

NESTLING SEX RATIOS IN THE YELLOW-NAPED AMAZON: NO EVIDENCE FOR ADAPTIVE MODIFICATION

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Abstract. Many birds, including some parrots, may adjust the sex ratio of their offspring in relation to the relative fitness benefits of sons and daughters. We investigated nestling sex ratios in Yellow-naped Amazons (*Amazona auropalliata*) using a molecular sexing technique that amplifies intronic regions of the CHD-W and CHD-Z genes in birds. We examined all nestlings in 37 complete clutches comprising 77 chicks. The overall nestling sex ratio did not differ from unity. Sex allocation was not associated with hatch date, sequence of hatching, or clutch size. We also found no difference in sex ratio between two regional dialects. Female Yellow-naped Amazons may be unable to control their hatchling sex ratio. Alternatively, there may be no fitness benefits to females producing more of one sex in relation to the factors we measured here.

Key words: *Amazona auropalliata*, *CHD gene*, *molecular sexing*, *sex ratio*, *Yellow-naped Amazon*.

No Existe Evidencia que Indique Modificaciones Adaptativas de la Proporción de Sexos en la Progenie de *Amazona auropalliata*

Resumen. En muchas aves, incluyendo los loros, la proporción de sexos en la progenie puede ajustarse en relación a los beneficios relativos de adecuación biológica de hembras y machos. Dichas tasas fueron investigadas en *Amazona auropalliata* por medio de una técnica molecular de determinación sexual por la cual se amplifican regiones intrónicas de los genes CHD-W y CHD-Z de aves. Se examinaron todos los pichones de 37 nidadas completas, constituidas por 77 pichones. La proporción de sexos total no resultó diferente a uno. La asignación sexual no estuvo correlacionada con la fecha de eclosión, la secuencia de eclosión, ni el tamaño de la nidada. Tampoco se encontraron diferencias en las proporciones de sexos entre dos dialectos vocales regionales. Las hembras de *A. auropalliata* podrían no tener la habilidad de controlar la proporción de sexos de su progenie. Alternativamente, es posible que en términos de adecuación

biológica, no haya diferencia en el beneficio de producir una progenie enriquecida en un sexo determinado con respecto a los factores medidos en este estudio.

Evolutionary theory predicts diverse benefits to parents that control their relative investment in male and female offspring (Ellegren and Sheldon 1997). Evidence that some vertebrates can adjust their offspring sex ratios in an adaptive manner has accumulated in recent years (Komdeur et al. 1997, Kruuk et al. 1999, Whittingham and Dunn 2000). In the past, relatively few studies were conducted on sex-ratio evolution in birds. This dearth of studies is likely related to difficulties in sexing large numbers of individuals before dispersal, as well as the absence of external sex organs in birds. However, recent advances in molecular genetics have largely mitigated these difficulties (Ellegren and Sheldon 1997).

Many studies on sex allocation in birds have focused on species with extreme sexual dimorphism in such factors as size (Wiebe and Bortolotti 1992), plumage coloration (Heinsohn et al. 1997), or paternal ornamentation (Saino et al. 1999). One of the most extreme examples of biased sex allocation in birds was reported for captive Eclectus Parrots (*Eclectus roratus*), which breed cooperatively and exhibit reverse sexual dichromatism (Heinsohn et al. 1997). Additionally, a highly skewed adult sex ratio has been found in the sexually size-dimorphic Kakapo (*Strigops habroptilus*), a ground parrot native to New Zealand (Trewick 1996). In such parrots one would predict a sex-ratio bias arising from gender-specific costs due to extreme sexual dimorphism (Svensson and Nilsson 1996). In sexually monomorphic parrots, one might expect sex-ratio variation in relation to other differential costs. Local resource competition, for example, arises when one sex competes directly with parents for resources through differential philopatry or timing of dispersal (Clark 1978). This scenario predicts selection pressure on progeny sex ratio that causes the parents to produce fewer of the more costly philopatric sex (Gowaty 1993). Sex ratio has also been shown to vary with such factors as sequence of hatching (Ankney 1982), clutch size (Heg et al. 2000), or date of hatching (Dijkstra et al. 1990), presumably due to the relative costs and benefits to the parents.

