

No evidence of sex ratio manipulation by Galápagos mockingbirds in response to environment

Sabrina M. McNew^{1,2}, Sarah A. Knutie^{1,3}, and Dale H. Clayton¹

¹School of Biological Sciences, University of Utah, Salt Lake City, Utah 84112-0840, USA

²Cornell Lab of Ornithology and Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14850, USA

³Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269, USA

Corresponding author: Sabrina M. McNew, School of Biological Sciences, University of Utah, Salt Lake City, Utah 84112-0840, USA. E-mail: sm983@cornell.edu

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Abstract

Birds can adjust investment in reproduction by altering the sex ratios of their broods. When one sex is more costly to raise than the other, producing either more male or female offspring can lower the costs of reproduction. Biasing sex ratios can also be advantageous if the expected fitness of male versus female offspring differs. Here, we investigated whether Galápagos mockingbirds (*Mimus parvulus*) bias sex ratios in response to variation in rainfall. Female mockingbirds are smaller and thus females may reduce the costs of reproduction by biasing sex ratios in favor of female offspring. We quantified sex ratios in 131 mockingbird broods over four years with variable rainfall (2012, 2013, 2015 and 2016). We found no evidence that mockingbirds adjusted sex ratios in response to environmental conditions. Mockingbirds may not adjust sex ratios either because they lack the ability to do so, or because the costs of manipulation outweigh its benefits.

Keywords: Sex allocation, reproductive investment, rainfall

Introduction

Even sex ratios of offspring were long thought to be maintained through frequency dependent selection (Fisher 1930, Bull and Charnov 1988). However, this prediction assumes that the costs and benefits of different sexes are equal, which is often not the case (Trivers and Willard 1973). Male offspring typically have the potential to sire many more offspring than their sisters, and can thus confer a higher inclusive fitness benefit for their parents (Trivers and Willard 1973). At the same time, however, the costs of raising one sex may be higher than the the cost of raising the other sex. In sexually dimorphic species, the larger sex requires more energy to raise and is thus more costly to produce (Myers 1978). As a result, various scenarios predict adaptive sex ratio manipulation to maximize inclusive fitness and/or minimize costs of reproduction.

Environmental conditions may mediate the adaptive value of sex ratio manipulation (Myers 1978). In general, the costs of raising male versus female offspring scales with the degree of sexual size dimorphism (Anderson et al. 1993, Krijgsveld et al. 1998). In poor environmental conditions, parents should favor the less costly sex (Ligon and Ligon 1990, Pryke and Rollins 2012). Conversely, in good environmental conditions parents may opt to invest more in sons, even if they are larger, because the inclusive fitness benefit of a high quality son is greater than that of a high quality daughter (Hasselquist and Kempenaers 2002, Robertson et al. 2006, Pryke and Rollins 2012). For example, conservation biologists discovered that heavy supplemental feeding of the critically endangered kakapo (*Strigops habroptilus*) unexpectedly led females to produce heavily male-biased clutches (Robertson et al. 2006). Kakapo have a lek mating system, which means that a small number of males monopolize a majority of the mating opportunities. Consequently, when environmental conditions are favorable and female kakapo are in a position to produce high-quality offspring, they maximize their inclusive fitness by producing sons instead of daughters. With a more moderate supplemental feeding scheme, kakapo produce an equal sex ratio of offspring (Robertson et al. 2006). These results are consistent with the hypothesis that environmental conditions can drive sex ratio bias by changing the cost-benefit ratio of male versus female offspring.

Here, we investigated whether Galápagos mockingbirds (*Mimus parvulus*) manipulate sex ratios of their broods in response to environmental conditions. Galápagos mockingbirds adjust reproductive investment in different ways in response to climatic variation: in dry years, in which food is limited, they lay small clutches or fail to reproduce altogether. In wet years, they attempt more clutches (Curry and Grant 1990).

Fledgling dispersal is sex biased with females more likely to disperse (Curry and Grant 1990). Sex ratios have not been investigated in this species; however, in a congener, the northern mockingbird (*Mimus polyglottus*), nestling sex ratios differ between populations (Schrand et al. 2011). Northern mockingbirds may overproduce females (the dispersive sex) in high density populations to avoid competition between parents and offspring for food (Schrand et al. 2011).

Galápagos mockingbirds (hereafter “mockingbirds”) may similarly manipulate sex ratios of their broods. Male mockingbirds on Santa Cruz Island are larger than females (adult male mean mass: 46.5 g, female mean mass: 42.3 g, ~ 10% difference, N = 129, unpublished data); thus, raising males should require a larger provisioning investment from parents. In dry years, when resources are limited, mockingbirds should lay female-biased broods. Female offspring are likely less costly to raise and are also more likely to disperse if they fledge, reducing parent-offspring competition. In contrast, in wet years parents should produce male-biased broods, which have the potential to provide greater inclusive fitness to their parents.

