Evolutionary Ecology Research, 2008, **10**: 907–912

Unbiased sex ratio among nestling echo parakeets *Psittacula eques*

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ABSTRACT

Question: Is the male-biased sex ratio observed in wild adult populations of the critically endangered echo parakeet potentially a consequence of inbreeding?

Study subjects: DNA samples were analysed from chicks over a 3-year period.

Method of analysis: Molecular screening enabled the primary sex ratio to be determined in a sample of sexually monomorphic nestlings.

Conclusion: No bias in sex ratio was observed in chicks, suggesting that the departure from equality observed among adults is due to differential mortality. Sex ratio bias can occur due to a number of environmental and physiological factors. The implications to the current conservation management of this species in relation to sex ratio bias are discussed.

Keywords: conservation management, echo parakeet, *Psittacula eques*, sex ratio.

INTRODUCTION

The echo parakeet *Psittacula eques* is a critically endangered bird endemic to Mauritius, and is thus listed in CITES Appendix I. Its conservation was considered a priority in the IUCN Action Plan for Parrots (Snyder *et al.*, 1999). Threats to this species include loss of native habitat, seasonal shortages, and natural disasters (Thorsen *et al.*, 1998). By 1986, the population was at an all-time low, estimated at between 8 and 12 individuals, of which only three birds were female (Jones, 1987; Jones and Duffy, 1993). Following intensive conservation management by the Mauritian Wildlife Foundation, the population of echo parakeets began to recover, substantially so since 1995, to an estimated wild population of between 276 and 302 in April 2005 (J. Malham, personal communication). Historically, counts of wild birds have recorded a consistent bias towards males in the adult sex ratio (Thorsen *et al.*, 1998). The data presented in this paper relate to the wild nestling and adult population, prior to the release of captive-bred birds aimed at supplementing the wild population (Harper *et al.*, 1999).

Biased sex ratios have been recorded both in nature and in experimentally inbred populations (Jarrell, 1995; Dermitzakis *et al.*, 2000). Since the echo parakeet population has

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undergone a severe genetic bottleneck, the possibility exists that the observed bias may have been a consequence of inbreeding. If this was the case, then the bias may be apparent in the primary sex ratio of the chicks. Although adult echo parakeets are sexually dimorphic, it is not possible to determine the sex of young birds externally. This study employed a molecular technique to establish the sex of the phenotypically monomorphic nestlings using blood and feather samples.

Fisher's equilibrium theory of sex ratio suggests that parents should place equal investment in offspring of both sexes where they are equally costly to produce (Fisher, 1930). Traditional sex ratio theories were generally based on insects that have little or no parental care. Modifications to Fisher's theory have been developed that are more applicable to birds. These theories are complicated since several environmental and physiological factors may influence the sex ratio (Komdeur and Pen, 2002; Pike and Petrie, 2003) and these may vary for an individual species under different environmental conditions. In addition to inbreeding depression, the sex ratio may be influenced by a variety of parameters, including the external environment (e.g. season), parental quality, the social environment (e.g. cooperative breeding), and sexual dimorphism (Komdeur and Pen, 2002).

One facet of echo parakeet breeding biology that might be relevant to the observed sex ratio is its tendency to polyandry, or the formation of breeding groups containing multiple males. For most parrots, monogamy appears to be the rule, although there is some evidence to suggest that echo parakeets may not be monogamous (T.D. Taylor and D.T. Parkin, in preparation). In 1998, of the 14 breeding groups of wild echo parakeets, only 5 comprised a traditional breeding pair – one male and one female. The other nine each consisted of one breeding female and between two and four adult males (Thorsen *et al.*, 1998), with one male in each group appearing dominant. This may be relevant because if cooperative behaviour does occur, which has not been determined, it has been shown that the 'helping' sex usually predominates in any bias in sex ratio (Faaborg and Bednarz, 1990; Komdeur *et al.*, 1997; Reyer, 1990). Another possible reason for the presence of the auxiliary males is that the biased sex ratio could lead to a shortage of female partners, although it is unclear whether on average they help or hinder breeding, since there is evidence they may do both (Jones, 1987).

