Complex sex allocation in the laughing kookaburra

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In groups of the cooperatively breeding laughing kookaburra (*Dacelo novaeguineae*), offspring sex varied with the type of social group and with hatch rank. Groups with female helpers, especially if all helpers were female, had male-biased clutch and fledging sex ratios. Groups without female helpers (unassisted pairs or male-only helpers) had female-biased clutch and fledging sex ratios. Breeding females responded facultatively to increases in the number of female helpers in their group by producing more male eggs. These biases may occur if breeding females try to limit the number of daughters recruited into their group because unlike male helpers, female helpers depress the breeding success of their parents. Across all nests, two-thirds of first-hatched young were male, two-thirds of second-hatched young were female, and the sex ratio of third-hatched young was even. Hatch rank sex ratios also varied dramatically between different types of social groups, from 16.7% for second-hatched nestlings of unassisted pairs to 100% for first-hatched nestlings of groups with only female helpers. A corollary of the relationship between hatch rank and sex was that hatching sex sequences were distributed nonrandomly: all groups avoided hatching a daughter first followed by a son (FM). Sibling competition is aggressive and sometimes fatal. Since females grow to be 15% larger than males the hatching sequence of sexes could affect nestling growth and mortality. However, an exhaustive analysis found little evidence that growth or survival of males was compromised if hatched after a sister. The small number of FM sequences may only have occurred in nests that were able to ameliorate any negative consequences. Alternatively, when clutch size is small and fledging success unpredictable because of brood reduction, the preferred brood sex ratio may be contingent on the number of fledged young, making it advantageous to order the sexes in the brood. *Key words:* cooperative breeding, kingfisher, reverse dimorphism, sex allocation, sex ratio, siblicide. *[Behav Ecol 12:524–533 (2001)]*

F isher (1930) showed that when males and females have equivalent reproductive value, parents should invest equally in sons and daughters. However, situations exist where one sex may be more profitable than the other, and parents could maximize their fitness by adjusting their sex allocation (Frank, 1990). Examples of nonrandom sex ratios in birds have accumulated only recently, but the types of biases and their proposed adaptive values have been very varied (reviews by Hardy, 1997; Sheldon, 1998). In species where individuals interact with kin, opportunities arise for competitive and/or cooperative effects to alter the relative values of each sex. Sex differences in dispersal may cause one sex to associate more intensively with siblings or parents, potentially bringing them into competition over resources and effectively making them more "costly." The "local resource competition" hypothesis and its variants therefore predict overproduction of the dispersing sex (Clark, 1978). Alternatively, if cooperative interactions between kin increase the fitness of breeders, parents may preferentially produce the philopatric sex. A version of "local resource enhancement," the repayment model, has been applied to cooperatively-breeding birds, many of which live in family groups with related helpers of one sex (Emlen et al., 1986; Lessells and Avery, 1997).

Species that breed cooperatively should be excellent models for evaluating the importance of enhancement and competitive effects, but there are still few examples. A population-wide bias towards males in red-cockaded woodpeckers (Gowaty and Lennartz, 1985) was interpreted as support for enhancement effects because helpers are usually male, but this result was not repeated in a later study reporting on a larger sample from the same species (Walters, 1990). Enhancement and resource competition models may be more usefully applied at the level of individual families, rather than across populations of cooperative-breeders (Koenig and Walters, 1999), since enhancement and competitive effects probably operate to varying extents among families within a population. Sex ratio variation between families has been reported for red-cockaded woodpeckers (Gowaty and Lennartz, 1985), green woodhoopoes (Ligon and Ligon, 1990), wild dogs (Malcolm and Marten, 1982), eclectus parrots (Heinsohn et al., 1997), but most convincingly for Seychelles warblers (Komdeur et al., 1997). Daughters in this species are most likely to help and can have a positive effect on the reproductive success of their parents. However, too many helpers can reduce nest success, particularly when food resources are poor. Breeding females produce daughters in their single-egg clutch when the territory is of high enough quality to support more birds, but produce the dispersing sex (sons) when the natal territory is "saturated."

One of the more common patterns of sex ratio bias reported in the literature is a sequence effect within the clutch, where the sex of eggs relates to their position in the laying sequence. Such sequence effects could cause considerable skews in the sex ratio across clutches, if the "switch" between sexes was moved up or down the sequence, or if tempered with order-related mortality or clutch size variation (Krackow, 1999). For example, if a cooperative breeder hatches the helping sex (say male) at the start of the clutch sequence, and brood reduction is common or the clutch size is curtailed in groups lacking male helpers, the brood sex ratio in these groups becomes relatively male-biased. In contrast, if groups with helpers have larger clutches or less brood reduction, they will produce clutches with a balanced or female-biased sex ratio.

Sequence effects may also have important consequences for

nestling interactions in dimorphic birds, particularly if there is sibling aggression. The sequence of sexes could affect nestling condition, nest productivity, and fledging sex ratios (Bortolotti, 1986; Dzus et al., 1996; Edwards and Collopy, 1983; Edwards et al., 1988; but see Drummond, 1991). In mixed sex clutches, hatching the smaller sex second may give it the double disadvantage of hatch rank and sex, leading to reduced growth and/or reduced productivity from those nests, especially if siblicide is possible. Alternatively, hatching the smaller sex first could increase sibling competition in the nest if shifting size asymmetries destabilize dominance hierarchies. In siblicidal species, an older nestling destined to be smaller than its nest mate may kill its younger sibling preemptively, to eliminate anticipated conflict.

Here we describe offspring sex ratio patterns in the laughing kookaburra (*Dacelo novaeguineae*) in relation to two major features of its complex social and breeding biology. First, kookaburras breed in nuclear family groups where a monogamous pair are assisted by up to six offspring-helpers (Legge and Cockburn, 2000; Parry, 1973). Dispersal, helping behavior and the effect of that help depends on the sex of the helper. Daughters disperse at a younger age than sons, sometimes leaving their natal group before they have helped at all (Legge and Cockburn, 2000). The contribution of helpers during nesting allows breeders to reduce their workload, with potential long-term effects on survivorship and reproductive success. However, females are "poor" helpers, feeding young at a much lower rate than their brothers. Thus, whereas males have a neutral effect on the success of each nesting attempt, female helpers actually reduce nest success (Legge, 2000a,b). Breeders may be sensitive to the number of males and females already in the group, for example producing sons if male helpers are lacking, and avoiding too many daughter-helpers at once. Consequently, we examine the clutch and fledging sex ratios from groups of different size and composition.

