Do sex ratio and development differ in sexually size-dimorphic shiny cowbirds (Molothrus bonariensis) parasitizing smaller and larger hosts?

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Two possible patterns of bias in primary sex ratio have been proposed for size-dimorphic brood parasites that do not evict host chicks: (1) larger males should be laid at greater frequency in hosts larger than the parasite because they compete better (increasing their survival) than females with large host nest-mates, and (2) more costly males (i.e. the larger sex) should be laid at greater frequency in hosts smaller than the parasite because, in these hosts, parasite nestlings are provisioned at a higher rate and grow faster than in larger hosts. We tested these hypotheses in two hosts of the sexually size-dimorphic shiny cowbird, Molothrus bonariensis, one smaller (house wren, Troglodytes aedon) and one larger (chalk-browed mockingbird, Mimus saturninus) than the parasite. We measured: (1) sex ratio at laying; (2) development of sexual differences in body mass during the nestling stage; and (3) chick survival and sex ratio of chicks before fledging. In both hosts, we found sexual differences in body mass of nestlings from 7 days of age onwards, although we did not find a bias in the sex ratio of eggs laid and chicks fledged. The results of the present study do not support the hypothesis that shiny cowbird females benefit from biasing the primary sex ratio depending on the size of the hosts they parasitize. © 2013 The Linnean Society of London, Biological Journal of the Linnean Society, 2013, ••–••.


INTRODUCTION

Individuals are expected to vary the sex ratio of their offspring in relation to the specific fitness benefits of producing sons and daughters (Charnov, 1982). In birds, several studies indicate that these benefits may depend on a variety of ecological and social factors, including maternal body condition (Nager et al., 1999; Clout, Elliott & Robertson, 2002), the presence of helpers (Komdeur et al., 1997; Doutrelant et al., 2004), and the quality of mates (Ellegren, Gustafsson & Sheldon, 1996; Kölliker et al., 1999), and studies also show that parents could adaptively skew the primary sex ratio (the sex ratio at the time of laying) by adjusting the sex composition (Nager et al., 1999) or the sex sequence (Blanco et al., 2002) of the clutch. In addition, in sexually dimorphic birds, the cost of rearing sons and daughters may differ as a result of sexual differences in food intake or energetic expenditure (Teather & Weatherhead, 1988), growth rate (Martins, 2004), and/or energetic needs (Magrath et al., 2007). These differences can lead to greater mortality of the larger sex (Benito & González-Solís, 2007), resulting in a skewed secondary sex ratio (i.e. the sex ratio at the time of fledging).

Interspecific obligate brood parasites, such as cowbirds and cuckoos, lay eggs in nests of other species, the hosts, which provide parental care for the eggs and chicks (Rothstein & Robinson, 1998; Davies, 2000). Brood parasites are a very good model for studying the possible patterns of primary sex ratio manipulation because the observed sex ratio depends directly upon what the female produces and is not confounded by any form of parental care (Kasumovic et al., 2002).
Two possible patterns of bias in primary sex ratio have been proposed for parasites that do not evict host chicks depending on the size of the host relative to that of the parasite. Weatherhead (1989) suggested that, in sexually size-dimorphic parasites, larger males should be laid at a greater frequency in nests of hosts larger than the parasite because they compete better than females with large host nest-mates and this could lead to a higher survival of the larger sex. By contrast, Kasumovic et al. (2002) suggested that the more costly sex (e.g. males) should be laid at greater frequency in nests of hosts smaller than the parasite because in these hosts parasite nestlings are provisioned at a higher rate and growth faster than in larger ones. This is because host nestlings stimulate parents to increase provisioning to the nest and larger parasite nestlings outcompete smaller host nestmates for food (Kilner, Madden & Hauber, 2004; Gloag et al., 2012). These patterns of sex ratio bias are only possible if parasite chick dimorphism appears early during the nestling period because, otherwise, no competitive or survival differences between sexes could arise.

Previous studies found no evidence in support of skewed sex ratios in brood parasitic brown-headed cowbirds (Molothrus ater) and common cuckoos (Cuculus canorus) raised by different hosts (Weatherhead, 1989; Kasumovic et al., 2002; Fossay et al., 2012). However, these studies did not investigate whether there were sexual differences in growth and survival of parasite chicks when hosts smaller or larger than the parasite raised them. To test the hypothesis that brood parasites adaptively bias the primary sex ratio depending on the size of the host, it is also necessary to determine whether parasitic males had a higher survival than females in hosts larger than the parasite, as suggested by Weatherhead (1989), or if males had a higher growth rate in hosts smaller than the parasite than in larger ones, as suggested by Kasumovic et al. (2002).