Previous research also suggests that some birds may be able to control segregation of the sex chromosomes and thus adjust the sex of their offspring (Dijkstra *et al.*, 1990; Nager *et al.*, 1999). The eclectus parrot *Eclectus roratus* is a member of the same family, Psittaculidae, as the echo parakeet, and appears to be able to manipulate the sex of its offspring (Heinsohn *et al.*, 1997). In another parrot, the kakapo *Strigops habroptilus*, male bias among offspring has been associated with females that receive supplementary feeding (Clout *et al.*, 2002), a management technique that has been used in the conservation of echo parakeets. Conversely, in the crimson rosella *Platycercus elegans*, female bias was associated with seasonal environmental factors (Krebs *et al.*, 2002). Sex ratio bias may be more common than expected for this family, as 5.6% of 89 parrot species examined showed a significant bias towards males (Taylor and Parkin, 2008).

METHODS AND MATERIALS

The sex of adult echo parakeets can be determined from bill coloration. In females, both mandibles are black, whereas in males the upper mandible is red. Nestlings and juveniles have orange/red mandibles that turn black at about 4–5 months of age; in males, the upper mandible subsequently turns red at approximately 2 years of age. Figures for the number of wild males and females observed in the wild population were taken from Thorsen *et al.* (1998) and represent the adult wild population at the beginning of 1998, the end of the 1997–98 breeding season. These data relate to the adult population prior to the release of captive-bred birds, biased towards females, to supplement the wild population (Harper *et al.*, 1999).

Samples for chicks represent offspring from the three preceding years, and were analysed by year. To determine the sex of chicks, DNA was extracted from blood or tissue samples collected over a number of seasons as part of routine management and for a separate study (Groombridge, 2000). DNA extraction was performed using either standard Phenol/Chloroform or Chelex methodologies (Taylor, 2001).

DNA was amplified by polymerase chain reaction (PCR) using the avian sexing primers P2 and P8 (Griffiths *et al.*, 1998). PCR amplification was in 20-μl reactions containing approximately 20–50 ng DNA template, 1× reaction buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl, 0.01% Tween20), 1 U *Taq* polymerase (Red Hot *Taq*, Advanced Biotechnologies), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 100 μM of each dNTP, and 10 μg BSA. Amplification was by MJ PTC-200 Peltier Thermal Cycler consisting of an initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C/45 s, 48°C/45 s and 72°C/45 s, and a final extension at 72°C for 5 min. PCR products were run on a 2% agarose gel at 100 V. The significance of sex ratio bias was analysed via the probability that the number of observed males was significantly different from equity, calculated using chi-squared and the normal approximation of the binomial distribution. A chi-squared heterogeneity test was undertaken to ensure homogeneity across the separate years in which offspring were sampled.

RESULTS

The sex ratio and the percentage of males among adult and young birds are provided in Table 1. The sex ratio among the adults in the wild breeding groups differed significantly from parity. However, the ratio among nestlings sexed by molecular analysis was close to unity for each of the previous three years and significantly different from the adult birds. Homogeneity of samples obtained for the offspring over the three years was observed (heterogeneity χ^2 not significant, $P = 0.248$, d.f. = 2).

Table 1. Number and ratio of male and female echo parakeets

	Female	Male	Male : female sex ratio	% Male	χ^2	χ^2 P	Binomial distribution
Wild adults observed in 1998	15	34	2.27	69.4	7.39	0.007	0.009
Offspring 94–95	3	8	2.67	72.7	2.36	0.124	0.227
Offspring 95–96	4	5	1.25	55.6	0.22	0.637	1.000
Offspring 96–97	13	10	0.77	43.5	0.43	0.510	0.678
All offspring	20	23	1.15	53.5	0.23	0.630	0.761

Note: Binomial and χ^2 analysis showed the sex ratio of wild male adults to be significantly greater than that of females. There was no significant difference in the nestling offspring sex ratio.

DISCUSSION

The sex ratio of nestling echo parakeets was close to parity, whereas the wild adult population, prior to additional birds being released, showed a significant excess of males. Near equality in the sex ratio of the nestlings implies that the bias is unlikely to be related to inbreeding, since this would affect the primary sex ratio. However, it cannot be assumed from this that inbreeding does not occur, as its impact may occur at a different stage in the life cycle. Despite the recent history of the echo parakeet, a good degree of genetic variation appears to have been retained in the wild population (T.D. Taylor and D.T. Parkin, in preparation), which might also argue against problems relating to inbreeding and/or genetic drift. The question remains, therefore, when does differential mortality between the sexes occur?