Here we report the results of a study on nestling sex-ratio variation in the Yellow-naped Amazon (*Amazona auropalliata*), a large (550 g), highly social, sex-

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ually monomorphic Neotropical parrot. These data represent only the second estimate of sex ratios in wild Neotropical parrot populations (Miyaki et al. 1998), and the first attempt to associate sex allocation in parrots with environmental and life history variables. Indirect measures of female dispersal based on mitochondrial control region sequence variation indicate that long-range dispersal of females is common in the Yellow-naped Amazon, but the extent of sex-biased dispersal is unknown (Wright and Wilkinson 2001). Given the potential for male philopatry in this species, we predicted that the overall offspring sex ratio should fall below 50% male, because male offspring may compete more with their parents for local resources (Gowaty 1993). Yellow-naped Amazons have an extended breeding season (December to April) and asynchronous hatching of chicks; both factors could allow parents to alter the timing of production of the more costly sex. We also examined whether sex allocation was associated with sequence of hatching, clutch size, or date of hatching. Additionally, populations of these parrots exhibit regional vocal dialects that may correspond to underlying environmental boundaries (Wright 1996). Since differential resource dispersion may influence competition between parents and offspring in the two dialect regions, we tested whether the offspring sex ratio differed between them.

METHODS

We obtained genetic samples from 45 wild clutches comprising a total of 95 nestling Yellow-naped Amazons at nine sites spanning two dialects in Costa Rica (Wright and Wilkinson 2001). We excluded eight clutches comprising 18 nestlings from further analysis because they were incompletely sampled or exhibited evidence of nest poaching. We extracted DNA from either feather tips or blood samples preserved in lysis buffer using DNeasy[™] tissue extraction kits (Qiagen, Valencia, California). We determined the sex of all nestlings using a polymerase chain reaction (PCR) that amplifies two different-sized products from the CHD-W and CHD-Z genes in females but only one from the CHD-Z gene in males. The PCRs were performed in 15- μ L volumes consisting of 1.5 μ L 10X Sigma[®] PCR buffer, 1.5 μ L 8 mM dNTP, 1.5 μ L 8 mM MgCl₂, 0.6 μ L each of the primers P2 (5'-TCTGCATCGC-TAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGA-GRAAYTG-3'; Griffiths et al. 1998), 0.1 μ L Sigma[®] Taq polymerase, 1.0 to 7.0 μ L genomic DNA and distilled water to volume. A cycle of 95°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec was repeated 35 times. The PCR products were visualized on ethidium bromide-stained 2% agarose gels and scored male (one band) or female (two bands).

We used goodness of fit tests to examine departures from 1:1 in the sex ratio of the entire population of nestlings, for all first, second, and third-born chicks, and between the two population dialects (North and South, Wright 1996). We used a two-tailed independent *t*-test to examine departures from unity of the mean percentage of males per brood. We also used a Fisher's exact test to examine departures from the expectation that each sex was produced with equal probability in those 13 clutches with two nestlings. We

TABLE 1. Numbers of female and male Yellow-naped Amazon nestlings by hatching order in two populations with different vocal dialects.

Hatch order	North Dialect		South Dialect	
	females	males	females	males
First	13	10	8	7
Second	8	9	5	3
Third	2	6	0	3
Fourth	1	1	1	0
Total	24	26	14	13

used binary logistic regression to examine the sex of individuals in relation to date of hatching, order of hatching, and clutch size (Sokal and Rohlf 1995). We employed a significance level of $P < 0.05$ for all statistical tests. We estimated hatching date of each chick to the nearest week for the regression analysis based on its weight at sampling and a growth curve for this species (Joyner and Brice 1994). We estimated hatching date of each chick to the nearest day to determine the hatching order of chicks within clutches, and calculated the median and range of the number of days between hatching of subsequent chicks within a clutch. All analyses were conducted using SPSS for Windows, version 10.0.0 (SPSS Inc. 1999).

