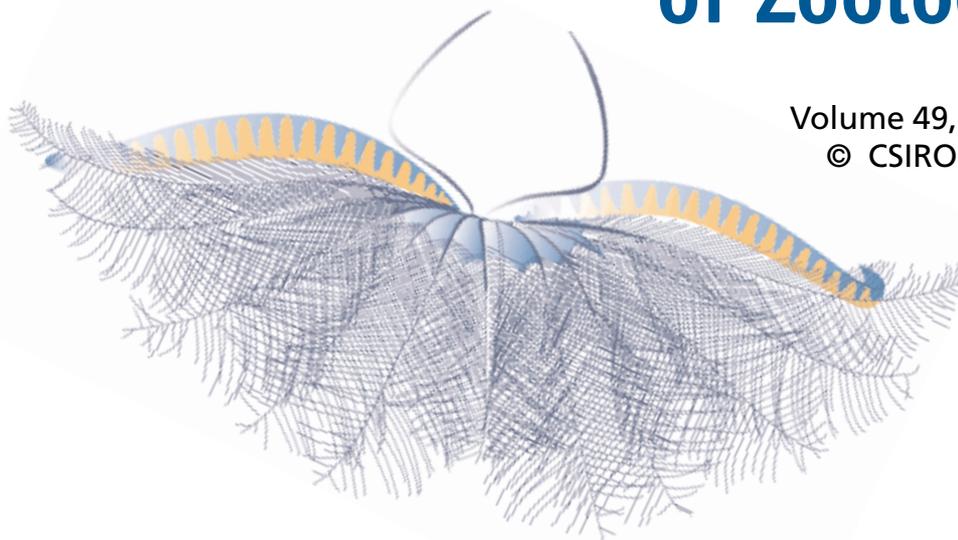


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## Latitudinal variation in the sociality of allodapine bees (Hymenoptera: Apidae): sex ratios, relatedness and reproductive differentiation

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### Abstract

Variation in sociality of two Australian allodapine bees was investigated over a latitudinal range of 10°, spanning subtropical to cool temperate forests in eastern Australia. *Exoneura robusta* is univoltine and singly brooded in southern populations but is able to produce two broods per season in northern populations, leading to opportunities for sib rearing by older brood members. In contrast, sympatric colonies of *E. angophorae* show no such variation with latitude, and all populations exhibit some colonies where opportunities for sib rearing arise. Patterns of ovarian differentiation, relatedness and sex allocation were examined over a one-year period for these two sympatric species in four populations. Within each species, the timing of ovarian development and the degree of reproductive skew were similar at all sites, but the onset of egg-laying and brood development was earlier in *E. angophorae* than in *E. robusta*. Relatedness and sex allocation patterns in northern populations of these species were not markedly different from southern populations. Selection for female-biased sex allocation and high reproductive skew in *Exoneura* probably stems from strong benefits from cooperative nesting, and our results suggest that opportunities for sib rearing (and eusociality) in these species may depend on latitudinally mediated brood development rates, and are unrelated to levels of sex bias and relatedness.

### Introduction

Recently, it has become clear that sociality in insects need not evolve toward a specific end point (e.g. eusociality), but may exhibit evolutionary reversals to more ‘primitive’ forms of social behaviour, or express different behavioural phenotypes in response to environmental conditions (review in Wcislo and Danforth 1997). There are now numerous examples of species that exhibit multiple forms of sociality, in the form of variation between or within populations (Sakagami and Munakata 1972; Packer 1990; Mueller 1996; Eickwort *et al.* 1996; Banschbach and Herbers 1996; Cronin and Schwarz 1999a, 1999b) or social variation over time (Yanega 1993; Richards and Packer 1995; Herbers and Banschbach 1999). Behaviourally polyphenic species provide a useful tool for assessing the selective pressures that influence the expression of social behaviour (West-Eberhard 1989; Banschbach and Herbers 1996), and may be important in its evolution. Whereas the role of genetic factors has received most attention in studies of social evolution, the potential importance of ecological and demographic factors has received less attention (Lin and Michener 1972; Evans 1977; Queller 1989; Gadagkar 1990, 1991; Keller and Reeve 1994; Schwarz *et al.* 1997; Wcislo 1997). An understanding of the relative importance of ecological, demographic and genetic factors in the expression of social behaviour is critical for comprehending the factors underlying the evolution of complex societies.

Allodapine bees are ideal for the study of factors affecting the expression of social behaviour as sociality varies both within and between species (Schwarz *et al.* 1988). The

Australian *Exoneura* group includes examples of mostly solitary species (Michener 1964; Neville *et al.* 1998), complex semisocial societies (*sensu* Michener 1974; Schwarz 1986; Melna and Schwarz 1993), and eusocial colonies exhibiting morphological castes (Houston 1977). Broods are progressively reared in undivided burrows in stems and branches, a trait that is likely to make them particularly susceptible to fluctuations in environmental variables such as predation, weather and availability of forage. Southern montane populations of *Exoneura robusta* (= *E. bicolor* prior to 1997, see Schwarz *et al.* 1998) have been the subjects of extensive study over the last decade. New nests are founded by up to 8 females and are quasisocial (*sensu* Michener 1974), whereas colonies in reused nests are semisocial (Schwarz 1986). Relatedness is high ( $r \approx 0.6$  in newly founded and  $r \approx 0.5$  in reused nests: Schwarz 1987), and sex ratios are female biased (Schwarz 1988a, 1994), two factors that could potentially facilitate selection for sib-directed altruism if constraints limiting colonies to a single brood were removed. Southern montane populations of *E. angophorae* (= *E. richardsoni* prior to 1997, see Schwarz *et al.* 1998) are usually singly brooded, but some colonies may also produce two broods in a season, leading to opportunities for older sibs to help rear young immatures (Schwarz *et al.* 1996; Cronin and Schwarz 1997). Hence, colonies may be semisocial, quasisocial or eusocial (Cronin and Schwarz 1997). Intra-colony relatedness is generally high ( $r \approx 0.75$  in newly founded nests, and  $r \approx 0.5$  in re-used nests: Schwarz *et al.* 1996), and sex ratios are female biased (Cronin and Schwarz 1997).