Obtaining data relating to the mortality of many species of birds between fledging and maturity is difficult because of their dispersal and the difficulty of observation outside the breeding areas. Estimates of mortality of adult echo parakeets indicate that it may be about 4% per annum; for juveniles it is about 9.5%, and it may be higher in sub-adults (Thorsen *et al.*, 1998). In New Zealand, introduced predators have been implicated in the increased vulnerability of female kaka during the nesting season (Moorhouse *et al.*, 1999), and there is some evidence that female echo parakeets are also more susceptible to predation during the nesting period (Harper *et al.*, 1999).

Other factors may be relevant to an elevated female mortality. Seasonal shortages of food have been identified as a problem for the echo parakeet, and may impair the physical condition of females prior to nesting (Jones and Duffy, 1993). In the kakapo, supplementary feeding has been implicated as a cause of male bias (Clout *et al.*, 2002). Breeding females that received supplementary food were in better physical condition and produced 72% male offspring; those not fed produced only 26.7% males. Evidence from the relationship between sex and laying order suggested this occurred at the primary sex ratio (Clout *et al.*, 2002). Historically, the wild echo parakeet population took little of the supplementary foods offered. However, more recently released birds have been trained to use the feeding stations and the number of wild birds learning to use the feeding hoppers has increased each season (Malham *et al.*, 2003).

The existence of multi-male breeding groups may be a consequence of small population size. However, since 'helping' occurs in some, but not all, breeding groups, monitoring the sex ratio of offspring may be valuable during the species' ongoing period of managed recovery. Furthermore, comparison of the sex ratio of clutches from pairs and groups may be informative.

Other studies (Faaborg and Bednarz, 1990; Komdeur *et al.*, 1997; Reyer, 1990) have shown that the 'helping' sex is usually the one that predominates in the bias. If helping behaviour is natural to the echo parakeet and does affect the sex ratio, this would have implications for its conservation management, as captive managed birds are usually kept as pairs. This might also be relevant to the wild management of the population, in that the sex ratio of the wild population was brought closer to equity through the release of a greater number of females than males (Harper *et al.*, 1999). Monitoring the sex ratio of clutches of pairs and groups of natural and released birds could clarify this.

Subsequent to the release of captive birds to supplement the wild population, estimates of the sex ratio of the wild 'breeding groups' were approximately even: 49% females in 2003–2004 [calculated from data in Malham *et al.* (2004)]. However, of the 35 'breeding groups' present in 2003–2004, only 12 were conventional pairs; the remaining groups ranged from 1 female and 9 males to 5 females and 2 males, and 'dominant' and 'sub-dominant' males were identified in some of these groups (Malham *et al.*, 2003).

The sex ratio of nestlings that produced the historical adult population cannot be examined. We only know that the offspring being produced at the time of this study was not biased. It appears the survival of the two sexes is similar up to fledging, so any differential mortality presumably occurs after the young fledge. With the current intensive conservation and breeding programme of both the wild and captive populations, the sex of all chicks produced should be ascertained (including eggs that fail to hatch) to establish whether the primary sex ratio continues to be unity. Individual colour ringing of known-sex nestlings and their continued monitoring through to adulthood is critical to determine the stage(s) at which any increased female mortality occurs. It may then also be possible to identify the factors responsible for this and implement a programme of alleviation, should this be deemed appropriate. Clearly, much remains to be discovered about the breeding system of this species, especially the functional significance of the variable breeding groups.

CONCLUSION

Molecular genetics enables the sex of an individual to be determined at any stage of its life and is especially useful in determining sex prior to the development of sex-specific morphology. In this study, molecular genetics was used to determine the sex of chicks and embryos that might never reach adulthood, and showed that the nestling population does not mirror the significant excess of males seen among wild adults. From our knowledge of the breeding ecology and conservation management of this species, there are a number of aspects of the behavioural ecology and reproductive behaviour of the echo parakeet that highlight the importance of monitoring the sex ratio of both captive and wild populations. Factors that influence sex ratio bias should be examined with a view to improving the general fitness of this endangered species.

ACKNOWLEDGEMENTS

This work formed part of PhD research by T.D.T. sponsored by a NERC (CASE – LGC Ltd) supervised by D.T.P. and Dr. P. Debenham (LGC). Thanks go to Jim Groombridge, who provided access to many of the samples, and members of the Mauritius Wildlife Foundation, especially Kirsty Swinnerton, Lance Woolaver, and Jason Malham for helpful advice.

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