Methods

The study was conducted January - April of 2012, 2013, 2015 and 2016 at the El Garrapatero field site on Santa Cruz Island, Galápagos. Daily rainfall was measured at the Charles Darwin Research Station, approximately 10 km from the field site (Charles Darwin Foundation 2018). Total annual rainfall is a common way to characterize year to year variation in conditions in the Galápagos (Grant and Grant 2014). However, we wanted to focus on the conditions that individual pairs experienced before and during breeding. Therefore, we quantified the cumulative amount of seasonal rainfall (from December 1 through the day of first egg hatch). Conditions during nesting attempts in 2012 and 2013 were wetter than those during 2015 and 2016 (Table 1).

Mockingbirds reproduce during the rainy season (typically January – May) in the Galápagos. They construct their nests primarily in *Acacia rorudiana* trees or *Opuntia echios* tree cacti. Males and females both construct the nest, after which females lay between one and five eggs (Knutie et al. 2016). Eggs are incubated by the female for about 15 days. Once the eggs hatch, both parents feed nestlings for about 15 days until they fledge. Nestlings are fed a diet consisting primarily of larval arthropods (Knutie et al. 2016). Dispersal generally occurs following the breeding season, although some fledglings may stay with their parents through the following year (Curry and Grant 1990, SMM pers. obs.). Mockingbirds are year-round residents at our

field site; however, in contrast to populations on other islands (Curry and Grant 1990), pairs do not generally defend territories outside of the breeding season (SMM pers. obs).

We searched for nests following the onset of the rainy season. Nests were checked every other day until nestlings hatched. Nestling mockingbirds were weighed and banded at 9-11 days of age with a numbered monel band and a unique combination of color bands. Fledging success was determined by observing color-banded nestlings after they left the nest. When nestlings were 9-10 days old in 2012, or 5-6 days old in subsequent years, a small blood sample was collected via brachial venipuncture. Samples were kept on wet ice in the field and later centrifuged to separate erythrocytes and plasma, for a separate immunological study, then frozen separately. Samples were kept in a -20°C degree freezer in the Galápagos. After the field season, the samples were transferred with a liquid nitrogen dry shipper to the University of Utah, where they were stored in a -80°C freezer until they were used for molecular sexing of nestlings.

Genomic DNA was extracted from frozen nestling erythrocytes using DNEasy kits (QIAGEN). Nestlings were sexed using PCR amplification of the CHD-W and CHD-Z genes, which differ in size on the W and Z chromosomes. Genes were amplified using primers specifically designed for *Mimus* mockingbirds: 5'-GAGRAAYTGTGCRAAA-CAGG-3', 5'-PET-GAGAYKGAGTCACTATCAGATC-CAG-3'; (Hoeck et al. 2009). PCR reactions were run in a total volume of 25 μl containing 2.5 μl of 10X standard reaction buffer, 500 μM of each dNTP, 0.2 units Taq polymerase (all reagents, New England BioLabs), 0.1 μM of each primer and 2 μl of genomic DNA. PCR conditions were as follows: an initial denaturation of 95°C for 15 min, 32 cycles of 94°C for 30 s, 60°C for 90 s, and 72°C for 1 min, followed by a final elongation step of 60°C for 25 min. Fragments were visualized on an agarose gel, where one fragment corresponded to a male nestling, and two to a female (the heterogametic sex). Each plate was run with both a negative and positive (known adult female) control.

Analyses were run in R (version 3.5.1). Variation in sex ratio among years was analyzed using generalized linear models (GLMs) with binomial errors using the R packages “lme4” and “lmerTest” (Wilson and Hardy 2002, Crawley 2012, Dunn and Smyth 2018). Sex ratio, the dependent variable, was expressed as two vectors composed of the males and females in each brood, which accounts for variation in brood size and is the typical way to encode proportional data in binomial GLMs in R (Zuur et al. 2009, Crawley 2012). Model estimates are presented as “odds ratios,” where values higher than one indicate that the predictor

increases the odds that nests were male-biased. Post-hoc comparisons between years were performed using Tukey HSD tests in the package “emmeans”. The relationship between sex ratio and rainfall was analyzed using a generalized linear mixed effects model (GLMM) with cumulative rainfall as a fixed effect and year as a random effect. Difference in pre-fledging mass between male and female nestlings was analyzed with LMM with sex as a fixed effect and nest as a random effect. Differences in fledging success between male and female nestlings was analyzed with a binomial GLMM with sex as a fixed effect and nest as a random effect.