Recent studies on geographic variation in the lifecycle of *Exoneura robusta* suggest that variation in climate and habitat between geographically separated populations leads to intraspecific social variation in this species (Cronin and Schwarz 1999a, 1999b). Southern montane populations are limited to a single brood per season (Schwarz 1986), and can be either semisocial or quasisocial, whereas a proportion of colonies in northern montane and southern heathland populations of *E. robusta* are able to produce a second brood, giving rise to opportunities for sib rearing and eusociality. In contrast, opportunities for sib-rearing are common in *E. angophorae* throughout its range (Cronin and Schwarz 1999b). Sib-rearing behaviour has been shown to occur in orphaned nests of several Australian allopapine species, including *E. nigrescens* (Bull and Schwarz 1997), *E. robusta* (Flavel 1999), *E. angophorae* (Schwarz *et al.* 1996) and *E. setosa* (Neville *et al.* 1998). We examined sex allocation ratios, relatedness and reproductive differentiation in four populations of sympatric *E. robusta* and *E. angophorae* along a latitudinal gradient. The interaction between these factors and the observed intraspecific social variation between populations is investigated.

## Materials and Methods

### *Study sites and collections*

Four sympatric populations of *Exoneura robusta* and *E. angophorae* were studied along the eastern coast of Australia from April 1995 to February 1997. The sites used were Mooball State Forest Park (28°25'S, 153°35'E), Washpool National Park (29°30'S, 152°20'E), Kioloa State Forest Park (35°30'S, 150°20'E) and Gembrook National Park (37°55'S, 145°40'E). For details of climates at these localities, see Cronin and Schwarz (1999b). Sampling was carried out at bimonthly intervals: 9–12 April 1995, 9–12 June 1995, 17–21 August 1995, 22–25 October 1995, 15–19 December 1995 and February 15–19 1996. Additional samples were taken on 12–20 December 1996 and 17–24 February 1997 from northern sites (Mooball and Washpool) to examine intra-colony relatedness, as comprehensive data already exist for southern populations (Schwarz 1987; Schwarz *et al.* 1996; Cronin and Schwarz 1997). Sites were located in montane forests where bees nested predominantly in dead fronds of the tree fern *Cyathea australis*. Collections were made at dawn or during inclement weather when bees were inactive and it could be assumed that all

occupants were within the nest. Intact nests were collected by plugging nest entrances with cotton wool and removing whole fronds from the field. Samples were kept at  $\approx 8^{\circ}\text{C}$  in insulated boxes until nests were opened and contents recorded. Adults were placed into Kahle's solution prior to dissection, or frozen at  $-70^{\circ}\text{C}$  for subsequent electrophoresis. Wet pupal weights were measured for all pupae recovered from the December 1996 collection from Washpool to examine sex investment ratios in this northern population.

#### *Trap nests*

Trap nests were used to assess founding behaviour. Setting of traps used the methods of Schwarz (1986) and consisted of applying spots of paint to dead fern fronds that did not contain nests and could be collected on subsequent trips when occupied. In this manner, when nests were occupied, it was certain that nests were newly founded and not re-used from the previous season.

#### *Determining nest age*

New nests of *E. robusta* and *E. angophorae* were difficult to discern from re-used nests as new nests were often cofounded by more than one female. Moreover, newly eclosed females were often indistinguishable from overwintered females soon after eclosion, as differences in pigmentation soon disappeared and some older females may show few signs of aging such as wing wear. Nest age could be determined to some extent by the colouring of the nest lumen, as nests from the previous season were stained by food and faecal deposition, but colouration varied with location and season and was not always a reliable guide. It was therefore possible to be sure of nest age only when data were from trap nests, with the age of other nests always the subject of some doubt. Nests in the latter category were termed 'nests of unknown age' and most likely included both newly founded and re-used nests.

#### *Electrophoresis*

Adult bees were frozen at  $-70^{\circ}\text{C}$  until used for electrophoresis. Bees were homogenised in 0.1% mercaptoethanol for allozyme electrophoresis. In total, 269 females from 81 nests were assayed from Mooball and Washpool. Bees were assayed for esterase (*Est*), dipeptidase (*PepA*) and proline dipeptidase (*PepD*) on horizontal starch gels using RSL discontinuous buffer system (Ridgway *et al.* 1970). Staining techniques are described in Richardson *et al.* (1987). Relatedness was estimated using the Relate 4.2 program of Queller and Goodnight (1989).

#### *Dissection techniques*

Females were dissected to assess ovarian development and insemination status following the methods of Schwarz (1986). Wing length, ovary size (mean length of the three largest oocytes), insemination status and presence of internal parasites were recorded.