Second, kookaburra broods hatch asynchronously, producing a size hierarchy that facilitates siblicide. The youngest nestling dies in nearly half of all nests because of severe aggression from older siblings (Legge, 2000c). Since kookaburras are also reverse size dimorphic (adult females 15% larger, fledgling females 7% larger), the order of sexes in the hatching sequence may influence the outcome of sibling aggression. Consequently we examine the relationship between hatch rank and sex, the distribution of sex sequences, and the effect of sex sequence on fledging success and nestling growth.

METHODS

The data presented here were collected between December 1994 and February 1998 from a closely monitored population of laughing kookaburras in Canberra Nature Park, a eucalypt woodland reserve in southeast Australia. About 35 groups in territories of 16–224 ha occupied the 20 km² study area each year. Kookaburras breed in the austral spring and summer, between September and January. Females lay a single clutch per season, usually of three eggs, in a naturally occurring tree hollow. The clutch hatches asynchronously, with intervals from a few hours up to 3 days between successive eggs. Hatch order reflects lay order. Nestlings emerge blind and naked, but use a specially modified "beak hook" to attack their siblings. The youngest nestling was killed in nearly half of nests where three young hatched (46%; Legge, 2000c). Groups fledged an average of 1.4 young (SD = 1.1, n = 131 group-years) 32–40 days after hatching. The young remain dependent on adult group members for food for another 8 weeks (Legge and Cockburn, 2000; Parry, 1973).

Group size was defined as the total number of birds attending a nest, including the breeding pair. Groups were also classified into four "helper types": groups could have no helpers (the breeding pair is unassisted), all-female helpers, all-male helpers, or helpers of both sexes. For details of censusing techniques and general field methods see (Legge, 2000a; Legge and Cockburn, 2000).

Nestling sex

Nestling sex was determined from DNA. A small blood sample (10–20 μl) was taken within 48 h of hatching by puncturing the brachial vein. DNA was extracted from the blood, and a molecular method was used to identify sex (see Griffiths et al., 1998 for details). The sex of 21 dissected birds was correctly predicted using this method. Unhatched eggs were checked for embryonic material, which was collected for DNA extraction and molecular sexing (successful for 10/24 eggs in 10 clutches).

Sex sequences

Near the hatch date (estimated previously by candling eggs) nests were visited daily to match hatchlings to eggs and assign them sequential hatch ranks A, B, and C. Nestlings were marked temporarily by tying soft, colored embroidery thread around the tibia. They were given permanent metal colored bands on the last nest-visit when the A nestling was 32 days old. Nestling sex sequences describe the sex of nestlings in order of their hatch rank. For example, the sex sequence 'MF' for A and B nestlings means that a male hatched first followed by a female second. In some clutches one or two eggs failed to hatch (24/257 eggs in 22/101 clutches). In these clutches the observed sex ratio and sex sequences of hatchlings may differ from the sex ratio and sex sequence of eggs. Within clutches, eggs could be ranked in order of egg volume [cm³, calculated as $0.00051 \times$ Length $\times$ (Width)²; Hoyt, 1979]. Hatch rank nearly always matched egg volume rank: only 6% of 180 eggs hatched earlier than their rank assigned from egg volume (Legge, 2000c). The hatch rank of unhatched eggs was deduced from their volume, and the ranks of successfully hatched young were adjusted accordingly. Adjusted hatch ranks and sex sequences are italicized (*A, B, C, AB,* etc.) to distinguish them from the hatch rank and sex sequences assigned to nestlings that had actually hatched.

Nestling growth

After all eggs had hatched, nests were visited at weekly intervals (usually days 11, 18, 25, and 32; day 0 is when nestling A hatches) to measure nestling mass, tibia, and flattened wing chord (nearest g, 0.1 mm, and mm respectively). Mass gives a general indication of size and condition, tibia reflects skeletal size, and wing-length indicates feather growth. Fledging success was defined as the number of young present in the nest on the final visit, when the A nestling was 32 days old.

Analysis

In descriptive statistics, heterogeneity, goodness-of-fit tests (χ^2) and the binomial test were used to check for departures from equal sex ratios. Other analyses were carried out with a statistical modeling approach using Genstat 5 release 4.1 (Genstat Committee, 1993). Some data were potentially nonindependent because groups were represented between one and four times, and broods contained up to three nestlings. To account for repeated sampling with an unbalanced design, mixed models were fitted incorporating random factors ("group"

and "brood," as required) as well as the fixed effects of interest.

Factors affecting clutch and fledging sex ratios

Variation in clutch and fledging sex ratios was analyzed by defining brood sex ratio as the binomial response variable (number of males over brood size) in generalized linear mixed models with a binomial error distribution (Genstat Committee, 1993). Estimates of the variance components and fixed effects were initially obtained using the restricted maximum likelihood procedure (REML). As the standard errors of the estimates for the random term proved to be large compared to the estimates, indicating negligible dependency associated with "group," the models were simplified by omitting the random term and using generalized linear models. The significance of terms was assessed using the change in deviance (which approximates to a χ^2 distribution) associated with dropping that term from a fuller model. Variables of interest were year, hatch date, group size, and helper type. Group size and helper type are correlated (e.g., groups without helpers must have group size two), and were therefore added to models separately. Broods were only included in the clutch sex ratio analysis if the sex of the entire clutch, including unhatched eggs, was known ($n = 66$ clutches; 38 groups). Similarly, broods were only used in the analysis of fledging sex ratio if the sex of all fledglings was known ($n = 82$ broods; 46 groups).

Effect of nestling sex on fledging success and nestling growth

When analyzing the effect of brood sex ratios, the sex of siblings and sex sequences on fledging success and nestling growth, we used the ranks and sexes of young that had actually hatched (i.e., not adjusted for unhatched eggs), since the growth and survival of nestlings can only be affected by extant siblings.

Fledging success was specified as the response variable in a linear mixed model with "group" as the random term. Variables of interest were year, hatch date, group size, helper type, brood sex ratio, sex of hatched nestlings A, B, and C, and sex sequences of hatched nestlings AB, BC, AC, and ABC. Again, group size and helper type were assessed in separate models. The AB sequence was also correlated with group size and helper type (see Results), and was therefore assessed separately. Information on each variable was not available for every nest, so sample sizes in this analysis vary depending on the variables being considered.

