Extreme bias in sex allocation in *Eclectus* parrots

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SUMMARY

We investigated extraordinary patterns of sex allocation in captive eclectus parrots (*Eclectus roratus*). These birds are extremely unusual as they show reverse sexual dichromatism, they are the only cooperatively breeding parrot, and they are one of the few birds with nestlings that are easily sexed. They lay two eggs per clutch, but often only fledge one young, and the sex ratio of 209 fledglings did not differ significantly from parity. However, when two young are fledged together they are very likely to be of the same sex, and some females produce long unbroken runs of one sex (the maximum was 20 males) before switching to the other sex. Monte-Carlo simulations show that these runs of same-sex clutches defy expectation if we assume that the sex of chicks within each clutch is independent of the previous clutch. We use further simulations to show that the sex bias must occur at fertilization (i.e. the primary sex ratio), although the female may make further adjustments via infanticide. Control over sex allocation in eclectus parrots is one of the most extreme reported from birds.

1. INTRODUCTION

Theory predicting benefits to parents that control relative investment in male and female offspring has received support from studies on plants, invertebrates and some vertebrates. However, evidence that birds control the sex of their offspring remains scarce (Clutton-Brock 1991; Frank 1990), partly because assigning sex to nestlings is usually difficult (Lessells & Mateman 1996). Despite their rarity, the few reported examples of sex allocation biases in birds suggest diverse adaptive benefits. For example, biased sex allocation has been reported when the reproductive value of sons and/or daughters varies either with laying date (Dijkstra *et al.* 1990) or laying order (Ankney 1982; Leroux & Bretagnolee 1996). Females may also adjust the sex ratio of their offspring according to the attractiveness or condition of themselves or their mates (Ellegren *et al.* 1996; Wiebe & Bortolotti 1992). Alternatively, competitive and cooperative interactions between parents and offspring of either sex may alter the benefits of producing one sex over the other (Komdeur *et al.* 1997; Gowaty & Lennartz 1985).

The mechanisms that produce the sex bias are equally as interesting as the adaptive benefits (Krakow 1995). Possibilities include manipulation of the primary sex ratio at fertilization, selective infanticide, or unequal allocation of resources to offspring depending on their sex, or siblicide of young. There are too few examples to evaluate which mechanisms predominate. In Seychelles warblers, *Acrocephalus sechellensis*, skews in the sex ratio of offspring arise through biased production (i.e. the primary sex ratio), rather than through

differential mortality (Komdeur *et al.* 1997). Ellegren *et al.* (1996) also suggest that female collared flycatchers, *Ficedula albicollis*, are able to adjust the sex ratio of eggs ovulated in response to the phenotype of their social mate.

Eclectus parrots (*Eclectus roratus*) are ideal for the study of sex allocation because unlike the vast majority of birds, their nestlings develop sex-specific beak and plumage coloration soon after hatching. Unusual sex allocation patterns in these birds were brought to our attention by anecdotal reports from captive breeders. For example, Low (1986, p. 143) reports that 'a predominance of chicks of one sex is not at all unusual... One Eclectus in Chester Zoo produced 30 sons before the first daughter was produced'. Eclectus parrots are also unusual in other ways. First, they are the only parrot known to breed cooperatively; breeding groups of up to ten birds are comprised mostly of males, and probably persist into the non-breeding season and between years (Forshaw & Cooper 1989, personal observations). Second, they show reverse sexual dichromatism, with the vermillion females and green males so different that for decades they were thought to be separate species (Forshaw & Cooper 1989). This reverse dichromatism is not associated with reversed sex roles (see examples in Andersson (1994)) as, in typical parrot fashion, the male provides food for the incubating female, and later for nestlings (Forshaw & Cooper 1989).