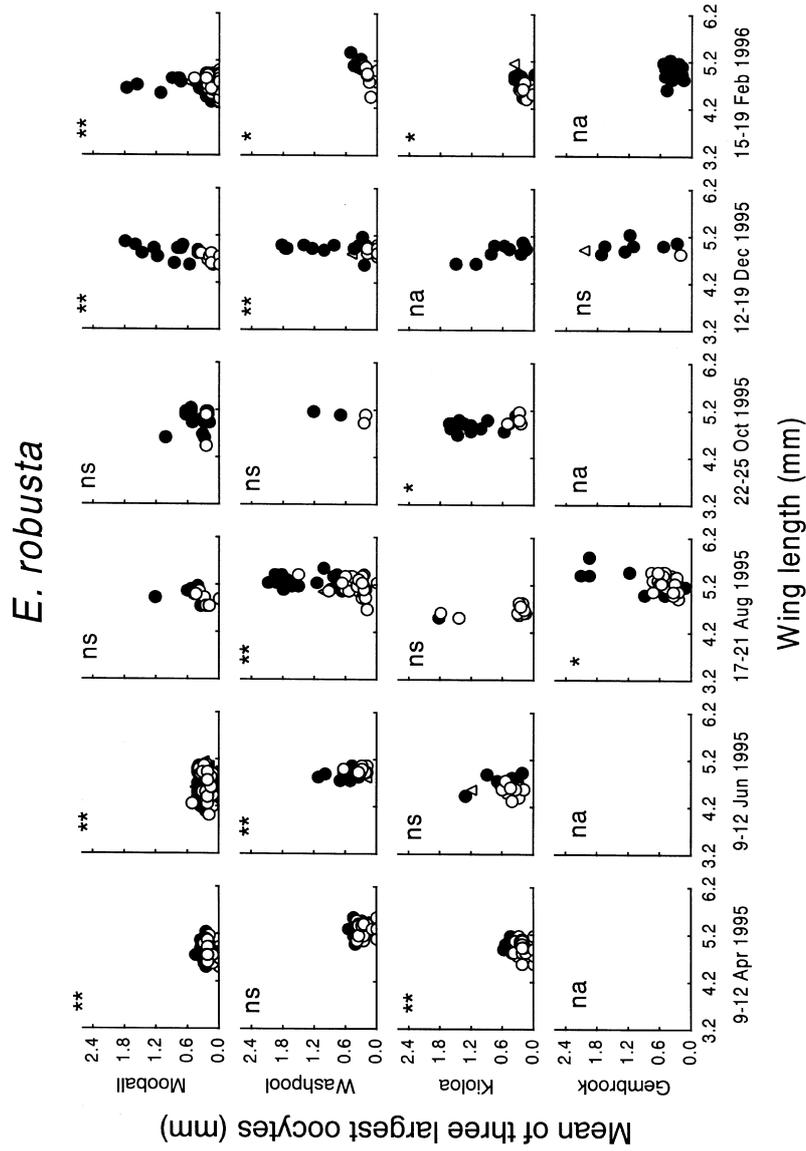
## **Results**

### *Reproductive differentiation in re-used nests*

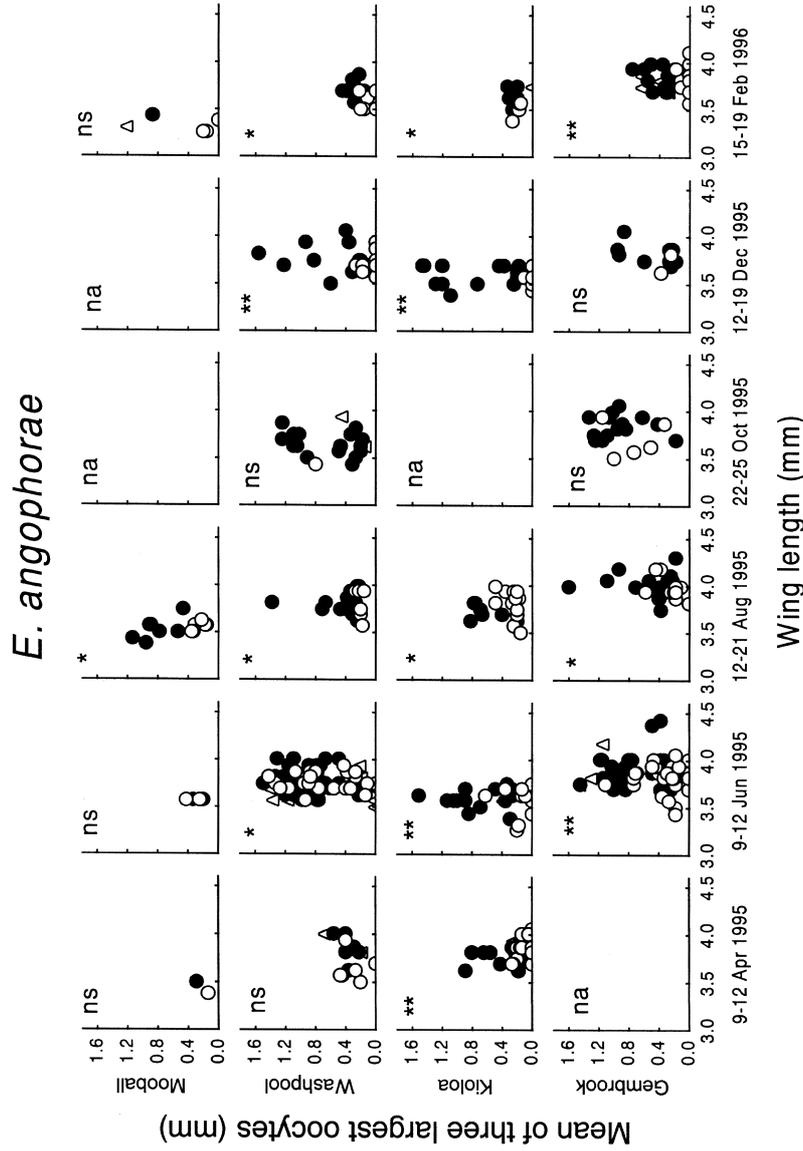
Reproductive differentiation was investigated by examining relationships between wing length (used as a measure of body size: Schwarz 1986), insemination status and ovary size in multi-female nests. These data are summarised for all sites and sampling dates in Fig. 1 for *E. robusta* and Fig. 2 for *E. angophorae*. It was not always possible to determine insemination status, and females for which insemination status could not be determined were excluded from statistical analyses but are included in Figs 1 and 2. The relationship between insemination status and ovary size was examined using Mann–Whitney *U*-tests, comparing ovary sizes between inseminated and uninseminated females. Because repeated tests were undertaken (i.e. repeated samples from each site) a sequential Bonferroni adjustment of  $\alpha$  levels (Rice 1989) was employed. The results of these tests are indicated in Figs 1 and 2. Specific results are discussed in the following sections.