To summarize nestling growth, logistic curves of the form $[a/(1 + e^{-K(age - i)})]$ were fitted to the mass and tibia measurements of nestlings that survived to fledging age (Ricklefs, 1971). The three parameters of the equation describe the asymptote (a), the growth constant (K), and the inflection point of the curve (i). Using parallel curve analysis, curves were fitted in steps of increasing complexity to assess first whether asymptotes differed between nestlings, then whether the nonlinear parameters (growth constant and inflection point) differed (Genstat Committee, 1993). Fitting separate asymptotes for each nestling significantly improved the model, but adding separate nonlinear parameters for each nestling did not. Thus, nestlings that achieve a higher asymptote do so by growing more quickly, reaching their "target" in the same time as a smaller nestling (Ricklefs, 1971). Logistic curves were inappropriate for wing-length because wings were still growing on the final nest-visit. Instead, the mean rate of length increase (mm/day) was calculated from the linear phase of growth, between day 10 and the final nest visit (day 30 to 32, depending on hatch rank of nestling).

The three estimates of nestling growth (mass asymptote, tibia asymptote, wing growth rate) were used as response variables in linear mixed models. The REML procedure in Genstat was used to estimate fixed effects and variance components for the random terms "group" and "brood." The deviance explained by a full model was contrasted with that of a sub-model excluding the fixed effect of interest, and the change in deviance was used to assess the significance of terms (Genstat Committee, 1993). Data were available for 125 nestlings (61 broods, 39 groups). Variables of interest were nestling sex and hatch rank, and the number of male and female siblings. Other variables likely to affect nestling growth were also tested: year, hatch date, group size, and brood size. This analysis suggested the growth of focal nestlings was not affected by the sex of their siblings (see Results). A second analysis was performed to confirm this surprising result. Using only broods of three young ($n = 22$), the growth estimates of A, B, and C nestlings were modeled separately to see whether nestling growth of either sex at each hatch rank was affected by the sex or sex sequences of their siblings.

RESULTS

Overall sex ratios

Across all nests, including those where one or two eggs did not hatch, the sex ratio of 242 eggs from 101 clutches (51 groups) was 47.1% male. In 66 clutches (38 groups) where the sex of every egg ($n = 189$) was known, the sex ratio was also 47.1%. Ten eggs which failed to hatch had embryonic material suitable for sexing: six were males, four females. At fledging, 48.6% of 175 young were male (88 nests, 49 groups). Using the binomial test, none of these sex ratios differed from parity.

Clutch and fledging sex ratios

Group size had no effect on clutch or fledging sex ratios (Figure 1a; Eggs, $\chi^2_3 = 6.2$, $p = .1$; Fledglings, $\chi^2_3 = 3.1$, $p = .38$). Figure 1a suggested that unassisted pairs laid more female eggs and fledged more daughters than groups of three or more. However, the differences between unassisted pairs and all larger groups were also not significant (Eggs, $\chi^2_1 = 3.0$, $p = .08$; Fledglings, $\chi^2_1 = 1.5$, $p = .22$). In contrast, when helper type was examined in the model instead of group size, groups lacking female helpers (i.e., no helpers or all-male helpers) had female-biased clutches, whereas groups with female helpers (i.e., all-female helpers or helpers of both sex) had male-biased clutches (Figure 1b; $\chi^2_3 = 13.0$, $p = .005$). By fledging, groups with all-female helpers had very male-biased broods, but groups of other helper types had even or slightly female-biased broods (Figure 1b; $\chi^2_3 = 14.3$, $p = .003$). Clutch and fledging sex ratios were not affected by year or hatch date.

Since the presence of female helpers appeared to have a strong effect on the clutch and fledging sex ratios, we compared the number of males per clutch produced by the same breeding pair when the number of female helpers in their group had increased. These comparisons go forwards and backwards in time, removing the potentially confounding effect of breeder age. When female helpers were added to the group in 13 paired comparisons, the number of male eggs in the clutch increased in seven pairs, was unchanged in six, and never decreased (Wilcoxon paired-sample by rank test, $T = 14.0$, $p = .02$; seven paired comparisons go forward in time, six go backward). In 14 comparisons, the number of males in the brood at fledging increased in six pairs, was unchanged in seven, and decreased in only one pair ($T = 11.0$, $p = .09$; seven paired comparisons go forward in time, seven go backward). Although the sample is small, this is a powerful test of the facultative response of females to changes in the compo-
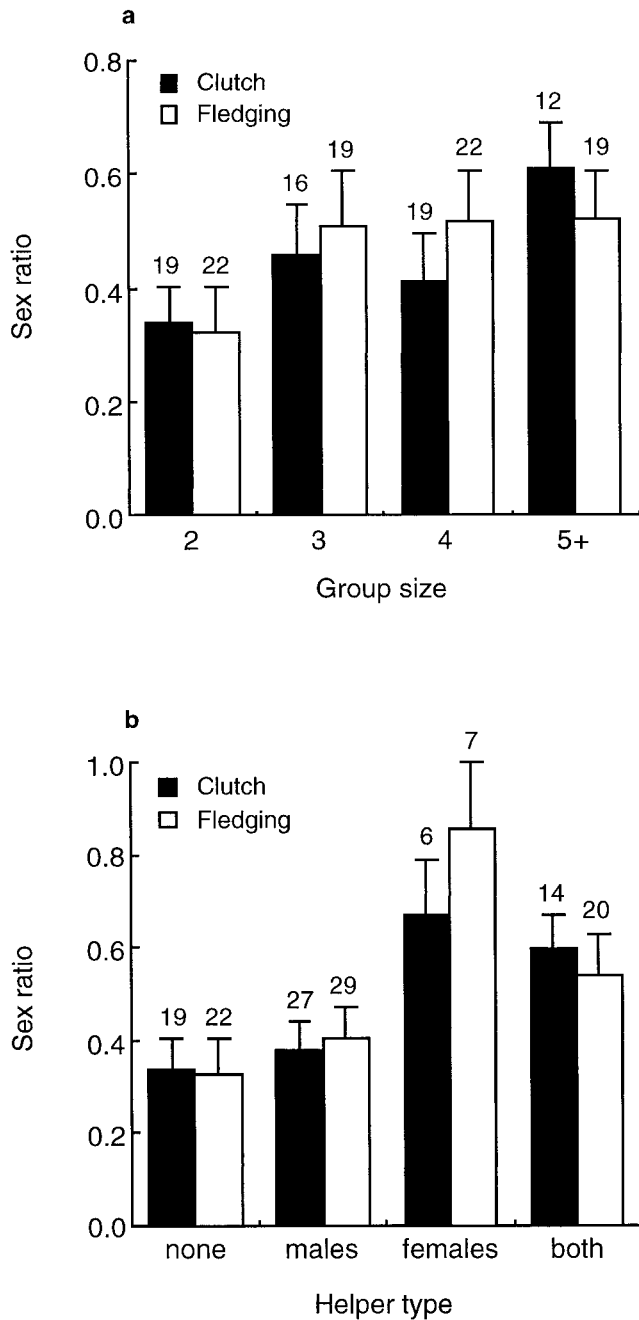
Figure 1
Sex ratio (proportion male) of clutches and broods at fledging for (a) groups of different size, (b) different helper types. Data are presented as means and standard errors, sample sizes above bars. Four groups with an unsexed helper could not be included in (b), hence the total sample is slightly smaller ($n = 78$) than for (a) ($n = 82$).