In this paper, we use long-term records from captive breeders to investigate sex allocation in eclectus parrots. We show that regardless of the mean offspring sex ratio, females have strong temporal control over the production of each sex. We identify a biased primary sex ratio

Table 1. *Sequences of fledglings produced by 12 female eclectus parrots, and tests for non-random sex allocation*

(These are (i) the probability that the female's particular distribution of runs of clutch types occurred by chance; (ii) the probability that her longest run of clutch types occurred by chance; and (iii) the probability that her number of runs occurred by chance if the 'unwanted' sex is killed. Female 8 (marked by an asterisk) was analysed using runs of individual chicks as her number of clutches was unknown. No tests were applied to females 9–12 due to insufficient sample sizes. Abbreviations: m, male; mm, male–male; f, female; ff, female–female; mf, male–female; u, unknown; na, insufficient data.)

female	sequence of clutches	number of clutches	number of fledglings	probability (i) (distance of runs)	probability (ii) (longest run)	probability (iii) (if infanticide)
1	f,f,f,f,f,f,f,f,m,f,ff,f,f,ff,m,f,m,f, m,f,f,f,f,ff,m,m,m,m,m,f,f,f,ff	36	40	0.040	0.304	0.130
2	m,m,m,m,m,m,m,m,m,m,m,m,m,m,m,m, mm,m,m,m,m,m,m,f,f,f,f,f,f,f,f,f, f,f,f,ff	28	33	<0.001	<0.001	<0.001
3	m,mm,mm,m,m,m,m,m,m,f,f,f,f, f,f,f,f,f	17	20	0.006	0.022	0.007
4	ff,ff,f,ff,mf,mf,m,m,m,m,m,ff,ff,ff	14	22	0.118	0.211	0.136
5	ff,f,f,mf,mm,mm,m,mm,mm,m, mm,mm	12	20	0.374	0.085	0.062
6	m,m,m,m,f,f,f,f,f,f,f	11	11	0.030	0.102	0.041
7	ff,mf,f,f,ff,f,mm,m,mm,f, ff	10	16	0.278	0.788	0.512
8*	10m, 12f	u	22	<0.001*	0.004*	na
9	mm,f,m,mm,f,ff	6	9	na	na	na
10	f,f,f,f,f,mf	6	7	na	na	na
11	mf,ff,m,ff,m	5	8	na	na	na
12	1f, 6m, 1f	u	8	na	na	na

as the probable mechanism of control, although secondary adjustments after hatching cannot be ruled out.

2. METHODS

We collected breeding records from five aviaries in three Australian states—the names and addresses of the aviculturists are available from the authors—and used the sexes of 209 fledglings from 12 females (subspecies *E. r. polychloros*) for which we have unbroken records ($n = 7\text{--}40$ fledglings per female, representing up to 12 years of reproduction for each). Most of the breeding females were still alive when the records were obtained from the aviculturists, so the data do not represent lifetime reproductive success. The success of all clutches was recorded by the aviculturists, and there were no complete failures. One breeder only recorded the number and sex of fledglings, but not the number of clutches, from two of his females. Eclectus parrots almost invariably lay a clutch of two eggs, but in our sample only 41 out of 145 broods fledged both young, whereas the remaining 104 produced only one fledgling. Because eclectus females are sensitive to intrusion when on eggs and nestlings (Low 1986), the aviculturists rarely examined them at this stage. Thus, all records pertain only to the sex of fledglings, and the sex of dead chicks (and, therefore, the sex ratio at hatching) is unknown. We test for departures from parity for the overall sex ratio, and the sex ratio produced by each female. We also examine the distribution of sex from clutches where both fledglings survive. We then use indirect simulation techniques to examine whether the runs of fledglings from each female conform to expectation if the sex of chicks in a clutch is independent of the previous clutch, and whether observed biases are produced at fertilization or after hatching (e.g. by infanticide).

(a) *Population and individual sex ratios*

We analysed both the overall sex ratio of offspring and that produced by each female. The pooled sample tests for a

population skew towards a particular sex, but the direction of bias may not be consistent for all individuals, and any such heterogeneity between females may be of further interest. However, we note that heterogeneity between females cannot be explored satisfactorily using this data set because later tests show that the sequences are not independent. Thus, there is the danger that biases may be detected that only reflect the period for which the female was sampled.