### *Exoneura robusta*

Ovarian development began in June (Fig. 1), as evidenced by the presence of a few females at Kioloa and Washpool that exhibited ovary sizes exceeding  $\approx 0.6$  mm. Most females had



**Fig. 1.** Scattergram showing mean ovary size (in millimetres) versus wing length (in millimetres) of *E. robusta* for all sites throughout a 1-year period. Localities are shown from most southerly at the bottom to most northerly at the top, and collection date runs from left to right. Insemination status is indicated by open circles (uniseminated), closed circles (inseminated) or unknown insemination status (open triangles). Significance of Mann-Whitney *U*-tests for relationship between insemination status and ovary size is indicated by \* (significant to the 5% level after Bonferroni adjustment of  $\alpha$  levels), ns (not significant) or na (no analysis could be performed).



**Fig. 2.** Scattergram showing mean ovary size (in millimetres) versus winglength (in millimetres) of *E. angophorae* for all sites throughout a 1-year period. Localities are shown from most southerly at the bottom to most northerly at the top, and collection date runs from left to right. Insemination status is indicated by open circles (uninseminated), closed circles (inseminated) or unknown insemination status (open triangles). Significance of Mann-Whitney *U*-tests for relationship between insemination status and ovary size is indicated by \* (significant to the 5% level after Bonferroni adjustment of  $\alpha$  levels), ns (not significant) or na (test could not be done).

ovary sizes  $<0.6$  mm at this time. Ovarian development at Mooball had not begun at this time, and no data were obtained from Gembrook.

By August, the ovaries of some females had reached the maximum size for the entire season (mean of three largest oocytes  $\approx 2.3$  mm), indicating that egg laying had begun, or was about to begin, consistent with lifecycle data (Cronin and Schwarz 1999b). This remained the case in October, though there was a greater proportion of females with enlarged ovaries at Kioloa. Data from all sites suggest that ovary sizes were divided roughly into two groups: one being a small proportion of individuals that possessed moderately or well developed ovaries ( $>0.6$  mm), and the other, comprising most individuals, that exhibited minimal ovarian development ( $<0.6$  mm). Ovary sizes in August at Mooball resembled that of Kioloa and Washpool from the previous sample, suggesting that ovarian development was delayed at the most northerly site. December and February data suggest that new adult eclosion was taking place at some sites, as evidenced by the increased presence of uninseminated females with extremely small ovaries (at Mooball in December and also at Kioloa and Washpool in February), which concurs with lifecycle data (Cronin and Schwarz 1999b). Many females still possessed very large ovaries during December, but only Mooball females retained enlarged ovaries in February.

Insemination rates at the start of the year are  $\approx 50\%$  in the northernmost sites, and  $\approx 25\%$  at Kioloa, and increase markedly during October (Fig. 1), coinciding with dispersal (Cronin and Schwarz 1999b). A decrease in insemination rates in December (at Mooball) and February (at Washpool and Kioloa) coincides with brood eclosing into adults (Cronin and Schwarz 1999b). However, all females at Gembrook were inseminated, indicating that brood emergence was yet to occur. Inseminated females showed significantly larger ovaries than uninseminated females at Kioloa and Mooball regularly during the season at all sites (Fig. 1).

#### *Exoneura angophorae*

In contrast to *E. robusta*, where most ovarian development occurred between June and August, some females of *E. angophorae* had enlarged ovaries ( $>0.5$  mm – *E. angophorae* is considerably smaller than *E. robusta*) in April. By the second sample in June, many individuals had achieved the maximum ovary size ( $\approx 1.5$  mm), suggesting that egg laying had begun, and lifecycle data show that eggs were present in nests at this time (Cronin and Schwarz 1999b). August, October and December samples indicate that many females retain well-developed ovaries ( $>0.5$  mm) throughout winter and spring (Fig. 2) at Washpool, Kioloa and Gembrook (data are lacking for Mooball). In contrast to *E. robusta*, ovary sizes form a continuum, rather than two distinct groups, and there is a greater proportion of females with enlarged ovaries. During December, there was an increase in the number of uninseminated females with extremely small ovaries at Kioloa and Washpool, signifying brood eclosing into adults. Similar to *E. robusta*, ovary sizes remained large until February, when they resembled overwintering levels.

Insemination rates in April were comparable to those of *E. robusta* (Fig. 2). Once again, inseminated females often exhibited significantly larger ovaries, though there were larger numbers of uninseminated females possessing enlarged ovaries than in *E. robusta*. As for *E. robusta*, data indicate an increase in the proportion of inseminated females in October, and a drop in overall insemination rates in December or February at sites for which data were available. This coincides with dispersal and adult eclosion of brood respectively (Cronin and Schwarz 1999b).

These data indicate that there is a high degree of ovarian differentiation in *E. robusta*, with a minority of females, usually inseminated, possessing enlarged ovaries. In *E. angophorae*, the degree of reproductive skew is apparently less, though inseminated females usually possess larger ovaries. Moreover, the degree of ovarian differentiation in both species does not differ markedly between populations.