sition of their group. Individual breeding females respond to the addition (or removal) of female helpers in their group by altering the sex ratio of their clutch, and this probably affects the sex ratio of their brood at fledging.

Hatch rank and nestling sex

Across all nests, A nestlings were predominantly male (58/92 males; 63.0%; binomial test $p = .02$). B nestlings were predominantly female (26/82 males; 31.7%; binomial test $p =$
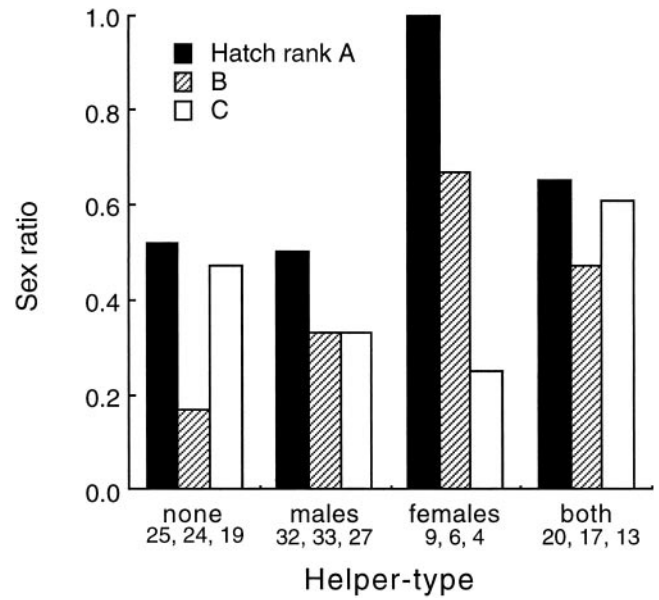

Figure 2
Sex ratio (proportion male) of nestlings in each hatch rank for groups of different helper type. Sample sizes are below each bar.

.002). In contrast, the sex of C nestlings did not deviate from parity (27/63 males; 42.9%; binomial test $p = .5$). These numbers incorporate the sex and deduced hatch ranks of unhatched eggs. Sample sizes dwindle for C nestlings because some were killed by their siblings before a blood sample could be taken for sexing, and some clutches had only two eggs.

Since clutch sex ratios varied between groups with different helper types, we examined whether the relationship between hatch rank and sex was consistent within groups of different helper type. In a generalized model with a binomial error distribution, nestling sex was affected by both hatch rank ($\chi^2_2 = 10.6$, $p = .005$) and helper type ($\chi^2_3 = 11.6$, $p = .009$), but these variables did not interact. Thus, groups of different helper types all maintain the pattern that A nestlings are more likely to be male than B nestlings (Figure 2), even though the clutch sex ratio and the mean sex ratio of A nestlings varies between helper types. Consequently, some helper type and hatch rank combinations displayed extravagant sex ratio biases. For example, the B nestling in unassisted pairs was male in only 16.7% of nests ($n = 24$), whereas the A nestling in all-female groups was male in 100% of nests, although the sample for this last category was small ($n = 9$).

Sex sequences

The sex ratio biases in hatch ranks A and B could arise if certain AB sex sequences are avoided or overproduced. The observed distribution of AB sex sequences was significantly nonrandom. In particular, the FM sequence was very rare ($\chi^2_3 = 15.6$, $p = .001$; see totals in Table 1). This distribution of AB sex sequences could result either because FM was avoided, or because breeders preferred particular sexes at each hatch rank. In this data set, it is impossible to distinguish between these two alternatives. For example, if breeding females avoid producing the FM sequence for the A and B nestlings, the expected overall sex ratios in hatch ranks A and B would be 67% and 33%, which is close to that observed (60% and 32% respectively).

The distribution of BC sex sequences appeared to be nonrandom, although the effect was nonsignificant ($\chi^2_3 = 6.9$, $p = .08$; see totals in Table 1). BC sequences leading with a

Table 1

Distribution of *AB* and *BC* sex sequences in groups with each helper type

Helper type	Sex sequence AB MM	MF	FM	FF	BC MM	MF	FM	FF	Hatch rank sex ratio (% male) A	B	B	C
No helpers	2	10	0	10	2	1	6	8	55	9	17	47
Females only	4	2	0	0	0	2	0	1	100	67	67	0
Males only	7	7	4	11	4	4	4	13	48	38	32	32
Both sexes	5	7	1	3	4	3	4	0	75	38	64	73
Totals	18	26	5	24	10	10	14	22	60	32	36	43

The overall distribution of *AB* and *BC* sequences was nonrandom, and the distribution was also heterogeneous among groups of different helper type (see text). Note the sample sizes are slightly smaller than illustrated in Figure 2 because the sexes of two consecutive eggs are required to compile the sex sequences in this table.

female were more common (FF and FM). However, this is expected since *B* nestlings were predominantly female.