The shiny cowbird (Molothrus bonariensis) is an extreme generalist brood parasite whose eggs have been found in nests of more than 250 species, with almost 100 of these hosts successfully rearing cowbird young (Lowther, 2011). These hosts have a wide range of body masses, some of them much smaller than the parasite (i.e. 10 versus 45 g) and others much bigger (i.e. 80 versus 45 g) (Ortega, 1998; De Mársico, Mahler & Reboreda, 2010). As a result, parasite chicks can be the largest or the smallest chick in the brood.

Shiny cowbirds are very appropriate to test possible patterns of bias in sex ratio depending on host size. They are sexually size-dimorphic (adult females, 45 g; adult males, 55 g; Mason, 1987). Although male nestlings reach an asymptotic weight larger than that of females (Tuero et al., 2012), it is not known when sexual size dimorphism arises during chick development. The survival of nestlings is higher in nests of small hosts than in those of large hosts (Fiorini, Tuero & Reboreda, 2009; Gloag et al., 2012a). This is because shiny cowbird chicks are outcompeted for food by host chicks in large hosts, although they outcompete host chicks for food in small hosts (Gloag et al., 2012a). However, it is unknown whether there are sexual differences in chick survival in hosts smaller or larger than the parasite.

In the present study, we investigated the primary sex ratio of shiny cowbirds in two hosts that differ markedly in body mass: the house wren (Troglodytes aedon; hereafter wrens), which is much smaller than the parasite (13 g versus 45 g) and the chalk-browed mockingbird (Mimus saturninus; hereafter mockingbirds), which is considerably larger than the parasite (75 g). In addition, we analyzed, in experimentally controlled conditions: (1) the development of size-dimorphism and sexual differences in growth rate and (2) the survival of shiny cowbird chicks raised in nests of wrens and mockingbirds. Sensu Weatherhead’s (1989), we expect a bias towards males in nests of mockingbirds because males would be the more competitive sex and, in this host, parasite chicks compete more intensely for food with host nest-mates (Gloag et al., 2012). Additionally, a higher survival of males than females during the nestling stage in mockingbird nests but not in wren nests must be observed. By contrast, sensu Kasumovic et al. (2002), we expect: (1) a bias towards males in wren nests because males are the larger sex and should be the more costly sex and (2) a higher growth rate of males in wren nests than in mockingbird nests.

MATERIAL AND METHODS

STUDY SITE AND SPECIES

The study was conducted at ‘Reserva El Destino’ (35°08’S, 57°23’W), near the town of Magdalena, in the Province of Buenos Aires, Argentina. Shiny cowbirds, wrens, and mockingbirds are common species in this area where they breed from October to January. At our study site, we followed approximately 50–60 nesting attempts conducted by approximately 40 pairs of wrens each breeding season. Frequency of shiny cowbird parasitism in wrens was 60% (Tuero, Fiorini & Reboreda, 2007). As for mockingbirds, each breeding season we followed approximately 80–100 nesting attempts conducted by 35–40 pairs. The frequency of shiny cowbird parasitism in mockingbirds was 70% and 2.2 eggs per parasitized nest, respectively (Fiorini et al., 2009).
DATA COLLECTION AND ANALYSIS

We determined primary sex ratio from 35 shiny cowbird eggs collected from 34 nests of wrens and 94 shiny cowbird eggs collected from 69 nests of mockingbirds during the 2004–2005 to 2006–2007 breeding seasons (Table 1). The eggs collected represented a relatively small fraction (15%–37%) of the shiny cowbird eggs laid in these hosts during the study period (94 eggs in 163 wren nests and 614 eggs in 256 mockingbird nests). The collection of eggs did not have a noticeable effect of the frequency of shiny cowbird parasitism on wrens and mockingbirds on subsequent breeding seasons (Gloag et al., 2012b; D. T. Tuero, unpubl. data). We artificially incubated eggs for 2–3 days at 37.5 ± 1 °C to ensure minimal embryonic development (Strausberger & Ashley, 2001) and then froze them. For processing, we defrosted eggs and extracted embryonic tissue. Shiny cowbird embryos were genetically sexed by amplification of a size-different intron within the highly conserved chromo-helicase-DNA binding protein gene located on the avian sex chromosomes (Ellegren, 1996; De Mársico et al., 2010).