We calculated the goodness-of-fit of both the total data set and that for each female to a 0.5 sex ratio. We also tested whether the distribution of sexes in the 41 clutches where two young were fledged conformed to expectation if each sex is produced with equal probability (i.e. 25% male–male, 50% female–male, and 25% female–female).

(b) *Runs of fledglings*

The data are striking because individual females often produce long runs of one sex before switching to the other sex (table 1). For example, in the most extreme case, one female produced a run of 20 males before switching to producing 13 females. We examined whether these single sex runs defy expectation by using two modified runs tests (Morgan 1984) which explore the likelihood of repeatedly producing clutches of the same sex. A feature of this data is that we have a sample of clutches, and then chicks within clutches. To reflect this characteristic, we classified the clutches of a particular female into male clutches (either male–male or single-male), female clutches (either female–female, or single-female), and mixed clutches (male–female) depending on the fledglings produced, and set the probability of each clutch type equal to the observed proportion of clutches for each bird. This allows for possible heterogeneities between females, and makes no assumptions about the sex ratio. While considering clutches is more complicated than considering the individual chicks, we believe it is more intuitive to treat the clutch as the event of interest. It also avoids the problem of male–female clutches where we do not know which

came first in the sequence. Unfortunately, it was not possible to perform this test on four females with insufficient clutches (females 9–12, table 1). Another female (female 8) with a large number of fledglings, but whose clutch records were incomplete, was analysed separately using runs of individual chick sexes rather than clutch types. This is not entirely satisfactory due to the complication of variable clutch sizes; however, it is obvious that the results of this test, and the test in which we know the clutch history, should be highly correlated.

In the first test, we explored the likelihood that each female produced her particular distribution of clutch run-lengths by chance. We tabulated the number of runs of clutch types of length 1, 2, ..., 7+ for each breeding female. Runs of seven and above were treated as a single category. Sequences of clutches for each bird were bootstrapped (sampled 5000 times with replacement up to the observed number of clutches) to determine a Monte-Carlo estimate of the sampling distribution of the runs statistic, assuming the ordering of clutches is in fact independent. A test statistic, T , for each female was then calculated as the quadratic form:

$$T = [\mathbf{Y} - U] \text{inv}(S) [\mathbf{Y} - U],$$

where $\mathbf{Y}$ is the vector of observed counts, U and S are the mean and variance of the bootstrap distribution of T , and $\text{inv}(S)$ is the matrix inverse of S . This statistic measures the distance away from the expected counts under the null distribution. The bootstrap sample was then used to generate the null distribution of T , and a p value was calculated by finding the proportion of samples larger than our observed statistic. Proportions less than 0.05 were considered to support the hypothesis that the observed runs of clutch type were non-random if produced with equal probability.

The previous test is conservative as it does not necessarily take into account the longest single sex run of each female (i.e. the largest run used is seven), but it has the important property of examining the distribution of runs. Its limitation is that it does not specifically test the nature of the non-randomness, only detect that it is present. To investigate whether the departure from non-randomness could be due to runs longer than usual, we also considered a second test. This examines the likelihood that the longest single sex run of each female could be produced by chance given her total output. We used the longest run of each female as the test statistic, and calculated the proportion of the 5000 bootstrapped samples that had a run greater than or equal to the longest run seen in the sequence. We again considered proportions less than 0.05 as significant.

(c) *Are observed biases due to the primary sex ratio?*

Finally, we investigated whether the observed departures from expectation result from biases at fertilization, or whether females modify their investment by behavioural strategies such as infanticide of the unwanted sex, or preferential feeding of the desired sex. For example, a large run of male fledglings might result from all fertilizations producing male chicks, or from the mother killing female chicks. Although we do not have information on the sex of all hatchlings, we can examine whether the observed runs of fledgling sex can be explained by differential mortality. If the data still do not conform to expectation once sex-specific mortality is incorporated into the simulations, the hypothesis of biases at fertilization is supported.