#### *Reproductive differentiation in new nests*

Data are limited for newly founded nests, and do not permit similar analyses to those performed on reused nest data. Hence, reproductive differentiation in new nests was investigated as follows: females were grouped into categories on the basis of ovarian development, with ovaries grouped into 6 even classes (between 0 and the maximum ovary size for an individual of that species; 0–2.12 mm for *E. robusta* and 0–1.59 mm for *E. angophorae*) to create an ovarian index based on the mean length of the three largest oocytes (1 being the smallest and 6 being the largest ovaries). These data are summarised in Table 1, which includes data on insemination and brood present in the nest. Results indicate that insemination rates in newly founded nests are high, with 84% of *E. robusta* foundresses and 100% of *E. angophorae* foundresses inseminated. Ovarian index data suggest that there is a varying degree of ovarian differentiation between colonies. That is, in some nests all females exhibit some ovarian development (index values >2), whereas in others there is distinct ovarian differentiation between cofoundresses (i.e. females with large ovaries and females with small ovaries in the same nest). This pattern can arise from two potential sources: (i) there is reproductive skew in these nests, such that some females are reproductively subordinate and do not develop large ovaries, or (ii) there is no reproductive skew, but there is some differential in timing of egg laying between females, such that some females have laid eggs, leaving only small ovarioles. There are a few trends to indicate that the latter of these is likely. Firstly, ovaries are generally smaller in nests that also have more advanced brood development; as would be expected in colonies where egg laying has ceased. For example, compare ovary sizes in *E. robusta* nests from Mooball (where brood are relatively advanced) with those at Washpool (where brood are mainly eggs). This suggests that small ovaries may result from completion of egg laying. Secondly, colonies where some females have very small ovaries (index = 1), generally have well developed broods (e.g. Mooball nests 140–147), such that one female may have completed laying eggs before the other(s).

#### *Sex investment ratios in northern populations*

Previous studies have reported sex allocation ratios of southern montane populations of *E. robusta* and *E. angophorae* (Schwarz 1987; Cronin and Schwarz 1997). Here, we examined the sex allocation ratios of a northern population of these species, using data from the December 1996 collection at Washpool (insufficient data were obtained to do similar analyses for all sites). The relationship between investment ratios and total brood size was investigated as follows: sex ratio data were grouped into five brood size categories based on all broods in the nest, these being: 1, 2–3, 4–5, 6–10, 11–15 and 16+ broods per nest (as per Cronin and Schwarz 1997). Mean investment ratios were then calculated for each brood size-group as {summed wet weight of male pupae / summed wet weight of all pupae}. These data were graphed against brood size categories for both species and are shown in Fig. 3, which indicates a high female bias for all brood size categories. Unfortunately, it was not possible to statistically test for the effects of nest age due to small sample sizes, but Fig. 3 indicates that trends are similar for all nest types. In *E. robusta*, bias is variable in the

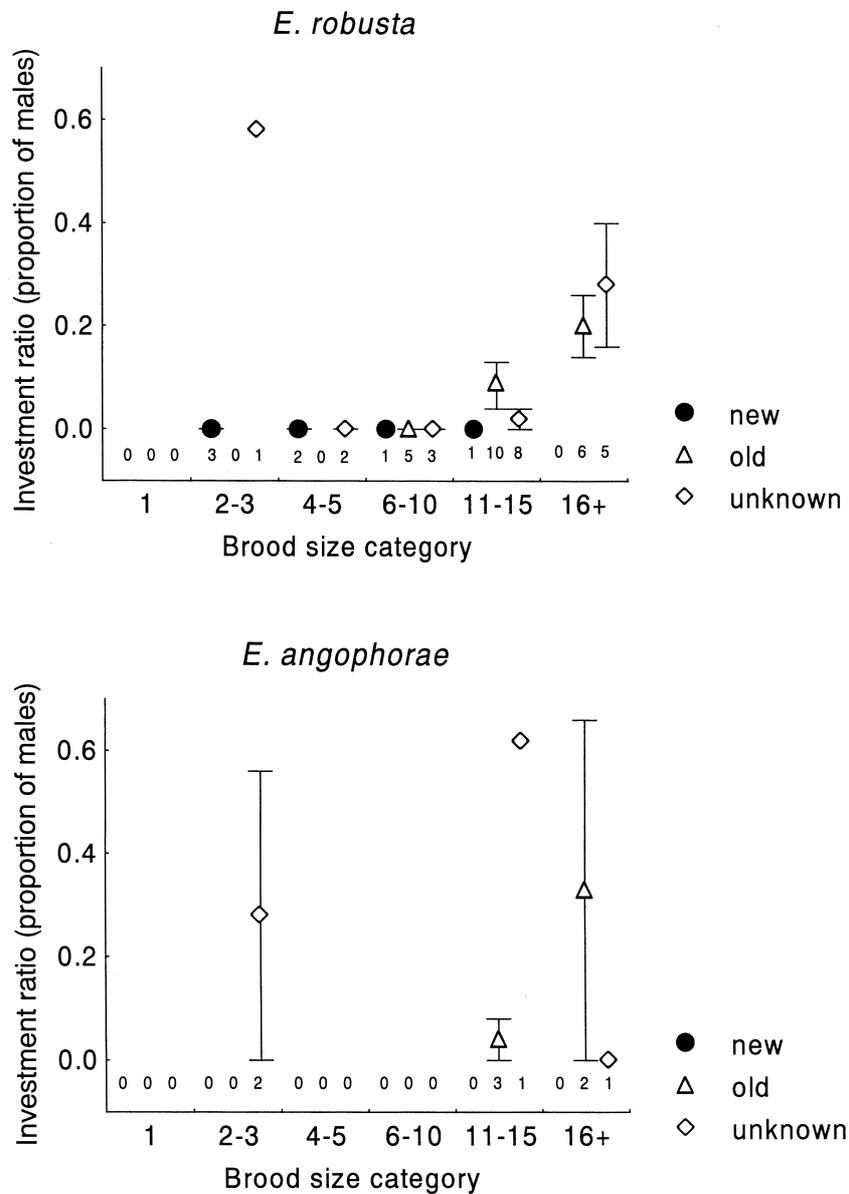
**Table 1. Summarised dissection data from newly founded nests of *E. robusta* and *E. angophorae***

'Nest ID' is the nest identification number and 'Col. size' is the number of adult females present in the nest. 'Ovindex/insem' indicates the ovarian index value (see text) and insemination status (y = inseminated, n = uninseminated) for each female in the nest. 'Brood data' indicates the brood present in the nest (e = eggs, s = small larvae, m = medium larvae, l = large larvae, pp = prepupae, p = pupae; after Cronin and Schwarz 1999a)

