

Life Cycle and Social Behavior in a Heathland Population of *Exoneura robusta* (Hymenoptera: Apidae): Habitat Influences Opportunities for Sib Rearing in a Primitively Social Bee

ADAM L. CRONIN AND MICHAEL P. SCHWARZ

School of Biological Sciences, Flinders University of South Australia, GPO Box 2100, Adelaide, South Australia 5001

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ABSTRACT Montane populations of the allodapine bee *Exoneura robusta* Cockerell in southern Victoria, Australia, have been the subject of numerous studies of sociality over the last decade. These populations are univoltine and colonies exhibit a semisocial/quasisocial polymorphism. Synchronous brood development and restricted periods of egg laying in these populations severely limit opportunities for sib rearing by older daughters. Here, we report the life cycle and social behavior of a conspecific subcoastal heathland population from southern Victoria. Colony sizes and intracolony relatedness are lower in the heathland population, possibly because the nesting substrate is relatively short-lived and spatially dispersed. Brood development is relatively rapid at the heathland locality, such that some colonies are able to produce a 2nd brood in late summer. As a result, opportunities for sib rearing frequently occur in heathland *E. robusta*, allowing for quite different forms of alloparental care than occurs in montane populations. The presence of opportunities for sib-rearing behavior in this species provides evidence of habitat mediation of sociality that is not caused by latitudinal variation.

KEY WORDS *Exoneura*, allodapine, bee, life cycle, habitat variation, sociality

KIN SELECTION (HAMILTON 1964) is often considered a primary factor in the multiple origins of eusocial behavior (sensu Michener 1974) in the Hymenoptera. This is because of the extraordinarily high levels of relatedness that can arise between full sisters. However, studies on many hymenopteran species in the last decade, facilitated by direct measures of relatedness, have elucidated numerous instances where relatedness between individuals was not as high as initially expected (reviews in Gadagkar 1985, Queller and Goodnight 1989), suggesting that other influences may also play a key part in the evolution and maintenance of helping behavior. Consequently, there has been an increased focus on various ecological (Lin and Michener 1972, Evans 1977, Rissing et al. 1989, Schwarz et al. 1997, Weislo 1997) and demographic (Queller 1989, Gadagkar 1990, 1991) factors liable to facilitate the evolution and maintenance of sociality, and a considerable body of literature now exists that reveals numerous examples of nongenetic influences on social evolution (see reviews in Gadagkar 1985, Queller and Goodnight 1989, Schwarz et al. 1997). This does not discount the role of genetic influences in social evolution (see for example Ross and Matthews 1989, Ross and Keller 1995). Indeed, these factors are likely to act in an interactive fashion to form 'causal mosaics' (Crespi 1996). However, the study of the influence of nongenetic factors on social evolution has been a field of relative neglect in the past.

Ecological factors thought to influence sociality include climatic effects, predation and parasitism pres-

ures, and local habitat and nesting conditions (Lin and Michener 1972, Evans 1977, Schwarz et al. 1997). The role of climatic factors has been studied in several halictine bees, and it is apparent that solitary behavior is more common at high latitudes and altitudes, whereas lowland/low-latitude counterparts tend to be eusocial (Sakagami and Munakata 1972, Eickwort et al. 1996, Mueller 1996). Despite inferences that local habitat conditions are likely to be a contributing factor to social variation, the literature predominantly consists of examples of the effects of broad-scale climatic variation, and analyses of local habitat effects and impact of predators and parasites are rare or absent (but see Yanega 1989).

Australian allodapine bees provide a unique tool for the study of factors affecting social variation in the Hymenoptera. They exhibit a diverse array of social behaviors (Michener 1990), ranging from largely solitary behavior in *Exoneura lawsoni* Rayment (Michener 1964), to highly eusocial behavior (sensu Michener 1974) in *E. tridentata* Houston (Houston 1977, Schwarz et al. 1997), and complex semisocial behavior in the subgenus *Exoneura* sensu stricto (Schwarz 1986, Schwarz et al. 1997). Several environmental factors have been hypothesized to play key roles in the social evolution of Australian allodapines (Wilson 1971; Michener 1974, 1985, 1990; Schwarz and O'Keefe 1991; Schwarz et al. 1997), but analyses of social variation within species, and qualitative assessments of extrinsic factors likely to be important, have been scarce.

Studies of populations of *Exoneura robusta* (= *E. bicolor* [S. Reyes, personal communication; Schwarz et al. 1998]) in southern montane habitats of Australia have been extensive. In these areas, *E. robusta* exhibits a univoltine lifecycle and subsocial/semisocial/quasisocial organization (sensu Michener 1974). Brood is reared progressively, and adult females usually survive until their brood reach adult eclosion. A single brood is produced each year, and newly eclosed adults overwinter in their natal nest until the following spring when they either disperse to cofound nests with up to 8 nestmates (Schwarz 1986), or remain in their natal nest. Opportunities for sib rearing are largely precluded by a lack of generational overlap at a time when mothers are still reproductive; local conditions are probably responsible for limiting activity and development rates because of low temperatures and a short active season (Schwarz et al. 1998). Colonies of *E. robusta* in reused natal nests have a complex semisocial system with a well-developed, pheromonally mediated reproductive hierarchy based on order of adult eclosion (O'Keefe and Schwarz 1990, Schwarz and O'Keefe 1991, Schwarz and Woods 1993). These colonies are characterized by distinct behavioral castes (Melná and Schwarz 1994). In contrast, newly founded nests are cofounded by up to 8 related females (Schwarz 1988b) and are quasisocial (Schwarz 1986). Relatedness among female nestmates in montane *E. robusta* is high in both newly founded ($r \pm SE = 0.597 \pm 0.062$) and reused ($r \pm SE = 0.485 \pm 0.060$) nests (Schwarz 1987), and sex ratios are female biased (Schwarz 1988a, 1994), 2 factors likely to facilitate selection for helping behavior if opportunities for sib rearing arise (Schwarz 1994).