To carry this out, we used as our test statistic for each of seven females with known number of clutches the number of runs in the sequence, as an observed number of runs less than expected implies biases at fertilization. We compared this to a

theoretical distribution constructed by assuming (i) that the birds always kill a particular sex, and (ii) that at least one chick always survives. Thus if the female always kills male chicks, and two males are hatched, then only one will die. If the female had k clutches, we randomly simulated k new clutches using a 50:50 sex ratio. This ratio was chosen to reflect the overall sex ratio in the samples, and to serve as a plausible null distribution. We then allowed the unwanted sex (if present) to be 'killed', and recorded the number of runs produced in the sequence. We repeated this process 5000 times, and calculated a probability by finding the proportion of simulated values less than or equal to the number of runs observed in the data.

In order to gain some insight into the test, consider the following. If no differential mortality is occurring, then the possible outcomes are MM, MF, FM, FF, with a probability of 0.25 for each. If males are being killed, we observe M, F, F, and FF in each case. Thus, there is a probability of 0.75 that a particular run of females continues. By assuming that a female always kills a particular sex in our simulations, we ensure that the number of runs is minimized compared to that produced if the female switches the sex it kills. Thus, a significant result implies that the runs are highly implausible, and supports the hypothesis that processes beyond infanticide are involved.

3. RESULTS

(a) *Sex ratio at fledging*

Table 1 shows the sequences of offspring produced by each female. Overall, the sex ratio of fledglings was 46% male (97 out of 209), which did not represent a significant departure from parity ($\chi^2 = 2.24$, d.f. = 1, $p > 0.10$). On closer inspection, two females had sex ratios that differed significantly from parity on their own (female 1, $\chi^2 = 12.1$, d.f. = 1, $i < 0.001$; female 5, $\chi^2 = 5.0$, d.f. = 1, $p < 0.05$). As mentioned previously, we present these results tentatively, and reiterate that the lack of independence between data points (shown below) makes conclusions about sex ratios from individual females difficult due to the problem of truncated records.

The subset of clutches ($n = 41$) where two young were fledged consisted of 18 female–female pairs, 17 male–male pairs, and only six male–female pairs. This differed markedly from expectation ($\chi^2 = 20.6$, d.f. = 2, $p < 0.001$).

(b) *Runs of fledglings*

Four out of seven females with sufficient sample size and known clutch histories showed distributions of single sex runs that differ from expectation if each clutch type was produced independently (i.e. $p < 0.05$, test 1, table 1). From the binomial distribution, the probability of observing four or more such results out of seven is less than 0.001. The remaining female with sufficient sample size but with an unknown clutch history also had an unexpected distribution of single sex runs ($p < 0.001$, table 1).

In the second test, the longest run of same-sex clutches for two out of seven females defied expectation given the total output of each ($p < 0.05$, table 1). The probability of obtaining two or more such results out of seven is low (binomial test, $p = 0.044$). The length of

the longest run also defied expectation in the remaining female for which clutch history was unknown ($p = 0.004$). Collectively, these tests provide strong evidence that at least some of the birds produce non-independent sequences, and that in some cases this is because the females produce runs that are longer than expected.

(c) *Primary sex ratio*

The number of runs of single sex clutches are fewer than expected for three out of seven females, even after allowing for the unwanted sex to be killed (table 1). The probability of observing three or more such results is 0.004. Thus, the data strongly support the hypothesis of biases at fertilization, but do not rule out further adjustments through infanticide or selective resource allocation.

4. DISCUSSION

Until recently, biases in offspring sex ratios in birds have been considered rare (e.g. Clutton-Brock 1991). However, new molecular techniques for sexing birds (Lessells & Mateman 1996) are bringing more cases to light, forcing a reappraisal of both the mechanisms and adaptive value of producing one sex over the other (e.g. Ellegren *et al.* 1996). Most reported cases of skew are small, but exceptional biases occur in the Seychelles warbler, where females without helpers on high and low quality territories produced 13% and 77% sons, respectively (Komdeur *et al.* 1997). Sex allocation is also extremely biased in eclectus parrots. The early development of sex-specific plumage and bill coloration in these birds is unusual, and the ability to sex nestlings may allow females to adjust the sex ratio of their clutch after hatching. However, the data presented here show that females have at least some control over the primary sex ratio, even if later adjustments also occur.