Species	Location	Date	Nest ID	Col. size	Ovindex/insem	Brood data	
<i>E. robusta</i>	Mooball	Aug. 1995	130	2	2n, 1n	0	
			131	4	3y, 1n, 1n, 1n	0	
			140	2	3y, 1y	3m, 2l, 1pp, 2p	
		Oct. 1995	141	3	3y, 1y, 1y	3m, 3l, 2pp	
			142	3	2y, 1y, 1y	7m, 1l, 2pp	
			143	6	3y, 1y, 1y, 1y, 2s, 1m, 4l, 12pp	1y, 1y	
			145	2	3y, 1y	1l, 4pp, 3p	
			146	4	3y, 2y, 1y, 1y	2m, 9l, 2pp	
			147	3	3y, 2y, 1y	1s, 2m, 3l	
			148	4	4y, 4y, 4y, 3n	11e	
			Dec. 1995	73	3	2y, 1n, 1n	3m, 3l
				Washpool	189	2	2y, 1n
			195		2	5y, 2y	16e
		196	4		5y, 4y, 4y, 4y	9e	
		197	4		6y, 5y, 4y, 3y	11e	
	199	3	6y, 5y, 4y		7e		
	200	2	5y, 4y		5e		
	201	2	4y, 4y		4e		
	202	2	5y, 4y		6e		
	Dec. 1995	204	3	4y, 4y, 3y	9e		
		16	3	3y, 1y, 1y	1m, 3pp, 4p		
		17	3	5y, 1y, 1y	1m, 3l, 3pp, 1p		
		19	2	2y, 2y	2s, 2m		
108		2	4y, 3y	8e			
<i>E. angophorae</i>	Washpool	Oct. 1995	187	2	2y, 1y	4s, 4m, 4l, 2pp	
			203	2	2y, 2y	9e	
			205	2	2y, 2y	4e, 3s	
			209	2	2y, 2y	2e	
	Kioloa	Oct. 1995	268	2	2y, 2y	8e	
	Gembrook	Oct. 1995	271	2	2y, 2y	0	
		Dec. 1995	109	2	2y, 2y	6e, 2s	

smallest colonies, strongly female biased in medium-sized broods, and increasingly less female biased in the largest broods. In *E. angophorae*, sample size was small, and there are no trends to indicate a decrease in investment in females with increasing brood size. The potential for female bias to decrease with increasing brood size was investigated statistically using Spearman's correlation coefficient between investment ratio and brood size, with each individual nest as a single datum point. These tests indicated a highly significant relationship for *E. robusta* ( $r_s = 0.449$ ,  $n = 47$ ,  $P = 0.002$ ), but no such relationship for *E. angophorae* ( $r_s = 0.045$ ,  $n = 10$ ,  $P = 0.902$ ).

Estimates of sex ratios may be affected by protogyny (earlier eclosion of females) or protandry (earlier eclosion of males), as it was not possible to determine the sex of larvae



**Fig. 3.** Relationship between sex ratios and brood size for *E. robusta* (above) and *E. angophorae* (below). The proportion of investment in males is shown against brood sizes, grouped into 1–3, 4–6, 7–9, 10–15, and 16+ broods per nest. New nests are indicated by filled circles, old nests by open triangles and nests of unknown-age by open diamonds. Figures below each point indicate the number of colonies.

present in the nests. To test for protogyny and protandry, we performed *t*-tests on numerical sex ratios between December 1996 and February 1997 collections (there were no investment ratio data from the February collection). Analyses revealed no significant difference in numerical sex ratios between the two samples for either species ( $t_{66} = -0.47$ ,  $P = 0.640$  for *E. robusta*;  $t_{16} = 0.25$ ,  $P = 0.803$  for *E. angophorae*). However, other studies

of *E. robusta* (Schwarz 1994) and *E. nigrescens* (Bull, unpublished) have found evidence for protogyny in these species. If data here are misleading, and protogyny does occur, then there may be some overestimation of the degree of female bias in some colonies. However, as larger nests are more likely to contain more unsexed brood (and hence more concealed males) protogyny is likely to result in an overestimation of female bias in larger nests. Hence, the difference in sex investment ratios between large and small colonies may be larger than the data suggest.

Because opportunities for sib rearing arise at the colony, rather than population, level, the relationship between sex allocation and opportunities for sib rearing was investigated to determine whether female bias was greatest where opportunities to rear sibs were more frequent. A *t*-test was used to test for differences in sex allocation ratios between colonies that did or did not exhibit opportunities for sib rearing. These tests indicated no significant difference in sex allocation ratios between these groups for either species ( $t_{45} = -0.9$ ,  $P = 0.38$  for *E. robusta*;  $t_7 = 0.64$ ,  $P = 0.55$  for *E. angophorae*).

#### *Estimates of relatedness in northern populations*

*E. robusta* exhibited 2 polymorphic, monomeric esterase loci, and dimeric loci assayed as *PepA* and *PepD*. Only a single esterase locus could be scored reliably in *E. angophorae* in addition to *PepA* and *PepD*. Males were excluded from analyses. The allele frequencies of loci used are shown in Table 2. *PepA* exhibited allele frequencies of the most common allele greater than 0.9 in *E. angophorae* and hence was not considered useful for estimates of relatedness and excluded from analyses. Intracolony relatedness between adult females was estimated by jackknifing individual colony estimates over all loci. Estimates of relatedness are shown in Table 3; relatedness estimates from previous studies of southern *Exoneura* (Schwarz 1987; Schwarz *et al.* 1996) are included for comparison. Relatedness estimates used data from December samples of *E. robusta* from Washpool and Mooball, and *E. angophorae* from Washpool, and indicated a high level of kin association for both species. These estimates were then compared against values of  $r = 0$  and  $r = 0.75$  using single-sample *t*-tests. The results of these tests are summarised in Table 3 and indicate that,