Cronin and Schwarz (1999) showed that in populations of *E. robusta* from subtropical habitats, opportunities for sib rearing arose in a number of nests where 2nd broods were produced within a single season. Similarly, Silberbauer and Schwarz (1995) have shown that in a heathland population of *E. nigrescens* Friese (formerly referred to as heathland populations of *E. bicolor* [S. Reyes personal communication; Schwarz et al. 1998]), a proportion of double-brooded colonies occurs. It should be noted that the existence of 2 more or less distinct broods does not refer to 'voltinism': in both subtropical colonies of *E. robusta* and populations of *E. nigrescens*, females that emerge in summer are never able to begin egg laying in the same season, so the life cycles still comprise only 1 generation per year.

Here, we investigate the lifecycle and social behavior of a heathland population of *E. robusta*, which is sympatric with the *E. nigrescens* population studied by Silberbauer and Schwarz (1995). If the difference in brood development rates between montane populations of *E. robusta* and heathland populations is the result of climatic or habitat-specific effects, then opportunities for sib rearing are also expected to occur in heathland colonies of *E. robusta*. We examine this possibility here and discuss the consequences of our findings for the role of proximate factors in mediating social organization in insects.

It should be pointed out that recent revision of the phylogeny of the *Exoneura* group has resulted in the reassignment of some species names (S. Reyes, personal communication). Previous analyses of montane *E. robusta* have been published under the name *E. bicolor* (Schwarz 1986, 1988a, b, c, 1994), and studies of heathland '*E. bicolor*' (Silberbauer 1992, 1997; Silberbauer and Schwarz 1995) assumed that this species was conspecific. It has been shown that the latter population represents another species, *E. nigrescens*. Hence, this study provides the 1st comparative analyses between heathland and montane populations of *E. robusta*.

Materials and Methods

Study Site and Nest Processing. A population of *E. robusta* was sampled fortnightly over the brood rearing season of 1996/1997. Colonies were found within unevenly dispersed stands of *Eucalyptus baxteri* in dense swampy heath of Lower Glenelg National Park, near Portland, Victoria, southeastern Australia (38° 77' S, 141° 35' E; elevation, 26.8 m). Aggregations of nests were common in dead and partly rotted vertically oriented branches of *E. baxteri*. Intact nests were removed from the field by cutting off branches containing nests, plugging nest entrances with cotton wool, and returning nests to the laboratory in insulated boxes on crushed ice.

An initial sample was taken on 11 July 1996, and regular sampling began on 10 October 1996 and continued every 2 wk until the final sample on 10 February 1997. Collections were made in the early morning or during inclement weather when it could be assumed that all nest occupants were present.

Nests were opened in the laboratory at 10°C and the numbers of all nest occupants (adults and brood) and inquiline bees (genus *Inquilina*) were recorded. Larvae were scored as small (1st instar), medium (2nd and 3rd instar), or large (4th instar). Efforts to assess nest age (of nontrap nests) by the coloring of the nest lumen (Schwarz 1986) were confounded by the nature of the nesting medium (rotted wood), which was often mottled in color in both newly founded and reused nests. Hence the majority of nests were scored as of unknown age, with only a small proportion scored as reused or new with confidence.

Trap Nests. Trap nests were used to assess timing of dispersal and extent of cofounding. These consisted of lengths of balsa wood (25 by 25 by 300 mm) attached to trees adjacent to dead or rotting wood and marked with colored tape. Such balsa trap nests have been used successfully to trap dispersing colonies of *E. robusta* in Lower Glenelg National Park (M.P.S., unpublished data). A total of 300 trap nests was set on 11 July 1996 and checked for occupants on all subsequent visits. Trap nests were retrieved when occupied, as evidenced by easily identifiable nest entrances.

Electrophoresis. All adult bees and pupae were frozen at -70°C until used for electrophoresis. Wings and metasomas were removed and placed in Kahle's solution for subsequent dissection. Thoraces were ho-

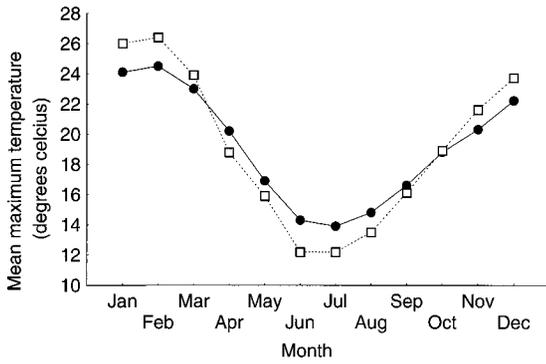


Fig. 1. Mean daily maximum temperatures for heathland (filled circles) and montane (open squares) sites for each month, showing the similarity in climate between localities. Data are from climate records for nearest weather stations at Heywood (heathland) and Healsville (montane).

we recorded wing-length, ovary size (mean length of the 3 largest oocytes), insemination status, and presence of internal parasites.

