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Pre-ovulation control of hatching sex ratio in the Seychelles warbler

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Females of some bird species have a high degree of control over the sex ratio of their offspring at laying. Although several mechanisms have been put forward to explain how females might control the sex of their eggs, virtually nothing is known. As females are the heterogametic sex in birds, adjustment of the clutch sex ratio could arise either by pre- or post-ovulation control mechanisms. The Seychelles warbler (Acrocephalus sechellensis) exhibits extreme adaptive egg sex ratio bias. Typically, warblers produce only single-egg clutches, but by translocating pairs to vacant habitat of very high quality, most females were induced to produce two-egg clutches. Overall, females skewed clutch sex ratios strongly towards daughters (86.6%). This bias was evident in the first egg, but critically, also in the second eggs laid a day apart, even when all absent, unhatched, or unsexed second eggs were assumed to be male. Although a bias in the first egg may arise through either pre- or post-ovulation mechanisms, the skew observed in second eggs could only arise through pre-ovulation control. Post-ovulation adjustment may also contribute to skewed hatching sex ratios, but as sex-biased release of gametes is likely to be a more efficient process of control, pre-ovulation mechanisms may be the sole means of adjustment in this species. High fitness differentials between sons and daughters, as apparent in the Seychelles warblers, may be necessary for primary sex ratio adjustment to evolve.

Keywords: egg sex ratio; pre-ovulation control; post-ovulation control; Seychelles warbler

1. INTRODUCTION

Sex allocation theory predicts that parents should benefit from the ability to control their relative investment in male and female offspring if their profitability sometimes varies (Charnov 1982). This idea has received strong support among taxa, especially some haplodiploid insects, that have precise and well understood mechanisms for the adjustment of offspring sex ratios (Hamilton 1967; Charnov 1982; Godfray & Werren 1996). However, an increasing number of studies show apparent control of sex ratios at birth in mammals (Clutton-Brock & Iason 1986; Werren 1980; Godfray & Werren 1996). Nevertheless, an adjustment of offspring sex ratios (Hamilton 1967; Charnov 1982). This idea has received strong support among taxa, especially some haplodiploid insects, that have precise and well understood mechanisms for the adjustment of offspring sex ratios (Hamilton 1967; Charnov 1982; Godfray & Werren 1996). However, an increasing number of studies show apparent control of sex ratios at birth in mammals (Clutton-Brock & Iason 1986; Werren 1980; Godfray & Werren 1996). Nevertheless, an adjustment of offspring sex ratios (Hamilton 1967; Charnov 1982).

Although several mechanisms have been put forward to explain how females might control the sex of their eggs, virtually nothing is known. As females are the heterogametic sex in birds, adjustment of the clutch sex ratio could arise either by pre- or post-ovulation control mechanisms. The Seychelles warbler (Acrocephalus sechellensis) exhibits extreme adaptive egg sex ratio bias. Typically, warblers produce only single-egg clutches, but by translocating pairs to vacant habitat of very high quality, most females were induced to produce two-egg clutches. Overall, females skewed clutch sex ratios strongly towards daughters (86.6%). This bias was evident in the first egg, but critically, also in the second eggs laid a day apart, even when all absent, unhatched, or unsexed second eggs were assumed to be male. Although a bias in the first egg may arise through either pre- or post-ovulation mechanisms, the skew observed in second eggs could only arise through pre-ovulation control. Post-ovulation adjustment may also contribute to skewed hatching sex ratios, but as sex-biased release of gametes is likely to be a more efficient process of control, pre-ovulation mechanisms may be the sole means of adjustment in this species. High fitness differentials between sons and daughters, as apparent in the Seychelles warblers, may be necessary for primary sex ratio adjustment to evolve.