We measured the survival and growth of cowbird chicks and sex ratio before fledging in wren and mockingbird nests found during the breeding seasons 2004–2005 to 2009–2010. We only considered nests that survived at least 10 days after hatching and did not suffer eggs losses as a result of cowbird female punctures. In these hosts, multiple parasitism is common (Tuero et al., 2007; Fiorini et al., 2009) and parasite eggs are deposited during laying and early incubation (Fiorini et al., 2009). To measure chick growth and survival, we standardized intra-nest conditions; in both hosts, we experimentally controlled for synchronous hatching of parasite and host eggs and for the number of host eggs. To obtain nests with only one cowbird chick and with similar synchronization between host laying and parasitism, we artificially parasitized wren (N = 34) and mockingbird (N = 66) nests with one shiny cowbird egg collected in nests of conspecifics and removed all other parasite eggs laid in these nests. Because the incubation period of shiny cowbirds (12 days) is 2–3 days shorter than that of wrens (14–15 days) but similar to that of mockingbirds (13 days), we parasitized wren nests 3 days after the onset of incubation, and mockingbird nests before the onset of incubation. In both hosts, cowbird chicks hatched 1 day before or the same day the first host chick hatched. This laying synchronization was observed in 44% and 74% of parasitized nests of wren and mockingbird nests, respectively (Fiorini et al., 2009). In addition, we created host clutches with the modal clutch size of parasitized nests (four host eggs in wren nests and two host eggs in mockingbird nests). In unparasitized wren and mockingbird nests, the modal clutch sizes are five and four eggs, respectively (Tuero et al., 2007; Fiorini et al., 2009). The experimental clutch sizes correspond to the natural competitive environment of cowbird nestlings in wren and mockingbird nests (Tuero et al., 2007; Fiorini et al., 2009). We visited wren and mockingbird nests daily or every other day until the chicks fledged. Immediately after hatching, parasite chicks were marked with waterproof ink on the tarsus. They were weighed with a digital portable balance (Ohaus LS 200) to the nearest 0.1 g. We collected blood samples (20–30 µL) when parasite chicks were 9 days of age and stored samples in lysis buffer for subsequent determination of chick sex.

To analyze the development of sexual differences in body mass in shiny cowbird chicks raised in wren and mockingbird nests, we adjusted their growth curves to a logistic equation and calculated the equation parameters (i.e. $A$, asymptotic weight; $K$, growth rate; $t_{max}$, age of maximum growth). Then, we used these parameters to estimate the weight of the chicks at different ages and to calculate the maximum growth rate as: $K_{max} = KA/t_{max}$ (Richner, 1991).

In this analysis, we included chicks that fledged and for which we had at least five measurements of body mass (29 shiny cowbird chicks from 29 wren nests and 39 shiny cowbird chicks from 39 mockingbird nests). We analyzed sexual differences in maximum growth rate and the age at which parasite chicks became sexually dimorphic in body mass by comparing body masses from hatching until they were 12 days of age (day 0 = day of hatching). We did not weight the chicks after they were 12 days of age to avoid inducing premature fledging.

Table 1. Sex ratio (male offspring/total offspring) of shiny cowbird eggs laid in nests of house wrens and chalk-browed mockingbird at Reserva El Destino, Province of Buenos Aires, during the 2004–2009 breeding seasons

<table>
<thead>
<tr>
<th>Year</th>
<th>Wrens</th>
<th>Mockingbirds</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>0.57 (23)</td>
<td>0.57 (28)</td>
</tr>
<tr>
<td>2005</td>
<td>0.57 (7)</td>
<td>0.52 (21)</td>
</tr>
<tr>
<td>2006</td>
<td>0.40 (5)</td>
<td>0.48 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>0.54 (35)</td>
<td>0.51 (94)</td>
</tr>
</tbody>
</table>

Numbers given in parenthesis indicate the total number of eggs.

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and year for eggs; host for chicks) were entered as fixed factors into the model and the response variable, sex ratio, was included as the ratio of male offspring to total offspring. To determine sex ratio departure from an expectation of 50 : 50, we analyzed the intercept of the null model (Wilson & Hardy, 2002).