Results

Climatic Variation Between Regions. Climate data were obtained from climatic records from the nearest weather stations at Heywood (38° 14' S, 141° 62' E; elevation, 26.8 m) for the heathland locality, and Healsville (37° 68' S, 145° 53' E; elevation, 131 m) for the montane locality. Heathland and montane localities were at similar latitudes, and climatic variation between sites was minimal (see Fig. 1). Temperature extremes were marginally greater at the montane locality; this is probably because of the subcoastal nature of the heathland habitat, such that the nearby location of the Southern Ocean probably buffers against temperature extremes.

mogenized in 0.01% mercaptoethanol for allozyme electrophoresis. Heads were not included in electrophoresis to preclude the possibility of mandibular gland secretions influencing enzymatic activity. In total, 146 females from 38 nests were assayed. Electrophoresis followed methods of Schwarz (1986) and were carried out on horizontal starch gels using the RSL discontinuous buffer system (Ridgway et al. 1970), and assayed for dipeptidase (*PepA*) and proline dipeptidase (*PepD*). Staining techniques are described in Richardson et al. (1986). Relatedness was estimated using Queller and Goodnight's *Relate 4.2* program (Queller and Goodnight 1989).

Founding Patterns. Of the 300 trap nests set out, only 12 contained nests during the collection period. Of these, 1 contained an inquiline (an undescribed species in the allopapine genus *Inquilina*) in addition to a single foundress, 7 were multifemale nests and the remaining 4 were solitarily founded (Table 1). In addition to trap nests, a further 5 nests in *E. baxteri* were classed as new at time of opening on the basis of coloration of the nest lumen. The 1st newly founded nest was found in November, and a sharp reduction in colony sizes in nontrap colonies after October (see below) indicates that dispersal was likely to occur at this time, and continued until January (Table 1). All trap nests recovered in February contained pupae or callow females and thus had not been founded recently. These nests were recovered from a trap-nesting location that was not visited for some months

Dissections. Females were dissected to assess variation in ovarian development and insemination status. Metasomas were dissected in 70% ethanol. Dissection procedures followed the methods of Schwarz (1986);

Table 1. Composition of trap nests and newly founded nests recovered over the brood rearing season

Date collected	Nest type	Length	Old females	Callow females	Eggs	Small larvae	Medium larvae	Large larvae	Prepupae	Female pupae	Male pupae	Inquilines
3 November 1996	T	10	1	0	0	0	0	0	0	0	0	0
3 November 1996	T	15	1	0	0	0	0	0	0	0	0	0
2 December 1996	T	135	3	0	19	2	0	0	0	0	0	0
2 December 1996	T	70	3	0	7	0	0	0	0	0	0	0
2 December 1996	T	80	1	0	8	0	0	0	0	0	0	0
2 December 1996	T	55	2	0	4	0	0	0	0	0	0	0
14 December 1996	T	110	1	0	4	12	0	0	0	0	0	0
14 December 1996	N	50	2	0	0	5	0	0	0	0	0	0
14 December 1996	N	45	2	0	0	4	1	0	0	0	0	0
11 January 1997	N	65	1	0	0	0	0	0	0	0	0	0
11 January 1997	N	30	1	0	0	0	0	0	0	0	0	0
10 February 1997	T	95	2	3	5	0	0	0	0	0	0	0
10 February 1997	T	80	1	2	0	0	0	0	0	2	0	1
10 February 1997	T	85	2	2	5	0	0	0	0	1	0	0
10 February 1997	T	140	2	4	0	0	0	0	0	2	4	0
10 February 1997	T	100	2	3	0	0	0	0	0	0	0	0
10 February 1997	N	50	3	2	0	0	0	0	0	1	0	0

Note opportunities for sib rearing in advanced nests recovered in February, where late stage brood coincide with eggs. T, trap nest (founded in balsa nests); N, newly founded nest (as classed by coloration of the nest lumen). Inquilines, number of female parities.

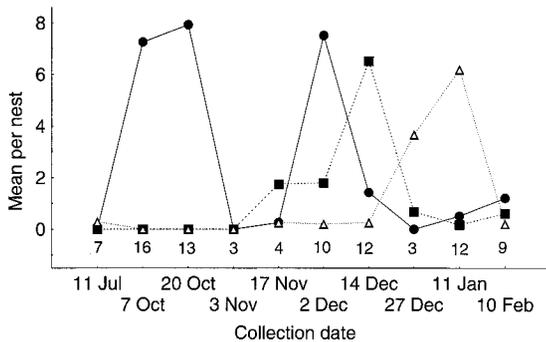


Fig. 2. Lifecycle of heathland *E. robusta*. Mean number of eggs (filled circles), larvae (filled squares) and pupae (open triangles) per nest are shown throughout the sampling period. Sample size is indicated for each sampling date.

because of inaccessibility, and hence may have been initiated several months before collection.