**Table 2. Frequencies of alleles assayed for in electrophoretic analyses for *E. robusta* and *E. angophorae***

Samples are from December 1996. Est 1 = esterase (first locus), Est 2 = esterase (second locus), PepA = dipeptidase, PepD = proline dipeptidase; only a single esterase locus could be scored for *E. angophorae*. Allele frequencies for each species are indicated by a–d for esterases (monomeric) and a and b for peptidases (dimeric)

Species	Location	Locus	a	b	c	d
<i>E. robusta</i>	Mooball	Est 1	0.354	0.371	0.275	0.000
		Est 2	0.049	0.546	0.311	0.093
		PepA	0.893	0.107	–	–
		PepD	0.518	0.482	–	–
	Washpool	Est 1	0.353	0.509	0.138	0.000
		Est 2	0.017	0.519	0.459	0.005
		PepA	0.819	0.181	–	–
		PepD	0.423	0.577	–	–
<i>E. angophorae</i>	Washpool	Est	0.000	0.641	0.359	0.000
		PepA	1.000	0.000	–	–
		PepD	0.712	0.288	–	–

**Table 3. Relatedness estimates and jackknife values (over colonies) between adult nest mates, for *E. robusta* from Mooball, and for *E. robusta* and *E. angophorae* from Washpool**

Data presented here are from December 1996; those from previous studies are for re-used nests. Details are also given of *t*-test comparisons between relatedness estimates and  $r = 0$  and  $r = 0.75$ ; *t* values are followed by significance values (*P*) of test statistics. These tests indicate significant differences in all cases except *E. angophorae* and  $r = 0.75$

Species	Location	Collection date	Mean relatedness ( <i>r</i> )	s.e. of <i>r</i> jackknifed over colonies	No. of colonies	No. of individuals	Test statistics when $r = 0$	Test statistics when $r = 0.75$	Source
<i>E. robusta</i>	Mooball	18.xii.1996	0.4886	0.0933	20	79	$t_{79} = 5.24, P < 0.001$	$t_{79} = -2.80, P < 0.005$	—
<i>E. robusta</i>	Washpool	18.xii.1996	0.4391	0.0600	48	132	$t_{132} = 7.32, P < 0.001$	$t_{132} = 5.18, P < 0.001$	—
<i>E. angophorae</i>	Washpool	18.xii.1996	0.4247	0.1705	13	58	$t_{58} = 2.49, P < 0.02$	$t_{58} = -1.91, P > 0.05$	—
<i>E. robusta</i>	Dandenong	30.xi.1985 – 9.i.1986	0.485	0.060	98	355	—	—	Schwarz 1987
<i>E. angophorae</i>	Dandenong	23.xii.1992 – 22.i.1993	0.498	0.152	30	79	—	—	Schwarz <i>et al.</i> 1996

**Table 4. Inbreeding estimates and jackknife values (over colonies) for *E. robusta* from Mooball and for *E. robusta* and *E. angophorae* from Washpool**

Test statistics are included for *t*-test comparisons between inbreeding estimates and zero, which indicate no significant difference from zero in all cases. All data were collected on 18 December 1996

Species	Location	Inbreeding estimate ( $F_{it}$ )	s.e. of <i>r</i> jackknifed over colonies	<i>t</i>	d.f.	<i>P</i>
<i>E. robusta</i>	Mooball	0.0328	0.0522	0.63	79	n.s.
<i>E. robusta</i>	Washpool	0.0181	0.0498	0.36	132	n.s.
<i>E. angophorae</i>	Washpool	0.1644	0.1742	0.94	58	n.s.

in all cases, estimates of relatedness were significantly different from 0 and in all but *E. angophorae* were significantly lower than 0.75. We also tested our relatedness estimates from Washpool samples against those obtained for reused nests (as these were the most frequent nest type in our collection) from southern populations (Schwarz 1987; Schwarz *et al.* 1996) using *t*-tests. These tests indicated no significant difference in relatedness between northern and southern populations of either species ( $t_{116} = 0.033$  and  $t_{144} = -0.541$  for Mooball and Washpool *E. robusta* respectively;  $t_{41} = 1.747$  for *E. angophorae*;  $P > 0.05$  in all cases).

#### *Inbreeding*

Inbreeding estimates were calculated using Wright's  $F_{it}$  statistic with means and standard errors jackknifed over colonies, and are shown in Table 4. Results were then tested against 0 using single-sample *t*-tests, which indicated that inbreeding was not significantly different from 0 in all populations. The results of these tests are included in Table 4.

### **Discussion**

#### *Labile and invariant traits among populations*

Opportunities for sib rearing arise in doubly brooded colonies in northern populations of *E. robusta* and all studied populations of *E. angophorae*. Although sib rearing *per se* has not been observed in all populations studied here, it has been experimentally demonstrated in orphaned colonies of *E. nigrescens* (Bull and Schwarz 1997) and observed in experimental colonies of *E. robusta* (Flavel 1999). Thus, colonies in which opportunities for sib rearing arise are probably eusocial (*sensu* Michener 1974), a form of sociality that is apparently precluded in southern montane *E. robusta* (Schwarz 1986). Selection for sib-directed altruism may be facilitated in two-brooded colonies by high relatedness and female-biased sex ratios in populations where opportunities for sib rearing arise. High intra-colony relatedness may in turn arise from high levels of reproductive skew. Although the available data (Cronin and Schwarz 1999b) indicate latitudinal variation in opportunities for sib rearing in *E. robusta*, there is little evidence here for corresponding variation in reproductive skew, relatedness or sex allocation between these populations. These factors are discussed separately below:

#### *Reproductive skew*

Skew theory (Reeve and Ratneks 1993; Keller and Reeve 1994) predicts that reproductive skew may vary depending on ecological constraints, and should be highest where there are

strong constraints to solitary nesting and/or dispersal. Some studies of insects and vertebrates have provided evidence in favour of this model (e.g. Bourke and Heinze 1994; Jamieson 1997). However, data presented here indicate that although there was some evidence that the timing of ovarian development varied between sites, with relatively late ovarian development in *E. robusta* at Mooball, there is no evidence to suggest that the degree of reproductive skew differed between populations of either species. This may indicate that ecological constraints are relatively consistent over the latitudinal range. Recent studies of *E. nigrescens* (Bull 1999) suggest that skew did not differ between sub-populations under varied ecological constraints (see also Cant and Johnstone 1999). The presence of reproductive skew in reused nests in all populations indicates that colonies are probably semisocial (or transiently eusocial depending on opportunities for sib rearing) throughout the species range, whereas newly founded nests are probably quasisocial (*sensu* Michener 1974). This concurs with previous studies of southern *E. robusta* and *E. angophorae* (Schwarz 1986; Schwarz *et al.* 1996).

#### *Relatedness and sex allocation*

Unfortunately, we were not able to obtain estimates of relatedness and sex allocation ratios for all populations in our study. However, data from the northern locality of Washpool permits a comparison to be made with previous data from studies of southern populations (Schwarz 1988a, 1994; Cronin and Schwarz 1997). Sex allocation ratios in southern *E. robusta* and *E. angophorae* are female biased, and exhibit decreasing bias with increasing brood size, as reported here for Washpool *E. robusta*. Our data for Washpool *E. angophorae* are limited, and do not permit analyses of brood size effects, but do indicate a strong female bias, as for southern populations. These data indicate that sex allocation patterns exhibit little variation between populations of *E. robusta*, regardless of differences in opportunities for sib rearing, and are probably not related to opportunities for sib rearing. Other studies (Cronin and Schwarz 1997) suggest that in southern *E. angophorae* opportunities for sib rearing are not always associated with the highest levels of female bias.

Previous studies have shown that female-biased sex allocation can arise in southern populations of *E. robusta* and *E. angophorae* via Local Fitness Enhancement (LFE: Schwarz 1988a, 1994; Cronin and Schwarz 1997). This model predicts fitness advantages for producing daughters (up to an optimal colony size of 3–5 females) through increased colony efficiency and improved colony defence (Schwarz 1994; Bull and Schwarz 1996). The patterns of variation in sex allocation with brood size in Washpool *E. robusta* are consistent with the expectations of this model, and LFE may explain female-biased sex allocation in this population. Unfortunately, small sample sizes do not permit similar analyses for Washpool *E. angophorae*.

Relatedness among adult female nestmates in northern *E. robusta* from Washpool indicates a high level of kin association. Unfortunately, it was not possible to estimate relatedness in newly founded nests and reused nests separately due to small sample sizes and difficulties in determining the ages of nests. Tests did not indicate that there was any difference in relatedness between northern and southern populations of either species, despite varied opportunities for sib rearing in *E. robusta*.

Previous studies of southern heathland populations of *E. robusta* have shown that relatedness is lower than for montane conspecifics, probably because of differences in nesting substrate distribution (limiting kin association during dispersal) and longevity (Cronin and Schwarz 1999a). Interestingly, opportunities for sib rearing are common at the heathland locality, but are precluded in the montane habitat (Schwarz 1986; Cronin and

Schwarz 1999a). Other studies of allodapines have shown that experimental manipulation of nesting substrate distribution may alter intra-colony relatedness (Hurst *et al.* 1997), indicating that nesting substrate characteristics probably influence intra-colony relatedness. In a study on ants, Herbers and Banschbach (1999) found that relatedness and sex allocation varied with experimental manipulation of food and nesting site availability. Kin confounding is common in montane colonies (Schwarz 1986, 1988b) because of benefits to cooperative nesting (Schwarz 1988a, 1994; Bull and Schwarz 1996), and kin association during dispersal in montane habitats is facilitated by the aggregated nature of nesting substrate (Schwarz *et al.* 1998). These latter factors may explain the relatedness conservatism between latitudinally dispersed montane populations relative to populations from other habitats.

#### *Importance of environmental factors on sociality*

Recent studies (Cronin and Schwarz 1999a, 1999b) suggest that brood development rates mediate opportunities for sib rearing in *E. robusta*. Southern montane populations of *E. robusta* are apparently precluded from opportunities for sib rearing by lack of sufficient time for the provisioning and development of multiple broods in a single season (Cronin and Schwarz 1999b). Similarly, Wcislo *et al.* (1993) demonstrate that a large disparity in brood development rates between two closely related *Lasioglossum* species limits one species to singly brooded (solitary) colonies. If relatedness and sex allocation bias in *Exoneura* are already sufficient to support sib rearing, eusociality may arise opportunistically depending on environmental conditions. Indeed, Wcislo and Danforth (1997) propose that solitary behaviour is probably facultative in many bee taxa that exhibit geographic variation in sociality (e.g. Wille and Orcozo 1970; Eickwort and Eickwort 1971; Sakagami and Munakata 1972; Packer 1990; Mueller 1996; Eickwort *et al.* 1996). In contrast to halictine and ceratinine bees, singly brooded colonies of *Exoneura* are usually quasisocial or semisocial rather than predominantly solitary. However, these colonies may readily express sib rearing if conditions are unusually favourable and facilitate the production of multiple broods. Similarly, the proportion of colonies that exhibit eusocial or semisocial behaviour in northern populations may fluctuate annually. Our data are too limited to analyse this properly, but do not differ markedly between years, with 30% of nests ( $n = 37$ ) exhibiting opportunities for sib rearing at Washpool in 1995, and 39% ( $n = 72$ ) in 1996. Further analyses of northern and southern populations are needed to elucidate annual variation, and may expose previously unrecorded behaviours during unusually favourable or unfavourable seasons.

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