To test for sexual differences in chick development, we adjusted the growth curve to a logistic equation using a generalized nonlinear mixed model (GNLMM; Pinheiro & Bates, 2000), considering repeated measurements of weight within an individual chick. We evaluated the effect of sex and host on logistic equation parameters using a GNLMM with Gaussian family distribution and identity-link function.

For testing the development of sexual differences in body mass of parasite chicks during the nestling period, we used a GLM with Gaussian family distribution and identity-link function. In both analysis, we used maximum growth rate and estimated chick weight as dependent variables, with sex and host as fixed factors.

For all analyses, we used a backward selection procedure, removing nonsignificant terms from the model one by one in decreasing level of interactions and in decreasing order of $P$ within the same level (Crawley, 2007). For statistical analyses, we used NLME in R software, version 2.15.1 (R Development Core Team, 2012). All tests were two-tailed. $P < 0.05$ was considered statistically significant. Data are reported as the mean ± SE.

RESULTS

SEXUAL DIFFERENCES IN BODY MASS
ACCORDING TO NESTLING STAGE

Males had a higher growth rate than females ($5.8 ± 0.1 \text{ g day}^{-1}$ versus $5.1 ± 0.1 \text{ g day}^{-1}$; GLM, $F_{1, 63} = 11.02$, $P < 0.01$), although there were no differences in growth rates between hosts (mockingbird: $5.5 ± 0.1 \text{ g day}^{-1}$, wren: $5.4 ± 0.1 \text{ g day}^{-1}$; GLM, $F_{1, 63} = 0.09$, $P = 0.76$), and the interaction between sex and host was not significant (GLM, $F_{1, 63} = 0.36$, $P = 0.55$). As for sexual differences in body mass during chicks' development, these developed when cowbirds were 7 days of age (males: $26.8 ± 0.7 \text{ g}$, females: $24.7 ± 0.4 \text{ g}$; GLM, $F_{1, 64} = 6.25$, $P = 0.01$) (Fig. 1). Either host or interaction between sex and host did influence body mass of cowbird chicks at day 7 or onwards. Males raised in both hosts reached a mean asymptotic weight of $43.1 ± 0.9 \text{ g}$, whereas the asymptotic weight of females was $38.0 ± 0.5 \text{ g}$.

SEX RATIO OF EGGS LAID AND CHICKS FLEDGED

There were no differences between hosts (GLM, $\chi^2 = 0.009$, d.f. = 1, $P = 0.92$) or among years (GLM, $\chi^2 = 0.81$, d.f. = 2, $P = 0.67$) in the sex ratio of eggs (Table 1). The interaction between host and year was not significant (GLM, $\chi^2 = 0.18$, d.f. = 2, $P = 0.91$). When we pooled the data across hosts and years, the overall ratio of males to females was $1.08 : 1$ (67 males and 62 females) and did not differ from 0.5 (GLM, $\chi^2 = 0.62$, d.f. = 128, $P = 0.54$). Similarly, the proportion of males within each host did not differ from random (GLM; wren: $\chi^2_{34} = 0.51$, d.f. = 34, $P = 0.61$; mockingbird: $\chi^2_{93} = 0.41$, $P = 0.68$).

The proportion of cowbird chicks that fledged in successful nests (i.e. nests at which at least one host or parasite chick fledged) was $97\%$ (33 of 34 chicks) in wrens and $67\%$ (44 of 66 chicks) in mockingbirds (GLM, $\chi^2 = 2.67$, d.f. = 1, $P = 0.01$). Almost $70\%$ of the
deaths of shiny cowbird chicks in mockingbird nests occurred before the development of sexual differences in body mass (15 when they were 1–6 days of age and 7 when they were 7–9 days of age). The proportion of males did not differ between mockingbirds (0.51; 21/41 chicks) and wrens (0.47; 15/32 chicks) (GLM, $\chi^2 = 0.37$, d.f. = 1, $P = 0.71$). When we pooled the data across hosts, the overall ratio of males to females was 0.97:1 (36 males and 37 females) and did not differ from 0.5 (GLM, $\chi^2 = 0.12$, d.f. = 72, $P = 0.91$). Similarly, the proportion of males within each host did not differ from random (GLM; mockingbirds: $\chi^2 = 0.16$, d.f. = 40, $P = 0.88$; wrens: $\chi^2 = 0.35$, d.f. = 31, $P = 0.72$).