Colonies were founded by 1 ($n = 6$), 2 ($n = 3$), or 3 ($n = 2$) females (data from new and trap nests, excluding those from February sample), indicating a higher proportion (55%) of singly founded nests than occurs in montane populations ($\approx 25\%$ [Schwarz 1987]). Four of the 7 singly founded nests did not contain any brood (although 2 of these were collected in November and may not have initiated egg laying), whereas all multifemale nests contained some brood.

Nest Architecture. Nests were only found in soft, dead trunks or branches of *E. baxteri*, and consisted of single, unbranched burrows, with a constriction at the nest entrance. Nest lengths ranged from 5 to 370 mm, which is considerably less than that of montane *E. robusta* where nests were up to 700 mm long (Schwarz 1986).

Life Cycle. Life cycle phenology was examined using the numbers of adults, eggs, larvae, and pupae in all nonparasitized nests over the collection period. Colony composition over this time (July to February) is summarized (Fig. 2). Egg production most likely began between late winter and early spring (July to October), as indicated by the large peak in egg numbers in October (Fig. 2), and eggs were present in at least some nests until the end of summer (February). The lack of eggs in the November samples was probably a result of small sample sizes ($n = 3$ and $n = 4$, respectively) and new nest initiation (hence some of the sample contained nests in which egg laying had not commenced). In the 3 November sample, 2 of the 3 nests collected were trap nests and the remaining nest contained a single female without brood, indicating that it too may have been newly founded. The 2nd November sample contained 4 nests; 2 contained larvae (1 of which was reused, as determined by coloration of the nest lumen), and the other 2 contained no brood. Hence, at this time, it is likely that brood in reused nests were being actively reared while egg laying had not yet begun in new nests.

An apparent 2nd peak of egg production occurred in December. At this time, there were no eggs in

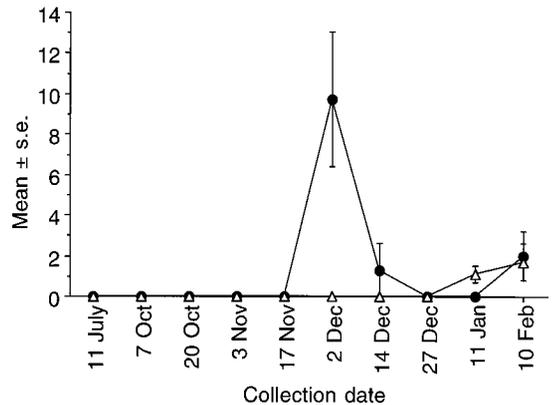


Fig. 3. Mean \pm SE number of eggs in new nests (filled circles) versus old nests (open triangles) over the sampling period. These data indicate that egg laying in newly founded nests corresponds to the 2nd peak in egg production in overall data (Fig. 2), whereas there are no eggs in old nests at this time.

reused nests, although again sample sizes were small ($n = 3$ for reused nests, whereas $n = 7$ and 15 for new and unknown age nests, respectively, in December overall). This 2nd peak is likely to have been a result of the initiation of egg laying in newly founded nests (whereas brood have developed into larval stages in reused nests), and does not represent a 2nd period of egg production in reused nests. Fig. 3 shows numbers of eggs in new and reused nests over the sampling period. The 2nd peak of egg production in Fig. 2 has a corresponding peak in new nests, but there were no eggs found in reused nests at this time. This suggests that in reused nests, egg laying occurred during or before October (before dispersal of some females in November), whereas egg laying in new nests commenced in December.

Larval development began in spring (November) for colonies in reused nests, and continued through to the end of December. Whereas there were 2 distinct pulses of egg production, there is only a single pulse of larvae and pupae in nests as a whole, indicating that brood development in newly founded nests soon catches up with reused nests. Comparatively accelerated brood development in newly founded nests also occurs in the montane population (Schwarz 1986). Pupation began in late December in both new and reused nests, and adult eclosion occurred in February.

In addition to the 1st pulses of egg production in reused and new nests in October and December, respectively, eggs were also found in both types of nests in January and February (Fig. 3). This indicates that in some nests at least, a 2nd batch of eggs was being produced. The proportion of nests that contained eggs in late summer was considerable; 25% of nests in January and 56% of nests in February contained eggs compared with 0% at the end of December. It is important to note that nests that contained eggs at this time did not contain any larvae (except for 2 nests in February where small larvae were present), although

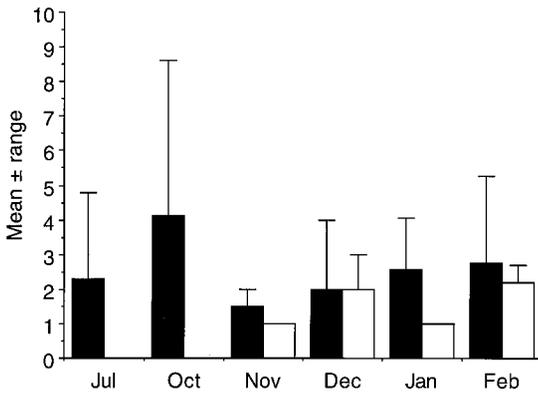


Fig. 4. Mean \pm range colony size in newly founded nests (white bars) and nests of unknown age (black bars) during the sampling period.

some pupae or prepupae, or both, were present. Hence, the presence of eggs in late summer indicates a discrete 2nd brood rather than the result of a single protracted period of brood rearing. In contrast, nests of montane *E. robusta* that contain eggs in January also contain all other brood stages, indicating a single, protracted brood.