Importantly, female birds are the heterogametic sex (producing W- and Z-bearing ova), so mechanisms of sex ratio adjustment could potentially function either pre- or post-ovulation (Hardy 1997; Oddie 1998). In the chicken (Gallus domesticus), sex is determined shortly before ovulation, during the first meiotic division, when segregation of the sex chromosomes leaves either the W or Z chromosome included in the developing ovum and the other sex chromosome consigned to part of the polar body (Sturkie 1986). It has been proposed that pre-ovulation (or primary) control could occur through segregation distortion at the first meiotic division or through differential provisioning of ova of different sex to influence the order in which they are released from the ovary (Ankney 1982; Krackow 1995; Oddie 1998, but see Krackow 1999). Post-ovulation (or secondary) control could operate through sex-selective re-absorption of the ova in the oviduct (pre- or post-fertilization) or dump laying of eggs of the ‘unwanted’ sex (Emlen 1997). A key difference
between pre- and post-ovulation mechanisms of adjustment is that post-ovulation control presumably requires skipping a day when an egg could have been laid and, in the case of dump laying, wasting the resources that were provisioned to that egg. Skipping day(s) at the start or during the ovulation sequence (Emlen 1997) would result in either delayed clutch completion or a smaller clutch. Females could also skip laying the last egg(s) of a clutch if they were of the ‘unwanted’ sex, resulting in a smaller clutch and delayed start of incubation. Pre-ovulation control by segregation distortion would not suffer from these costs, and control by pre-ovulation sex-differential follicle retardation would incur only minor costs when yolk could be re-absorbed. Therefore, in principle, pre-ovulation control of any kind would be the more efficient mechanism of control (Myers 1978; Oddie 1998; Komdeur & Pen 2002). In turn, more efficient mechanisms of control should broaden the scope for adaptive sex ratio adjustment, because the fitness differential between sons and daughters need not be as great to balance the costs of adjustment (Pen & Weissing 2002).

Previous studies that report hatchling sex ratio biases (see above) have not explicitly considered whether sex ratio adjustment occurred pre- or post-ovulation. In this study, we aim to distinguish between primary and secondary egg sex ratio adjustment in the Seychelles warbler, Acrocephalus sechellensis, a species known to exhibit extreme control of hatchling sex ratio (Komdeur et al. 1997). The Seychelles warbler is a rare island endemic, until 1988 known only to come from Cousin Island (29 ha) in the Seychelles. On this island, the warbler population has reached carrying capacity, and many breeding pairs are aided by helpers that are usually daughters from previous broods. Having helpers is beneficial to pairs on high-quality territories, because reproductive success is enhanced. However, on low-quality territories, helpers are costly because they deplete insect prey (Komdeur 1994). Breeding pairs maximize their inclusive fitness by modifying the sex ratio of the single-egg clutch towards sons (77%, n = 57), the dispersing sex, when breeding on poor territories, and towards daughters (87%, n = 32) when breeding on rich territories (Komdeur et al. 1997). However, because only single-egg clutches were produced by these pairs, it was not possible to distinguish between pre- and post-ovulation mechanism of egg sex adjustment.

In efforts to conserve the species, an additional population was established on the nearby Aride Island (68 ha) in 1988. Pairs transferred to this island started breeding immediately in very high-quality habitat and produced mainly two-egg clutches (Komdeur et al. 1995). The high quality of habitat and absence of helpers led females to skew brood sex ratios strongly towards daughters. This preference for daughters, coupled with the fact that most females produced two-egg clutches, allowed us to investigate mechanisms of egg sex ratio adjustment. A bias in the sex ratio of first-laid eggs could result from either pre- or post-ovulation processes, whereas a skewed sex ratio of the second egg laid within 24 h of the first could only arise through pre-ovulation adjustment. In this paper, we examine egg sex ratios of warbler pairs on Aride Island over 3 years to establish if there is evidence for pre-ovulation sex ratio adjustment in this species. In addition, we examine if there is any dependence or difference between the sex of first and second eggs, which could provide further insights into mechanisms of adjustment.

2. MATERIAL AND METHODS

(a) Study population and data collection

Following relocation, the entire warbler population on Aride Island was studied between September 1988 and October 1991. All warbler territories were situated in habitat where food abundance was on average 1.6 times higher than on high-quality habitat on Cousin Island (Komdeur 1996; Komdeur & Edelaar 2001). Furthermore, territories on Aride were initially 4.4 times larger than on Cousin, providing the warblers with access to an average of about seven times more insect prey than the warblers occupying high-quality territories on Cousin Island (Komdeur 1996). A total of 19 breeding pairs were studied. Initially, cooperative breeding was absent, but was observed in some pairs from November 1990 to October 1991. Because the presence of helpers has been shown to affect hatchling sex ratios (Komdeur et al. 1997), pairs were excluded from analysis once they had helpers on their territory. This only reduced the sample slightly because only three clutches produced by two of the study pairs were excluded. All nests of each pair were found and inspected daily, and eggs numbered with a permanent marker pen on the day that they were laid to determine the sequence of laying. Clutches were monitored daily for hatching. Conspecific egg dumping was unlikely because a subsequent study of Seychelles warblers on Cousin Island, using molecular genotyping, revealed that all eggs in multiple-egg clutches produced by breeding pairs were laid by the social mother (Richardson et al. 2001).