ABC sequences (Table 2) were not distributed nonrandomly, although the sample is reduced because we needed to know the sexes of three consecutive eggs rather than just two (χ^2_7 = 10.0, p = .19). However, the patterns revealed in *AB* and *BC* sequences were still reflected in the *ABC* sequences. The FMM and FMF sequences were rarest (i.e., those where *AB* is FM), and the MFF and FFF sequences were most common (i.e., those where *BC* is FF). An important point pertaining to the mechanism of sex allocation to eggs emerges from Table 2. The sex of sequential eggs in the clutch can switch from one sex to the other more than once because MFM and FMF are not absent sequences.

The distribution of dyadic sex sequences also differed between groups of different helper type (*AB* sex sequences, heterogeneity test χ^2_9 = 19.1, p = .02; *BC* sex sequences χ^2_9 = 21.3, p = .01, Table 1). Once again, this complements the observation that the mean sex ratio at each hatch rank differed between helper types (compare hatch rank sex ratios in Table 1 to Figure 2). Notably, in addition to the general paucity of FM for the *AB* sequence, the MM sequence was rare for pairs without helpers and the FF sequence was rare in groups with all-female helpers. Interpretation of the heterogeneous distribution of *BC* sequences is limited by the shortfall in some *AB* sequences (e.g., *BC* sequences starting with a male must be rarer). However, it appears that the FF sequence was overproduced by groups with all-male helpers (13/17).

Sex sequence and fledging success

The nonrandom distribution of sex sequences suggested that some sequences might influence competitive nestling interactions. Therefore we examined the effect of sex sequences

Table 2

Sequences of *ABC* sexes

ABC sex sequence	No. observed
MMM	5
MMF	7
MFM	6
MFF	9
FMM	3
FMF	2
FFM	8
FFF	11

The expected number of each sequence is 6.375 in each case.

on nestling growth and nest success. Fledging success was lower in groups with the AB sex sequence MF, and in groups where B was female, but these differences were not significant (AB sequence, χ^2_3 = 6.45, p = .09; B sex, χ^2_1 = 3.10, p = .09; Figure 3). However, note that the FM sex sequence for the AB dyad, which is the rare or "avoided" sequence, did not result in lower fledging success, although the sample for this sequence is small (n = 5). Brood sex ratio, the sex of A, C, and the sex sequences BC, AC, and ABC did not affect fledging success. Year, hatch date, and group size also failed to improve the model. However, fledging success differed between groups with different helper types, being lowest in groups with only female helpers (χ^2_3 = 9.3, p = .03; Figure 4).

Sex sequence and nestling growth

Although nest success was not compromised in nests with the taboo FM sequence for the AB dyad, sex sequence might still influence nestling growth. This could be important because fledging weight is a significant positive predictor of juvenile survival (Legge, 2000a). As expected, female nestlings attained greater mass and tibia length than males, but there was no difference in the rate of wing growth between the sexes (Figure 5 and Table 3). Nestling mass decreased with hatch
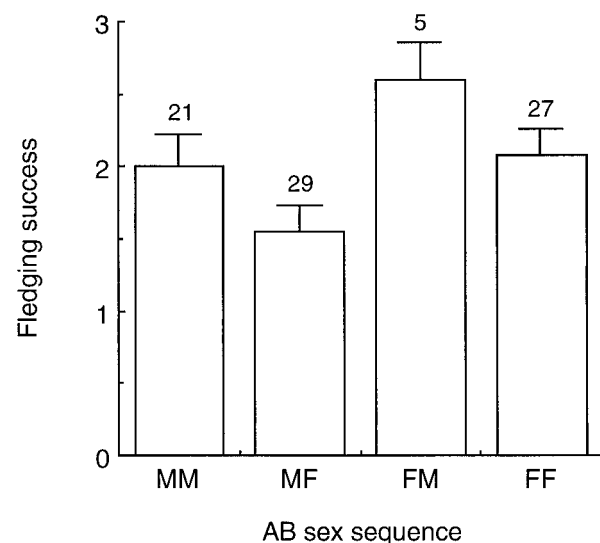

Figure 3
Fledging success from nests with different AB sex sequences. Sample sizes above bars.
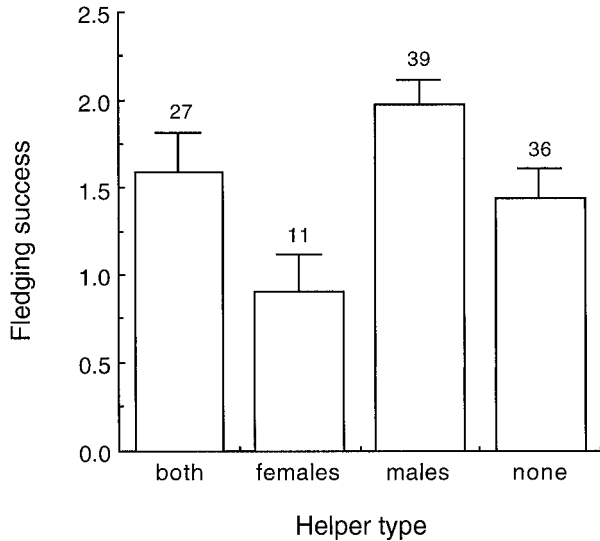
Figure 4
Fledging success for groups with different helper type. Sample sizes above bars. Note that the sample used here is larger than for Figure 3 because broods with unknown sex sequences are included in Figure 4. Means reported in Figure 4 are generally lower than for Figure 3, because Figure 4 includes all clutches, whereas clutches in Figure 3 here must have at least two fertile eggs.

rank, as did tibia length and rate of wing growth (Figure 5 and Table 3). Groups with at least one helper produced heavier and slightly larger nestlings than unassisted pairs (Tables 3 and 4), but again, wing growth was unaffected.

In an exhaustive analysis, there was no evidence that the growth of either sex was adversely affected by the sex of its siblings, or that either sex was a ''poorer'' competitor (Table 3). Figure 5a,b,c show the mass asymptote, tibia asymptote, and wing growth rates respectively for nestlings in each hatch rank, separated by sex. Although the data suggested that males performed poorly compared to females in C position for all estimates, the sex and hatch rank interaction was not significant in any model. Besides hatch rank, nestling sex did not interact with year, hatch date, group size, brood size, or the number of brothers or sisters in the brood. When the analysis was restricted to broods of three young, growth estimates for nestlings in each hatch rank were not affected by the sex of siblings in other hatch ranks, the sex sequence of other siblings, nor any interactions between these variables and the focal nestling's own sex. Again, males in C position did not fare worse relative to females, regardless of the sex of their elders.