Colony Size in New and Reused Nests. Determining the relative ages of nests from lumen coloration (Schwarz 1986) was problematic and only a small percentage of nontrap nests could be aged with any confidence ($n = 19$ out of a total of 79); the majority of nests ($n = 60$) remained of unknown age. Colony size throughout the season ranged from 1 to 10 females (Fig. 4). Colony sizes were highest before dispersal in November, and postdispersal nest sizes (including November, but before February) were considerably smaller. Multifemale nests constituted 73.3% ($n = 33$ of 45 total nests) of colonies before dispersal. After dispersal in November, proportions of multifemale nests differed significantly between newly founded and reused nests ($\chi^2_1 = 4.68$, $P = 0.03$), with 45.5% ($n = 11$) of newly founded nests and 90% ($n = 10$) of reused nests containing >1 female.

Social Organization and Ovarian Differentiation. The presence of behavioral and reproductive differentiation was investigated by looking at relationships between wing length (used as a measure of body size [Schwarz 1986]), ovary size, and insemination status in multifemale nests (Fig. 5). It was not always possible to determine insemination status because of the small size of spermathecae, and individuals that could not be classed were excluded from statistical analyses (but are shown in Fig. 5). Several samples not included in Fig. 5 contained insufficient numbers to provide statistically useful information. Samples not graphed were 3 ($n = 1$) and 17 ($n = 7$) November, 2 ($n = 7$), 14 ($n = 7$), and 27 December ($n = 0$), and 11 January ($n = 14$).

The July sample consisted of overwintering females and there was no clear relationship between wing length and ovary size. In October, brood data indi-

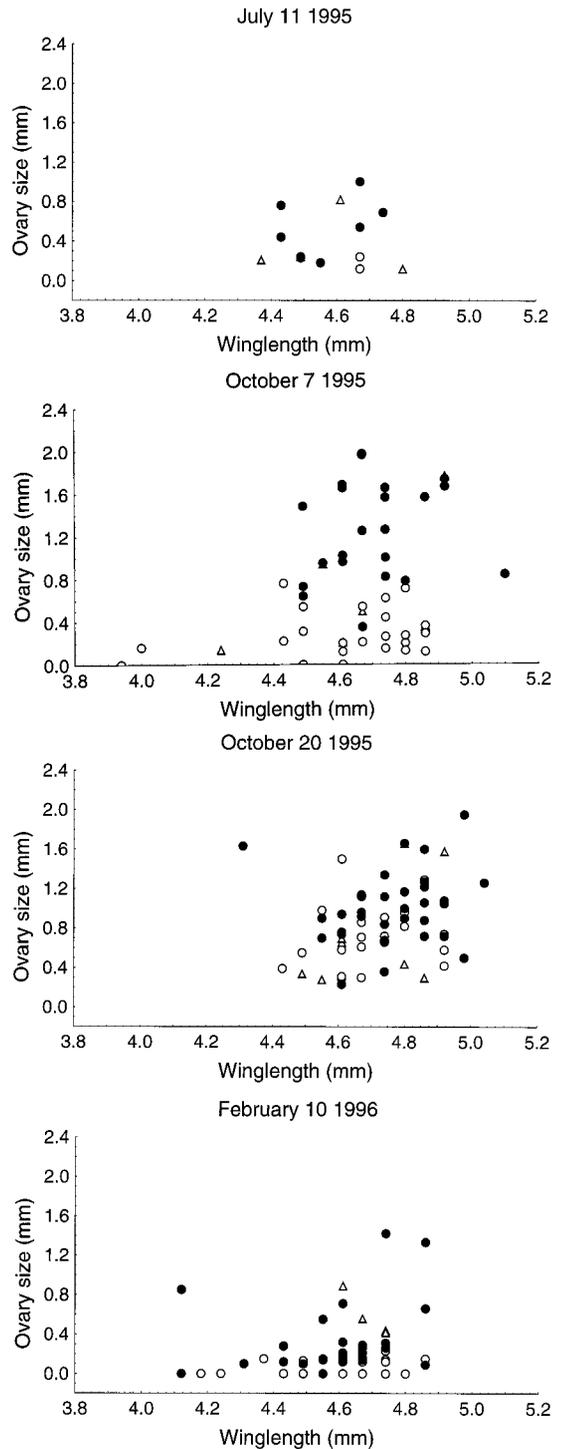


Fig. 5. Ovary size (mean of the 3 largest oocytes) versus wing length, for inseminated females (dark circles), uninseminated females (open circles) and females with undetermined insemination (open triangles) throughout sampling period.