Upon hatching, each nestling was given a unique marking on its legs and feet with a permanent marker and the egg number from which it hatched recorded. Hatching was asynchronous (usually 24 h apart in two-egg clutches) and it was always possible to determine which chick hatched from which egg in the clutch. The markings were renewed when necessary, until nestlings were fitted with coloured leg bands and a numbered metal band when between 5 and 9 days old. All nestlings survived to be re-trapped in their second year of life for sex determination. At the time, molecular sexing techniques were not available so we sexed all birds using the variables wing length and body mass in a multiple logistic regression model (χ² = 148.23, d.f. = 2, p < 0.001). The sexes of the warblers on Aride were separated using the function y = 1/(1 + e⁻ᶻ), where z = 79.88–2.72 × body mass–0.544 × wing. A bird was classified as female if y was close to zero or male if y was close to unity. A substantial proportion of these nestlings on Aride Island were subsequently bled as adults between 1993 and 1996 (58 males and 109 females) and sexed using random amplified polymorphic DNA markers (Lessells & Mateman 1996, 1998; Komdeur et al. 1997). Based on these individuals, all sexes assigned using the multiple logistic regression model were in agreement with the sex determined using this molecular technique.

(b) Statistical analyses

We first provide the binomial probability for the overall sex ratio of hatchlings to determine if the anticipated excess production of daughters was evident. Most of the 19 females contributed multiple clutches to the dataset (range 1–9). To avoid pseudoreplication and biased test statistics in small samples of broods of variable size (Krackow & Tkadlec 2001; Krackow et al. 2002), one sex ratio for first, second and all eggs combined.
was derived for each female in our sample by summing these eggs in all of her clutches. We then used Wilcoxon's signed rank test to evaluate deviations of overall progeny sex ratios and those of first and second eggs from parity. Heterogeneity between females was not analysed any further because the small number of clutches produced by most females afforded little statistical power and, furthermore, explaining variation between females was not the aim of this study.

To evaluate whether post-ovulation mechanisms could have been exclusively responsible for the sex ratio bias, we used the actual sex of second eggs found only if the second egg was laid on the day consecutive to the first egg laid. For those clutches that comprised only one egg, or where the sex of the second egg could not be determined, the second egg was assumed to be the ‘less adaptive’ sex—male, in this case. For those clutches where the second egg was laid more than one day apart, we assumed that this second-laid egg was really not the second egg to be ovulated and that the actual second unlaid egg was the ‘less adaptive’ sex—male in this case. The binomial probability of finding as few or fewer males in second eggs of this ‘worst case’ dataset estimates the likelihood that the bias in our observed sample was due only to post-ovulation mechanisms. This is a conservative estimate because any genuine single-egg clutch will erroneously inflate this probability. Clearly, this assertion rests on our assumption that the data represent a complete sample of clutches in the population (i.e. not over-representing clutches with non-delayed second eggs).

Finally, we tested if the sex ratio significantly changed between first- and second-laid eggs. In multiple-egg clutches, this was achieved with a McNemar test for change (Siegel & Castellan 1988), by calculating the two-sided binomial probability of the skew in incongruent pairs of clutches (i.e. those containing either males first and females second, or vice versa) that is appropriate for small numbers of such pairs (Siegel & Castellan 1988). In addition, Fisher’s exact probabilities were calculated in 2 × 2 contingency tables (i.e. first and second eggs were treated as independent samples) in comparisons not allowing for paired tests.

3. RESULTS

A total of 86 clutches was produced by 19 females over a three-year period between October 1988 and November 1991. These females produced an average of 4.53 ± 2.57 (s.d.) clutches with a range of 1 to 9. The majority of clutches contained two eggs (72%), but clutch size ranged from 1 to 4 (20 × 1 egg; 62 × 2 eggs; 3 × 3 eggs; 1 × 4 eggs). Over 90% of laid eggs were sexed (142 out of 157); of the remainder, 13 failed to hatch and were not sexed and two hatched but the young died before sexing. In two clutches, there was a period of two days in the laying sequence between the appearance of the first and second eggs.