DISCUSSION

Across the kookaburra population, the sex ratio of eggs and fledglings did not differ from parity. However, there is strong evidence that the sex of nestling kookaburras is nonrandom, and that offspring sex may be facultatively manipulated in response to two major factors: the helper type of the group and the hatch rank of the nestling. Sex ratios for combinations of helper type and hatch rank varied widely, ranging from 16.7% for the second-hatched nestlings in pairs without helpers to 100% male for the first-hatched nestlings of groups with only female helpers. Comparably large biases have only rarely been reported for other birds, most notably peregrines (Olsen P, personal communication), eclectus parrots (Heinsohn et al., 1997), and Seychelles warblers (Komdeur et al., 1997).
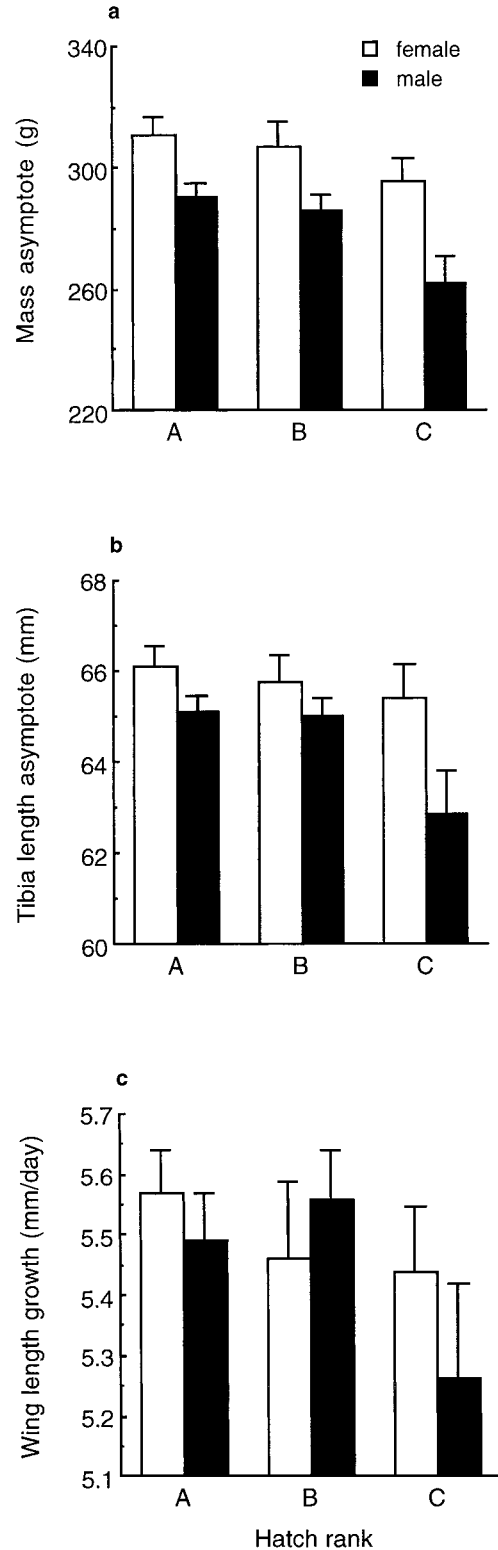

Figure 5
Estimates for (a) mass asymptote, (b) tibia length asymptote, and (c) rate of wing growth for male and female nestlings in each hatch rank. Bars are means and standard errors of estimates; $n = 28, 28, 12$ for females, and 29, 15, 13 for males, in hatch rank A, B, and C, respectively.

Table 3

Summary of analysis of nestling growth estimates, modeled using the REML procedure

Variable of interest	df	Mass asymptote χ^2	Mass asymptote p	Tibia asymptote χ^2	Tibia asymptote p	Wing growth rate χ^2	Wing growth rate p
(a) All nestlings							
Group size	3	**16.6**	**.001**	**8.5**	**.04**	5.7	.13
Sex	1	**23.7**	**.001**	**8.6**	**.003**	0.5	.48
Hatch rank	2	**12.5**	**.002**	**10.9**	**.004**	**6.0**	**.05**
Brood size	2	3.5	.17	2.5	.29	0.8	.67
Year	2	1.6	.45	4.7	.10	3.8	.15
Hatch date	1	1.0	.32	1.0	.32	1.5	.22
Number brothers	2	1.6	.45	0.2	.90	1.5	.47
Number sisters	2	3.9	.14	3.8	.15	0.7	.70
Sex × Hatch rank	2	0.7	.70	1.7	.43	2.7	.26
Sex × Brothers	2	1.4	.50	0.0	1	0.1	.95
Sex × Sisters	2	1.2	.55	3.0	.22	2.2	.33
Sex × Brood size	2	0.7	.70	2.8	.25	2.0	.37
Sex × Group size	3	5.8	.12	1.7	.64	3.4	.33
Sex × Year	2	2.3	.32	0.6	.74	1.7	.43
Sex × Hatch date	1	0.9	.34	0.6	.44	1.1	.29
(b) For A nestlings in broods of three							
B sex	1	2.4	.12	0.0	1	0.3	.58
C sex	1	0.0	1	0.1	.75	0.8	.37
BC sequence	3	5.5	.14	3.1	.38	1.5	.68
A sex × B sex	1	1.3	.25	0.6	.44	0.3	.58
A sex × C sex	1	0.0	1	3.2	.07	0.4	.53
A sex × BC	3	5.3	.15	3.3	.35	2.9	.41
(c) For B nestlings in broods of three							
A sex	1	2.6	.11	0.0	1	1.4	.24
C sex	1	0.6	.44	0.3	.58	2.6	.11
AC sequence	3	2.6	.46	0.4	.94	4.6	.20
B sex × A sex	1	0.3	.58	0.4	.53	2.0	.16
B sex × C sex	1	1.6	.21	0.4	.53	1.5	.22
B × AC	3	0.8	.85	0.6	.90	1.4	.71
(d) For C nestlings in broods of three							
A sex	1	1.1	.29	2.5	.11	0.9	.34
B sex	1	0.0	1	0.2	.65	0.1	.75
AB sex sequence	3	2.6	.46	4.2	.24	1.5	.68
C sex × A sex	1	0.5	.48	0.5	.48	0.1	.75
C sex × B sex	1	2.1	.15	2.1	.15	1.3	.25
C × AB sequence	3	2.0	.57	2.7	.44	0.7	.87

Model (a) includes all nestlings ($n = 125$), with random terms "group + brood." Models (b), (c), and (d) were fitted by restricting the data to nests that fledged three young ($n = 66$), and A, B, and C nestlings, respectively; the random term was group, brood now being unnecessary. The change in deviance is reported for each term. Significant terms are in bold type.