cated a major period of egg laying, and ovarian differentiation among nestmates at this time was well developed. In the 7 October sample, ovary size in inseminated females is generally larger than that of uninseminated females, although this distinction was less clear in the 20 October sample. We assessed whether ovarian differentiation was related to insemination status and wing length using a one-way analysis of covariance (ANCOVA), with insemination status as a factor and wing length as a covariate. 7 October data were $\log(x + 1)$ transformed to achieve homoscedasticity and ANCOVA revealed a highly significant effect of insemination status on ovary size ($F = 80.90$; $df = 1, 44$; $P < 0.001$), but no significant effect of the covariate ($F = 1.82$; $df = 1, 44$; $P = 0.19$). ANCOVA on untransformed 20 October data also indicated a significant effect of insemination status ($F = 6.60$; $df = 1, 51$; $P = 0.01$) and no significant effect of the covariate ($F = 1.19$; $df = 1, 51$; $P = 0.28$). These data suggest that ovarian differentiation is high and dependent on insemination status in both samples. However, Fig. 5 clearly shows that the differences in ovary size between inseminated and uninseminated females is less marked in the later part of October than in the 1st sample. This could be the result of a decrease in reproductive activity of earlier, inseminated females or onset of reproduction in secondary reproductives that have yet to become mated.

November and December sample sizes were extremely small. However, our limited data suggest that ovary sizes were generally smaller than those in October, despite continued egg laying (see above). The proportion of inseminated females in October was 49 and 62%, for 7 and 20 October, respectively. The proportion of inseminated females was relatively high (>85%) in November and December, and it remained so until adults from the 1st brood eclosed in February. In January, data from nest contents suggest a 2nd period of egg laying in $\approx 25\%$ of nests, although egg numbers were considerably smaller than in 1st broods for both reused and newly founded nests (Fig. 2). Examination of nest data indicated that in the 4 multifemale nests collected in January ($n = 2$ for 2 and 3 female nests), all contained a single female with enlarged ovaries whereas others had relatively undeveloped ovaries.

In February, eclosion of new adults resulted in an increase in numbers of uninseminated females with undeveloped ovaries (Fig. 5). A small proportion of females ($n = 8$) still possessed very large ovaries, whereas the majority had relatively small ovaries. February data could not be transformed to meet the assumptions of ANCOVA, and hence nonparametric methods were used for statistical analyses. A Kruskal-Wallis test showed no nest effects on ovary size ($H = 3.14$, $df = 11$, $P = 0.99$) and hence all nest data were pooled. The Spearman rank correlation indicated a significant relationship between wing length and ovary size ($r_s = 0.30$, $P = 0.013$, $n = 66$); and a Mann-Whitney U test indicated a significant effect of insemination status on ovary size ($U = 151.5$, $P < 0.001$, $n = 66$). Because new females are eclosing at this time and

have undeveloped ovaries, it is extremely likely that they are not able to lay eggs until the following season. Hence, at least some old females probably lay a 2nd clutch of eggs.

Ovarian Differentiation in New Nests. Individuals were dissected from 7 multifemale colonies that were either newly founded in *E. baxteri* branches or were retrieved from trap nests. Two of these were from December, and each contained 2 inseminated females. In both nests, 1 female possessed relatively larger ovaries than did to her nest mate. The remainder of these nests was collected in February, and ovary sizes were considerably smaller than in December. However, in all of these nests except 1 (which contained 2 females with similar-sized ovaries), there was a single female with relatively large ovaries. In addition, in each nest, 1 female with relatively small ovaries also had a large amount of wing wear, suggesting a foraging role. These data suggest that reproduction may be skewed in newly founded as well as reused nests, in contrast to data for montane *E. robusta* where newly founded nests are quasisocial and all cofoundresses have large ovaries.

Opportunities for Sib Rearing. The 2nd period of egg laying in some nests in late summer could lead to opportunities for sib rearing if 1st brood daughters eclose in nests containing feeding stage sibs. The occurrence of opportunities for sib rearing was investigated by searching for the presence of nests that contained both newly emerged adults (callow females) or female pupae, and eggs or small larvae. Nests that met these criteria (listed in Table 2) indicate that opportunities for sib rearing do arise in heathland *E. robusta*.

However, callow females are identified by a lack of pigmentation that lasts for a short duration only (Schwarz 1986) and hence some of these nests may consist entirely of same generation siblings, some of which have recently gained adult pigmentation. To take this into account we examined dissection data to determine if any noncallow females were indeed newly eclosed. These data indicated that of the nests in Table 2 that contained callow females, 3 contained a single (noncallow) female with extremely large ovaries and worn wings, suggesting the presence of 2 generations. Of the remaining 2 nests, nest 18 had a female with extremely worn wings but small ovaries, and nest 15 contained no females with enlarged ovaries. These data suggest that 3 of these nests were examples of nests with definite generational overlap (and opportunities for sib rearing), and 2 nests contained females of the same generation. However, the presence of eggs, and lack of enlarged ovaries in any remaining females in nests 15 and 18, suggests that these colonies may have been recently orphaned, and hence opportunities for sib rearing existed in all cases. There was a lack of intermediate stage brood in all these nests, indicating that 2 discrete broods were being produced rather than a single protracted brood. Interestingly, nests 15 and 18 are trap nests, indicating that these were founded at the beginning of the season, yet were still able to produce 2 broods.