**DISCUSSION**

We did not find support for the two possible patterns of sex-ratio bias proposed for sexually size dimorphic brood parasites that evict host chicks. On the one hand, our results do not support the hypothesis of brood parasites biasing the sex ratio towards males when they parasitize larger hosts (Weatherhead, 1989) because we did not observe a male biased sex ratio when shiny cowbirds parasitize mockingbirds (a host almost 50% larger in body mass than the parasite). We found that shiny cowbird chicks are sexually dimorphic, with males having a larger body mass than females once they are 7 days of age and reaching an asymptotic weight 15% larger than that of females. Despite sexual differences in body mass and the relatively high mortality of parasite chicks in mockingbird nests (approximately 33%), we did not detect any evidence of sexual differences in survival of cowbird chicks because the sex ratio of the chick at the time of fledging did not differ from random. Similarly, Weatherhead (1989) reported that the sex ratio of chicks of brown-headed cowbirds in nests of red-winged blackbirds (Agelaius phoeniceus, a host larger than the parasite) and yellow warblers (Dendroica petechia, a host smaller than the parasite) did not differ from random. Weatherhead (1989) concluded that the sexual size dimorphism seen in adult brown-headed cowbirds was not expressed prior to fledging (but see also Tonra et al., 2008) and therefore both sexes fledged equally well from hosts smaller and larger than the parasite. In addition, Weatherhead & Dufour (2005) did not find evidence of male-biased chick mortality after analysing a large data set of red-winged blackbird nests because the sex ratio at the time of fledging did not depart from 50:50. In our case, the more parsimonious explanation for the lack of sexual differences in chick mortality is that, in most cases, the death of the cowbird chick occurs when males and females do not differ in body mass (chicks 1 to 6 days of age) and, likely at this age, they do not differ with respect to their capacity to compete for food with larger host nest-mates.

On the other hand, we did not find support for the hypothesis of parasite females biasing the sex ratio towards the larger sex (e.g. males) when they parasitize hosts smaller than the parasite (Kasumovic et al., 2002) because the sex ratio in wrens, a host considerably smaller than shiny cowbirds, was not skewed towards males. Although Gloag et al. (2012a) reported that provisioning rates (number of feeds per hour) of shiny cowbird chicks were higher in wren than in mockingbird nests, we did not detect differences in growth rates between hosts, which was one of the assumptions of the hypothesis of Kasumovic et al. (2002).

Our results indicate that sexually size dimorphic shiny cowbirds do not skew the sex ratio when they parasitize either large or small hosts. One explanation for these negative results would be that there are no benefits in skewing sex ratios because chick survival does not differ between sexes in hosts larger than the parasite, as proposed by Weatherhead (1989), and males do not growth at higher rates in hosts smaller than the parasite, as proposed by Kasumovic et al. (2002). However, it is important to note that, even if there were benefits for parasitic adult females in skewing the sex ratio, this reproductive strategy imposes some constraints. For example, if female brood parasites are generalists at the individual level (i.e. each female parasitizes several hosts), regardless of the mechanism used to adjust the sex ratio (Pike & Petrie; 2003; Alonso-Alvarez, 2006), parasite females will know, 1–2 days in advance, which host they are going to parasitize to favour the more suitable sex for that host. This would not be a problem if parasitic females were specialists at individual level (i.e. each female parasitizes only one host). In some brood parasites, there is genetic evidence showing that females do not lay eggs at random among hosts (Gibbs et al., 2000; Mahler et al., 2007; Fossay et al., 2011; Spottiswoode et al., 2011), indirectly demonstrating host specificity. In particular, at our study site, the haplotype frequencies of mitochondrial DNA differ between females that parasitize wrens and mockingbirds (Mahler et al., 2007), which indicates that females tend to parasitize one particular host. The most accepted explanation for host specificity in brood parasites is that females imprint on their foster parents and, once mature, they search for nests of the same species in which to lay their eggs (Payne et al., 2000). In this case, however, a bias in the sex ratio towards males in a particular host would imply that the proportion of parasite females recruited by this host would diminish in time. Therefore, the more suitable host for males will not be used by females.
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