Group composition and the sex ratio

Groups with female helpers, especially if all helpers were female, had male-biased clutch and fledging sex ratios. In contrast, groups lacking female helpers had female-biased clutch and fledging sex ratios (Figure 1b). Paired comparisons showed that individual females responded facultatively to increases (or decreases) in the number of female helpers in

Table 4

Mass and tibia length asymptotes for different group sizes

Group size	Mass asymptote	Tibia length asymptote	n
2	278 ± 5.7	64.1 ± 0.49	37
3	309 ± 6.3	65.9 ± 0.54	27
4	303 ± 4.7	65.4 ± 0.37	40
5+	299 ± 6.8	66.0 ± 0.51	21

Data are presented as means and standard errors.

their group by producing more (or fewer) sons in their next clutch. Empirical examples of facultative biases in sex allocation in response to group composition in cooperative breeders are still rare. Some studies have demonstrated an enhancement effect, producing the "helpful" sex when group size is small (Komdeur et al., 1997; Ligon and Ligon, 1990), but this was not evident in kookaburras: although males are the more "helpful" sex, unassisted pairs produced female-biased clutches.

Female helpers, but not males, have a negative effect on the fledging success of their group (Legge, 2000a). Groups with all female helpers fledge fewer young than any other type of group, including groups with no helpers at all (Figure 4). This is partly because female helpers are poor contributors during breeding, but more complex interactions between group members could also be involved (Legge, 2000b). Breeding females may attempt to limit the number of female helpers in their group to avoid cumulative negative effects on their reproductive success, therefore producing sons if they already have daughter-helpers. During the study, groups seldom had

more than one female helper (10/132 group-years), and helpers were rarely all female (12/132 group-years).

If groups are constrained from producing daughters in some years, they may produce them whenever they have the opportunity, in other words when female helpers are not already present in the group. This could explain the female-biased clutches of groups that lack female helpers. Curiously, unassisted pairs who produce daughters risk becoming all-female groups the following year, which would lead to reduced breeding success. However, unassisted pairs live on the smallest territories of 16–50 ha (Legge and Cockburn, 2000), which may be unable to support additional birds. If resource competition is important, it may pay unassisted pairs to produce the sex that disperses at an earlier age. Although the data are limited, a comparison of dispersal behavior of sons and daughters produced by pairs versus groups is suggestive. Considering only those fledglings that were known to have survived to independence at two months, females hatched to unassisted pairs were more likely to leave their natal territory by nine months (60%, $n = 10$) than males hatched into pairs (17%, $n = 12$; $\chi^2_1 = 4.6$, $p = .03$). In contrast, the difference in sex-specific dispersal rates from groups of three or more birds was much less strong (31% of females leave, $n = 26$; 23% of males, $n = 40$; $\chi^2_1 = 0.6$, $p = .44$). Note that increasing group size through the production of philopatric sons would probably not lead to a corresponding acquisition of territory. Throughout the study, a group's territory changed little in size regardless of variation in group size (Legge S, unpublished data), probably because territory expansion requires a simultaneous retraction in territory area of neighboring groups.

Sex sequences and hatch rank

Nestling sex was strongly dependent on hatch rank. Overall, first-hatched nestlings were predominantly male, and second-hatched nestlings were predominantly female. The sex of third-hatched nestlings was unbiased, but this was unsurprising. Nest productivity is largely determined by competitive interactions between the oldest two nestlings and the third nestling is the least likely to fledge. In many groups the third egg is only laid as insurance against hatch failure of other eggs (Legge, 2000c). A corollary of the relationship between sex and hatch rank was that the distribution of sex sequences was nonrandom. In particular, the FM sequence was rare for the first and second nestlings, only occurring in five nests out of 73. This leads to the question of whether sex sequence affects nestling growth and survival.

FM—the double disadvantage?

It is intuitively appealing that sex sequence in kookaburras—a dimorphic, siblicidal bird—would affect sibling interactions and nest productivity. Could the avoidance of risky sequences underlie the observed relationship between hatch rank and sex? In reverse size dimorphic, siblicidal birds, the male in the FM sequence may suffer a double disadvantage of sex and hatch rank, resulting in elevated levels of siblicide and lower nest success (Edwards and Collopy, 1983). However, in kookaburras there was no evidence that the FM sequence was detrimental. First, nests with the FM sequence (in AB, BC, or AC position) did not fledge fewer young. Only one nest out of five with the taboo FM sequence for A and B nestlings suffered brood reduction, yet the background rate of siblicide is over 40% (Legge, 2000c). The remaining four nests fledged all their young, and fledglings in each brood were above average weight for their hatch rank (Legge S, unpublished data). Second, an exhaustive analysis revealed no convincing evidence that the growth of either sex was affected by the

number or sex of its siblings, or that either sex was a relatively poor competitor. In particular, B and C males did not suffer any additional disadvantage beyond their hatch rank when hatched after a sister.

However, differences in growth and mortality with sex sequence may be extremely small if breeding females only produce risky sequences when they can "afford" to. For example, all five of the FM broods occurred in groups with male helpers, and four occurred in groups of all-male helpers, which are the most able to raise young (see Figure 4). Also, although sex sequence apparently had no impact on nestling growth, the data presented in Figure 3 do hint that males may be poorer competitors than females, since they appear to perform slightly worse when hatched third. Finally, siblicide is particularly common in nests where the AB sex sequence is MF (Legge, 2000c). Hatching a fast-growing female after a male may destabilize the age-based dominance hierarchy, elevating levels of aggression in the nest, and leading to the death of the relatively vulnerable C nestling in the cross-fire. The preponderance of MF dyads in siblicidal nests implies that the sex sequence of nestlings does affect competitive interactions.