Table 2. Nests where opportunities for sib rearing arose in heathland *E. robusta*

Nest No.	Date collected	Nest length	Old females	Callow females	Eggs	Small larvae	Medium larvae	Large larvae	Prepupae	Female pupae	Male pupae	Nest type
5	11 January 1997	160	2	0	3	0	0	0	7	4	4	r
6	11 January 1997	240	4	0	2	0	0	0	2	7	8	r
7	11 January 1997	260	3	0	1	0	0	0	1	9	6	r
4	10 February 1997	110	2	7	3	2	0	0	0	0	0	r
10	10 February 1997	220	6	4	2	0	0	0	0	0	0	r
15	10 February 1997	95	2	3	5	0	0	0	0	0	0	t
18	10 February 1997	85	2	2	5	0	0	0	0	1	0	t
2	10 February 1997	150	2	4	1	0	0	0	0	0	0	u

These nests constitute 25% ($n = 12$) and 45% ($n = 11$) of the last collections, respectively. Nests were classed as new (n), reused (r) or of unknown age (u) based on residue left on the nest lumen. Trap nests (t) were those established in balsa wood traps.

Intracolony Relatedness. Relatedness between female nest mates during the brood-rearing season was estimated using data from 38 nests and a total of 146 individuals. The February sample was excluded from analyses because these nests were likely to contain newly eclosed adults that were not classed as callows (i.e., had recently become pigmented). Males were also excluded from analyses. Pep A and Pep D were both dimeric (allele frequencies shown in Table 3). Intracolony relatedness among adult females was calculated as $r = 0.336 \pm 0.111$, with standard error jackknifed over colonies (see also Table 3). Inbreeding estimates were calculated using the Wright F_{it} statistic as 0.144 ± 0.092 . The significance of inbreeding was tested using a single sample t -test, which indicated no significant difference between the estimate and zero ($t = 1.567$, $df = 168$, $P > 0.05$).

Discussion

Several differences exist between montane and heathland populations of *E. robusta*. Data from newly founded nests indicate that the number of cofoundresses is generally higher in montane (up to 8 females [Schwarz 1994]) than in heathland populations (up to 3 females). Montane *E. robusta* populations nest in dead fronds, which form densely aggregated skirts around the base of tree ferns. Hence, dispersal distances can be very small and nesting substrate is unlikely to be limiting (Schwarz 1986, Schwarz et al. 1996). In contrast, heathland *E. robusta* nests in sparsely distributed and partly rotted branches of *Eucalyptus*, where dispersal distances are much greater than for montane conspecifics (A.L.C., unpublished data). Smaller colony size in newly founded heathland

nests is likely to reflect difficulties in maintaining kin association (or locating nonkin) over long dispersal distances.

Colony size in reused nests in the heathland population was also smaller than for montane conspecifics. Silberbauer and Schwarz (1995) showed that colony size was significantly larger in more durable nesting substrate in heathland populations of *E. nigrescens*, and the relative fragility of nesting substrate used by heathland *E. robusta* may limit colony size in this population. Bull and Schwarz (1996) showed that pressure from enemies at the nest may select for increased colony size in *E. nigrescens*, and it is possible that variation in this pressure between habitats may influence colony size in *E. robusta*. However, further studies are needed before these possibilities can be properly addressed.

Montane populations of *E. robusta* exhibit high levels of relatedness in reused ($r \pm SE = 0.49 \pm 0.06$ [Schwarz 1987]), and newly founded nests ($r \pm SE = 0.60 \pm 0.06$ [Schwarz 1987]), respectively, indicating a high level of kin association during dispersal. We were unable to estimate relatedness separately for new and reused heathland nests because of difficulties in aging nests and small sample sizes, but an overall estimate of $r = 0.336 \pm 0.111$ was obtained. This result is not significantly different from relatedness in reused nests of montane *E. robusta* (t -test, $t = -1.181$, $df = 134$, $P > 0.05$) but was significantly lower than relatedness in newly founded montane nests ($t = -2.053$, $df = 124$, $P < 0.05$). In newly founded colonies of a sympatric population of *E. nigrescens*, which nests in dispersed scapes of the grass tree *Xanthorrhoea minor*, relatedness among cofoundresses varies with dispersal distance (N. J. Bull, unpublished data); where dispersal distances were low, mean relatedness among cofoundresses was high ($r \cong 0.7$), whereas relatedness was low ($r \cong 0.2$) where dispersal distances were high. Furthermore, Hurst et al. (1997) found that relatedness was high ($r \cong 0.6$) in experimental populations of *E. nigrescens* where dispersal distances were artificially small. In contrast, relatedness in a natural population of *E. nigrescens* where dispersal distances were large was not significantly different from zero (Silberbauer

Table 3. Gene frequencies for allozymes used in calculation of relatedness (Pep A and Pep D), relatedness estimate (r) \pm Jackknife of r over colonies, and inbreeding estimates (F_{it}) \pm standard error of F_{it}

Pep A	Pep D	$R \pm SE$	$F_{it} \pm SE$
A: 0.447	A: 0.954	0.336 ± 0.111	0.144 ± 0.092
B: 0.553	B: 0.046		

1992). If the suggestion of lower relatedness in heathland *E. robusta* is real, this may relate to difficulties in maintaining kin association during cofounding over relatively large dispersal distances at the heathland locality.