Overall, only 19 of the 142 sexed hatchlings were male (binomial test; p < 0.0001). The mean sex ratio of hatchlings produced per female was also strongly female-biased (Wilcoxon signed rank test: Z = 3.87, n = 19, p < 0.0001) and this skew was evident for both first (Z = 3.49, n = 19, p < 0.0001) and second, successively laid eggs (Z = 3.88, n = 17, p < 0.0001) (figure 1). The four third-laid eggs and one fourth-laid egg were all female. Critically, we found that the sex ratio of second eggs in the ‘worst-case’ dataset was also female-biased (binomial test; 31 males versus 55 females, p = 0.0063).

Comparing the sex ratio of first- and second-laid eggs, we found that second eggs were more female-biased than first eggs (table 1). The sex composition did significantly differ between first and second eggs when including all clutches, i.e. treating single eggs as first eggs (Fisher’s exact probability for all clutches: p = 0.044). The change of sex ratio within multiple-egg clutches did not reach significance (McNemar test: p = 0.065). This trend in multiple-egg clutches stems from the fact that mothers with female first eggs produced significantly fewer male eggs at second laying (2 in 47) than expected from the overall sex ratio of first eggs (13 in 61, table 1; Fisher’s exact probability: p = 0.012), while those with male first eggs did not (2 in 11, table 1; Fisher’s exact probability: p = 1.00).

4. DISCUSSION

Previous work has demonstrated that Seychelles warbler females adjust their hatchling sex ratio according to the quality of habitat within their territory (Komdeur et al. 1997). Consequently, when breeding pairs were relocated to the vacant, extremely high-quality habitat of Aride Island in 1988, it came as little surprise that female warblers were observed to skew clutch sex ratios strongly towards daughters (mean sex ratio = 0.12 ± 0.03 (s.e.), n = 19 females). Because of the low population density on Aride, most daughters dispersed from the natal territory, but remained unpaired for some time because breeding pairs were producing so few sons. The extreme high quality of the habitat on Aride also induced females to produce a predominance of two-egg clutches, so it was possible to show that the bias was evident in both the first, and more importantly, the second of eggs laid 24 h apart.

Even assuming that all missing eggs the day after the laying of the first egg were male, a highly significant over-representation of females was still evident in second eggs.
involved and that their relative in
effectively for second eggs, or that several mechanisms are
implies that the mechanism of control operates more
variation in female quality or condition, independent of
could re-
al.
steroid levels might cause segregation distortion (Petrie
1982; Oddie 1998), our current understanding of these
be completely ruled out. As pointed out by others (Ankney
(Sturkie 1986). As first meiosis (i.e. sex determination) in
domestic species takes place only shortly (0.5 to 3 h)
before ovulation, a pre-ovulation mechanism involving
differential resorption of ‘unwanted’ sex follicles would
seem unlikely because a delay in laying would be observed.
Most plausibly, pre-ovulation sex ratio adjustment in
Seychelles warblers results from distorted segregation of the
sex chromosomes at the first meiotic division (Ankney
1982; Krackow 1995). Although it is unclear how this
process could operate (Krackow 1999; Williams 1999), recent
evidence in peafowl (Pavo cristatus) suggests that maternal
studies (Ankney 1982; Oddie 1998), our current understanding of these
events is derived only from domestic species where biased
sex ratios are rarely observed and that are phylogenetically
very distinct from those species shown to exhibit appreciable
sex ratio variation. Clearly, there is a need for studies
of the processes of gamete formation and ovulation in
species where sex ratio adjustment is known to occur.
In this study, we found tentative evidence that the sex
ratio of the second, successively laid egg in multiple-egg
clutches was even more female-biased than the first. This
implies that the mechanism of control operates more
effectively for second eggs, or that several mechanisms are
involved and that their relative influence differs between
eggs. An adaptive advantage to a greater skew of the
second egg is unclear. However, if genuine, this pattern
could reflect a relationship between offspring sex ratio and
variation in female quality or condition, independent of
habitat quality.
Mechanisms of sex ratio adjustment should only evolve
where the benefits of control outweigh the costs. To what
extent do Seychelles warblers benefit from the ability to
control the hatchling sex ratio? Preliminary data indicate
that inclusive fitness benefits to females raising daughters
on high-quality habitat were 9.8 times higher than for
females raising sons (J. Komdeur, D. S. Richardson and
T. Burke, unpublished data), suggesting strong selection
for the evolution of sex ratio control in this population.
In principle, pre-ovulation control of sex ratio should
be more efficient than post-ovulation adjustment (see § 1).
Nevertheless, all mechanisms of sex ratio control are likely
to entail some cost. The threshold costs (percentage of
total reproductive resources wasted) of discarding the
undesired sex have recently been compared with the bene-
fit ratio of producing the desired sex versus the undesired
sex (Pen & Weissing 2002). In the Seychelles warbler,
after the 10-day period in the nest, parents continue to
feed the young for about a further three months after
hatching (mean: 88.5 ± 19.7 (s.d.); range 70–240 days;
n = 189) (Komdeur 1996). If we assume, for argument’s
sake, that investment in each egg represents 10% of total
maternal investment in parental care (almost certainly
larger than the real figure), the model of Pen & Weissing
(2002) predicts that a fitness ratio of only 1.5 is required
to sustain this level of wasted investment. This fitness dif-
erential is six times less than the 10-fold advantage esti-
imated for this population (see above). Furthermore, as
female warblers appear to exert pre-ovulation control,
which is potentially far cheaper than dumping eggs, the
cost of sex ratio control may be trivial relative to the
fitness benefits.
In theory, a more serious obstacle to the evolution of
pre-ovulation adjustment may be the potential conflict of
interest between mother and gamete (Reiss 1987;
Krackow 1999). It has been argued that the sex chromo-
somes of gametes have no genetic interest in sex ratio
adjustment, while gametic automoses should only agree
on sex ratio distortion if the fitness differential between
the sexes exceeded three (Reiss 1987). While this represents
a substantially higher threshold than the costs of wasted
resources, it is still well inside the estimated fitness differ-
tential for the warbler. Parent–gamete conflict is of parti-
cular relevance to pre-ovulation sex ratio control because,
after fertilization, the conflict is between parent and off-
spring, which is much reduced (Trivers 1974). While
surmountable in the Seychelles warbler, the
obstacles to pre-ovulation sex ratio control of parent–
gamete conflict coupled with resource waste may preclude