Although the sex ratio of offspring does not appear biased either in the pooled sample or for most individual females, we hesitate to draw conclusions due to the lack of independence between data points. Instead, we emphasize the implications of the sexes not being produced with equal probability at all times. First, when two fledglings are produced, they are very likely to be of the same sex, with only six out of 41 clutches producing male–female combinations. Second, females often produce long runs of first one sex and then the other, and our simulations have shown that both the distribution of these runs and the length of the longest run seen in each sequence are highly improbable if clutch types are produced independently. However, as most females only produce one fledgling, it was necessary to run further simulations to determine whether same sex broods and long runs of one sex result from biases at fertilization, or whether mixed-sex broods suffer differential mortality.

It is pertinent that the female alone incubates eggs and broods nestlings; even food brought by other group members is passed to the female at the entrance to the nest hole (Forshaw & Cooper 1989). The sex of surviving young therefore appears to be determined by the

breeding female or the nestlings themselves, and not by the other individuals in the group. If the female controls sex allocation, she may do so either by manipulation of the primary sex ratio (i.e. at fertilization), or after hatching through infanticide (i.e. direct aggression or starvation of one chick). If the nestlings are in control they must use siblicide. However, whereas infanticide may be directed at either the first or the second hatched chick, siblicide would be most likely to affect the younger chick because of the pronounced hatching asynchrony seen in these birds (Low 1986; Forshaw & Cooper 1989). Unless the female consistently produces the desired sex first in the hatching sequence, she would be less likely to produce long unbroken strings of the preferred sex. In either case, our third simulation model indicates that even if females kill offspring of the unwanted sex or siblicide occurs, the observed patterns are extremely improbable if the primary sex ratio is at parity. Thus, the most likely stage for bias to occur is at fertilization, possibly by controlling the sex of eggs ovulated (Krackow 1995). Although bias occurs at this early stage, we cannot rule out that females might also use infanticide or siblicide for fine-tuning. Indeed, one of the aviculturists who provided data suspects her breeding female of infanticide and cannibalism, and notes that she never finds the remains of dead nestlings.

Most information on wild eclectus parrots is anecdotal, although it is clear that they live in cooperative groups comprised mostly of males which defend the area around their nest holes in rainforest trees. Indeed, the whole adult population appears strongly male-biased (Forshaw & Cooper 1989, personal observations). If males are predominantly the helping sex, it may be adaptive for breeding females to produce males when they need help in reproduction and territory defense, and females when group size is large enough and any further helpers detrimental. This is precisely what has been shown to occur in the Seychelles warbler, in which females skew their single-egg clutch towards the helping sex (females) when their territory is of high enough quality to support more birds, and towards the dispersing sex when their territory is of low quality (Komdeur *et al.* 1997). However, all females in our data set were in pairs without helpers, and it is common avicultural practice to remove and sell young soon after fledging. None of the females showed the consistent bias towards males that would be expected if they were constantly trying to produce helpers.

In our sample, the lack of any overall bias towards male fledglings suggests that the male-biased adult sex ratios seen in the wild result from higher female mortality post-fledging. Further, the bizarre coloration of eclectus parrots indicates strong independent sexual selection on each sex. Together, these suggest that male and female offspring have different reproductive values, and that each sex should only be produced under particular circumstances (Trivers & Willard 1973; Leimar 1996). We cannot rule out that wild eclectus parrots respond to factors such as territory quality or social circumstances (e.g. Komdeur *et al.* 1997), but our data on captive birds breeding in pairs suggest that they choose which sex to produce according to other cues

(for example, their own health and condition; Trivers & Willard 1973). Although our data do not provide the adaptive explanation, eclectus parrots provide an exciting demonstration of a bird where females are capable of controlling the sex of the offspring they produce to an extraordinary degree, adding to the singular and challenging peculiarities of this species.

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