Ovarian data indicate that reproduction in heathland *E. robusta* is skewed in both reused nests and newly founded nests, and hence both fit the criteria for semisociality (sensu Michener 1974). These results differ from montane populations where colonies in reused nests are semisocial, but newly founded colonies are quasisocial (Schwarz 1986). In montane *E. robusta*, dispersal occurs over a very short period, such that there is a relative abundance of potential cofoundresses, and dispersal distances are relatively small such that kin association is possible. In contrast, females dispersing in the heathland habitat have relatively limited numbers of available cofoundresses, because dispersal is asynchronous between colonies and dispersal distances are large. Hence, it is possible that females predominantly initiate nests solitarily in heathland populations, and joiners arrive later on. In this way, joiners may be forced to adopt subordinate roles by initial foundresses in much the same way that first eclosed females assume dominance in reused nests (O'Keefe and Schwarz 1990). Alternatively, skew theory predicts that skew should be higher where ecological constraints are also high (Keller and Reeve 1994). There is evidence to suggest that reproductive skew also occurs in newly founded nests of heathland *E. nigrescens* (N. J. Bull, unpublished data), and montane colonies of *E. angophorae* and *E. bicolor* (Schwarz 1988c), where dispersal also occurs over a long period. Closer analyses of dispersal behavior may clarify reasons for differences in reproductive skew in newly founded nests.

Our most interesting finding is that a proportion of heathland colonies are able to produce a 2nd brood in mid- to late summer, which is apparently precluded in montane populations (Schwarz 1986, Schwarz et al. 1996). Climate at both localities is very similar and unlikely to influence the time available for brood rearing. Egg laying occurs in late August in montane *E. robusta* (Schwarz 1986), and our data indicate that eggs were present in October in heathland colonies, but do not allow us to compare the time at which egg laying commences. Larval eclosion begins at similar times at both localities (early November [Schwarz 1986]), but pupation begins slightly earlier in heathland colonies (late December) than in montane colonies (mid-January [Schwarz 1986]). Moreover, the majority of pupae at the heathland locality have eclosed into adults by mid-February, and a 2nd brood is being laid in some nests, whereas adult eclosion has only just begun in montane colonies at this time (Schwarz 1986). These data suggest that brood development rates may be greater in heathland colonies.

The production of 2 broods gives rise to opportunities for sib rearing, and if sib rearing does occur in heathland *E. robusta*, this would represent a previously unreported form of alloparental care for this species. Silberbauer and Schwarz (1995) argued that

periods or transient eusociality occurred in heathland *E. nigrescens*, and Bull and Schwarz (1997) showed that sib rearing occurred in experimentally orphaned nests of that species. The life cycle of heathland *E. robusta* is similar to that of sympatric *E. nigrescens* and, given that newly eclosed females of heathland *E. robusta* are probably unable to lay eggs of their own until the following season, it is likely that they may take advantage of opportunities to rear sibs in the intervening period. Selection for sib rearing in newly eclosed females at this time could be facilitated by the potential for gaining assured fitness returns (sensu Gadagkar 1990, 1991).

Previous studies have stressed the importance of broad scale climate variation in mediating sociality (Michener and Bennet 1977, Packer and Knerer 1987, Packer 1990). Cronin and Schwarz (1999) showed that opportunities for sib rearing varied in latitudinally dispersed populations of *E. robusta*, and were most probably attributable to variation in climate. However, the heathland population studied here was at the same latitude as the previous Victorian montane studies and temperature regimes between the 2 areas are very similar. Temperatures during brood rearing may be slightly warmer at the montane locality, yet our data suggest that brood development is more rapid in heathland colonies. Hence, although climate could potentially affect brood development rates in *E. robusta*, this may be overshadowed by other habitat differences. Two possibilities that have not been addressed in previous studies of latitudinal variation and which require further investigation are differences in abundance and phenology of floral resources, and differences in microhabitat. Floral resources could influence duration of brood rearing through floral seasonality and if development rates are limited by the abundance of available forage. Previous studies on allodapines show that pollen brought into the nest is very rapidly consumed by larvae and most nests collected do not contain uneaten pollen (Rayment 1951, Michener 1974). There are currently no data on relative floral abundance and seasonality for the 2 populations, but anecdotal observations suggest that floral resources may be more abundant and occur earlier at the heathland locality (A.L.C., unpublished data). Differences in microhabitat could also influence daily activity patterns, foraging yields, and development rates. Montane regions have tall and dense overstoreys that would limit insolation for nests under tree ferns, whereas in heathland regions the overstorey is relatively open and locally patchy, and nests receive more direct sunlight. The latter condition would result in warmer microhabitats, which may result in more rapid brood development and longer periods of activity, and could explain differences in brood development rates between these populations. This study indicates that the effects of cross-population differences in microhabitat and floral resources could overshadow the impact of broad climate differences, and should be taken into account in future studies of geographic variation in sociality.

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