Evidence from other species

Evidence for sex sequence effects on growth or mortality from other dimorphic, facultatively siblicidal species is limited, and mixed. To our knowledge, the only other detailed study (besides the one reported here) assessing the effect of sex sequence on nestling growth concerns blue-footed boobies (Drummond, 1991). Like kookaburras, they found that the growth of male blue-footed boobies was not compromised by the presence of an older, and eventually larger sister. Indeed, female booby nestlings were more likely to die in times of food shortage (Torres and Drummond, 1997), as predicted by a model suggesting that mortality will be higher in the sex that devotes most energy to growth (Clutton-Brock et al., 1985). Unsurprisingly, no biases in sex sequence were found. A study of the growth of golden eagles suggested male growth might be adversely affected if hatched after a sister, but the sample was very small (3/5 males had an older sister; Collopy, 1986). Golden eagle fledgling sex ratios became relatively more female-biased in poor food years, but there was no evidence males died from being outcompeted by sisters, or that sex sequence was manipulated (Edwards et al., 1988); see also (Bortolotti, 1989). The best support for the importance of sex sequence for nestling mortality comes from bald eagles. Breeding females avoided the risky sequence (MF in this case) when food was plentiful, but when food availability was low the risky sequence was more prevalent, possibly to encourage efficient siblicide (Bortolotti, 1986; Dzus et al., 1996).

Similarly, evidence for the effects of sex sequence on sibling rivalry in dimorphic but nonsiblicidal species is mixed. Several raptors have been observed to produce a preponderance of females early in the laying sequence, with males later (Dijkstra et al., 1990; Leroux and Bretagnolle, 1996; Olsen and Cockburn, 1991; but see Bednarz and Hayden, 1991). Although these sequence effects have been interpreted in the light of other hypotheses, the fact remains that these birds are producing sex sequences that should reduce nest productivity if the double disadvantage was a significant problem. In studies of nestling growth and/or mortality, the larger sex usually comes off worst when food is limited (e.g., Cooch et al., 1997; Griffiths, 1992; Howe, 1976; Roskaft and Slagsvold, 1985). In contrast, female American kestrels were able to out-compete their smaller brothers, but only under particular circumstances, when food was limited and monopolizable (Anderson et al., 1993). The ability of older nestlings to monopolize food

may explain variation in the consequences of sex sequence between species. Kookaburra nestlings are brought highly monopolizable food—items are 2–4 cm long, and swallowed immediately by a single nestling. Nestlings that use their age and/or sex advantage to control access to the premium feeding site at the hollow entrance should be at a distinct advantage.

Why order the sexes of offspring?

Besides the effect of sex sequence on nestling interactions, we can suggest one alternative explanation for ordering the sexes of offspring in a brood. Although kookaburras usually lay three eggs, brood reduction claims the C and sometimes the B nestling in nearly half of kookaburra nests between hatching and fledging (Legge, 2000c), and fledging success is generally low (1.4 fledglings per nest). Assuming all three eggs hatch, breeding females can be fairly confident of fledging one young, fledging two is possible but uncertain, and fledging three less likely. In small clutches with variable brood reduction, females may match sex to hatch ranks with a conditional strategy. That is, if she should only manage to fledge the A nestling, what sex should it be? If she succeeds in fledging B as well as A, what sex should B be, given the sex of A, and so on. The strength of sex biases in each hatch rank should be aligned with how badly the breeder ''wants'' a particular sex, and the probability she has of fledging successively-hatched nestlings. For example, kookaburra groups of all-female helpers have extremely male-biased clutches, indicating a general preference for sons. They are also likely to produce just one fledgling. Unsurprisingly, every first-hatched nestling from broods of all-female groups was a male. In addition, groups with all-male helpers were the most likely to fledge the C nestling, and these were the only groups that appeared to control the sex of the C nestling.

Heterogeneity between groups with different helper types

Because clutch sex ratios varied among groups of different helper type, there was heterogeneity in the hatch rank sex ratios (and therefore the distribution of sex sequences) between helper types. For the *AB* sequence, one strong pattern was superimposed on the general avoidance of FM sequence. In contrast to groups with helpers, unassisted pairs also avoided the MM sequence, nearly always hatching a female second (Table 1a). Siblicide is more common in broods of unassisted pairs, and the MF sequence is part of a suite of variables that characterize siblicidal nests (Legge, 2000c). Thus, it is unsurprising that MF dyads are common in broods of unassisted pairs. But this does not explain why FF is common, and MM rare. It may simply be an artifact of the general female-biased clutches of unassisted pairs, aiming to produce dispersive daughters rather than philopatric sons. The MM sequence may be rare because it would result in a clutch sex ratio of at least 67%. Although the sample for all-female groups is too low to draw firm conclusions, they may avoid FF for similar reasons: a clutch with a FF sequence is automatically female-biased, but all-female groups prefer to produce male-biased clutches.

Another strong pattern emerges from the heterogeneous distribution of *BC* sequences. The FF sequence is overrepresented in all-male groups, meaning C eggs are more likely to be female. The C egg has the highest chance of fledging in all-male groups, perhaps explaining why control of offspring sex extends further down the sequence than for other groups. Similarly, groups with helpers of both sex, which generally produce male-biased clutches, may avoid the FF sequence, thus producing more males in third position. After all-male

groups, groups with both sexes helping are the next most likely to fledge the C egg. However, the sample for this helper type is rather small.

Conclusions

The sex of kookaburra nestlings may be influenced facultatively by mothers in response to several selective pressures, demonstrating the potential complexity of sex ratio variation (see Cockburn, 1994 for a similarly complex example in mammals). Enhancement or repayment models at the level of individual families (Komdeur et al., 1997) do not explain the observed patterns. Local resource competition may be important in some contexts (i.e., in unassisted pairs). However, the make-up of the social group had a strong effect—breeding females avoid overburdening themselves with daughter-helpers by producing sons almost exclusively if daughter-helpers are already present in their group. Finally, there were very strong hatch rank effects, suggesting that the sequence of sexes in the brood affects the quality or quantity of young. Although there was little supporting evidence for this, experimental manipulations are required to create the risky sequence in nests that would otherwise avoid it.

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