RESULTS

Clutch sizes ranged from one to four (median = 2.0, mean \pm SE = 2.03 \pm 0.16). Overall, the sex ratio of nestlings was 51% male (39 out of 77), which did not represent a significant departure from unity ($\chi^2_1 = 0.01$, $P > 0.9$). The mean proportion of males per brood ($n = 37$) was 49%, which did not differ from unity ($t_{36} = -0.2$, $P > 0.8$), nor did the sex ratio of first-born (45% male, $\chi^2_1 = 0.4$, $P > 0.5$) or second-born nestlings (48% male, $\chi^2_1 = 0.04$, $P > 0.8$; Table 1). The sex ratio of chicks born third differed marginally from unity (82% male, $\chi^2_1 = 4.5$, $P < 0.04$; Table 1). The subset of clutches with two nestlings ($n = 13$) consisted of two male-male pairs, six male-female pairs, and five female-female pairs, which did not differ significantly from expectation ($\chi^2_2 = 2.0$, $P > 0.3$). Hatch date, as estimated from the growth curve, ranged from 2 February to 25 March 1999 (median = 7 March 1999). The number of days between hatching of chicks in the same clutch ranged from 0 to 10 days (median = 4). Sex of nestlings was not related to date of hatching ($\beta = -0.03 \pm 0.34$, $P > 0.3$), hatch order ($\beta = -0.05 \pm 0.3$, $P > 0.8$), or clutch size ($\beta = 0.41 \pm 0.88$, $P > 0.6$) in a logistic regression with all three variables as predictors ($n = 75$ nestlings). The sex ratio between North and South populations did not depart from unity (52% male North, 48% male South, $\chi^2_1 = 0.1$, $P > 0.7$; Table 1).

DISCUSSION

We found little evidence that the offspring sex ratio in wild populations of Yellow-naped Amazons differs from equality. Sex ratio was not influenced by hatch date, sequence of hatching, or clutch size. We also found no difference in sex ratio between two regional

dialects. These results contrast with studies on Australasian parrots and some other avian taxa. Eclectus Parrot females can produce long, unbroken series of one sex in captivity (Heinsohn et al. 1997). Additionally, the adult sex ratio of the Kakapo is highly skewed toward males in the wild (Trewick 1996). However, the sex ratio of monomorphic Hyacinth Macaw (*Anodorhynchus hyacinthinus*) chicks in the Pantanal, Brazil was close to 50:50 (Miyaki et al. 1998).

Evidence suggesting an influence of environmental variables on sex allocation has predominantly come from studies on sexually dimorphic species (Ellegren and Sheldon 1997). In American Kestrels (*Falco sparverius*), the proportion of the larger sex at hatching decreased as the food supply declined (Wiebe and Borolotti 1992). In another study on raptors, nestling Eurasian Kestrels (*Falco tinnunculus*) hatched earlier in the season were more likely to be the larger sex, and were also more likely to obtain breeding territories as yearlings (Dijkstra et al. 1990). Sex-biased natal dispersal rates may be another special case under which investment in the sexes is not equal, even for sexually monomorphic species. Some evidence does support a broad pattern of sex-ratio variation in relation to local resource competition between parents and the philopatric sex (Gowaty 1993). Under a scenario of differential natal dispersal one would expect the sex ratio of the philopatric sex to fall below 50%. We did not detect these patterns for Yellow-naped Amazons, despite high rates of female dispersal (Wright and Wilkinson 2001).

One possible explanation for the lack of effect of environmental variables on offspring sex ratio is that female Yellow-naped Amazons are unable to control their primary sex ratio. Sex differentiation in birds is poorly understood, and independent chromosomal segregation at meiosis may prevent sex-ratio adjustment (Ellegren 2000).

Alternatively, there may be no fitness benefits to females producing more of one sex in relation to the factors we measured here. Chicks hatching early in the season or before other chicks in the same clutch may not influence the fitness of their mother differently than those hatching later. For example, daily survival probabilities for breeding Green-rumped Parrotlet (*Forpus passerinus*) females vary little through the course of the nesting cycle (Stoleson and Beissinger 2001). Additionally, parents may not compete significantly more with their male offspring than female offspring, despite high female dispersal rates. Yellow-naped Amazons roost communally and forage in social groups (Wright 1996); the benefits of sociality may outweigh the costs of competition with offspring. Finally, the small clutch size of this species and the potentially low recruitment rates due to widespread nest poaching for the pet trade (Wright et al. 2001) may negate any potential benefits from sex-ratio manipulation through selective infanticide.

Subtle sex-ratio manipulation by Yellow-naped Amazon females may still occur under certain conditions. We were unable to test hypotheses suggesting optimal sex-ratio manipulation by individual parents according to territory quality (Komdeur et al. 1997), mate quality (Svensson and Nilsson 1996) or parental condition

(Whittingham and Dunn 2000). These latter hypotheses may prove difficult to test in many parrots because of the difficulty of obtaining data on parental condition. Further studies on sex allocation in birds are needed to determine how widespread biased sex ratios are among sexually monomorphic birds, and which factors most influence these biases.

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