<table>
<thead>
<tr>
<th>multiple-egg clutches</th>
<th>second egg</th>
<th>first-egg totals</th>
<th>single-egg clutches</th>
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<tr>
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<td>4 (31)</td>
<td>57 (55)</td>
<td>5</td>
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a One clutch.
b Two clutches with a period of two days between the first and second eggs.
such control mechanisms from evolving in all but a few bird species. In most studies that report sex ratio skew at hatching, it is unclear when the bias may have been produced. However, a range of other studies show skews in sex ratio related to position of the egg in the laying sequence. In some of these cases the bias appears to be confined to the first egg (e.g. Harris’s hawk (Parabuteo unicinctus); Bednartz & Hayden 1991). As discussed earlier, first-egg biases may arise through post-ovulation adjustment (Emlen 1997; Krackow 1999), while sex of the remaining eggs is left to chance. Nevertheless, mechanisms that result in non-random ovulation in first eggs only cannot be excluded in these cases. Other studies either show (lesser snow goose (Chen caerulescens), Ankney 1982; ring-billed gull (Larus delawarensis), Bortolotti 1986; zebra finch (Taeniopygia guttata), Kilner 1998; lesser black-backed gull (Larus fuscus), Nager et al. 1999) or imply (zebra finch, Clotfelter 1996; great tit (Parus major), Lessells et al. 1996; blue tit (Parus caeruleus), Sheldon et al. 1999; laughing kookaburra (Dacelo novaeguineae), Legge et al. 2001) a relationship between laying order and sex ratio, or a deviation from parity that continues beyond the first-laid egg. These studies certainly suggest the operation of pre-ovulation adjustment mechanisms, although because of the difficulty of obtaining these data, insufficient details on laying order, laying gaps and/or infertility of eggs were presented to rule out the operation of post-ovulation mechanisms. Further, some of these studies did not provide explicit tests for deviation from parity of eggs beyond the first. Still other studies show that male and female eggs within a clutch are provisioned differently, independent of the effects of laying order (white-crowned sparrow (Zonotrichia leucophrys), Mead et al. 1987; American kestrel (Falco sparverius), Anderson et al. 1997; house sparrow (Passer domesticus), Cordero et al. 2000). This implies either non-random ovulation or sex-specific recognition of fertilized ova coupled with control over egg provisioning. Clearly, further studies are needed at the physiological, genetic and ecological levels to develop an understanding of how some birds are able to adjust the sex ratio of their clutch in an apparently adaptive manner. In this study, we show that pre-ovulation control is evident in the Seychelles warbler, quite possibly through directed haplotype segregation at first meiotic division, though determination of the actual mechanism needs further investigation. The extent to which similar mechanisms may operate in other avian species remains to be explained.

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