We sexed 278 out of 362 nestlings (77%) during the study period (Table 1). Of the remaining 84 nestlings, most died before sampling. A small number of samples ($N = 13$; 4%) failed to amplify successfully. We account for potential effects of missing data in three ways: first we report results of analyses of sex ratios run with only nests where the entire brood was sexed. Second, we simulated missing data in which we assumed all missing nestlings were either male or female, and re-ran analyses to see if our conclusions changed. Third, we simulated missing data assuming that the sex of missing nestlings depended on rainfall; i.e. that rainfall caused differential early mortality of males vs. females (see Supplementary material for detailed methods). We created 1000 simulations and re-ran our analyses to see if the results changed qualitatively.

We also ran power analyses to calculate the power of our sample size under a range of effect sizes. We again used a simulation method, creating hypothetical broods based on our true sample sizes where the sex ratios depended on rainfall (see Supplementary material for detailed methods). We again ran 1000 simulations and calculated the proportion of simulations where we correctly identified an effect of rainfall on sex ratio.

Our data estimate the “secondary” sex ratio, because we determined the sex ratios after hatching (Komdeur and Pen 2002). It is possible that the “primary” sex ratio, (i.e. the sex ratio at conception) was different. However, hatching failure was very uncommon in our nests. Thus, we believe that there would be little difference in the sex ratios of the clutch vs. sex ratios of the brood.

Results

The mean sex ratio each year was close to 0.5 and did not differ significantly between years (Table 1, Table 2). The sex ratio of broods was not affected by rainfall (GLMM odds ratio estimate: 1.00, 95% CI: 1.00 – 1.00, $P = 0.84$). Alternative analyses to account for missing data did not change results of sex ratio

differences among years or with rainfall. When we analyzed the subset of nests for which we sexed the entire brood (“complete broods”, $N = 53$), there were no significant differences among years (all Tukey post-hoc comparisons $P > 0.7$, Supplemental Material). Likewise, assuming all missing nestlings were either male or female did not result in significant differences among years (all Tukey post-hoc comparisons $P > 0.4$, Supplemental Material). The effect of rain on sex ratio was not significant when only analyzing complete broods (GLMM odds ratio estimate: 1.00, 95% CI: 1.00 – 1.00, $P = 0.8$), assuming missing nestlings were all female (GLMM odds ratio estimate: 1.00, 95% CI 0.99 – 1.00, $P = 0.3$), or all male (GLMM odds ratio estimate: 1.00, 95% CI: 1.00 – 1.01, $P = 0.18$).

Most simulations of missing data assuming that early mortality was sex-biased depending on rainfall did not find an overall effect of rainfall on sex ratio (Supplementary material). However, under scenarios in which rainfall had a strong effect on sex-biased mortality (odds ratios > 5), 40% of simulations did find a significant effect of rainfall on overall sex ratios.

Close to fledging (at 9-11 days old) male nestlings were significantly larger than females (mean \pm SE male mass: 34.3 ± 0.80 g, female: 31.7 ± 0.53 g; LM estimate 2.63, 95% CI: 0.81 – 4.46, $P = 0.003$). The probability of fledging did not differ significantly between males and females (GLMM odds ratio estimate of difference between sexes: 0.87, 95% CI: 0.54 – 1.41, $P = 0.6$).

We tested our power to detect an effect of rainfall over a range of odds ratios reported in other studies of sex ratio adjustment in birds (Table 3). Simulations indicated that we had low power (< 0.4) to detect small effects of rainfall on sex ratio. However, at odds ratios greater than 3 we had power of 0.6, and for odds ratios greater than 4 our power was 0.8 (Supplementary material).

Discussion

Rainfall was twice as high during the first two breeding seasons as the second two seasons (Table 1). However, in each year the overall ratio of male to female nestlings was close to 1:1. Sex ratio did not vary significantly among years or with rainfall (Table 1). Male nestlings close to fledging were significantly larger than female nestlings, consistent with the hypothesis that males are more costly to raise than females. Nevertheless, we did not find evidence that mockingbirds manipulated sex ratios of their broods to adjust the costs of reproduction in response to environmental conditions.

Missing data from the 23% of nestlings that we did not sex could have affected our results if early mortality rates differed between males and females. Some previous studies have found higher nestling mortality of the larger sex in size dimorphic species, potentially due to their higher energetic demands (Cooch et al. 1997, González-Solís et al. 2005), while others report higher mortality of the smaller sex, perhaps because they have a diminished ability to compete with siblings for food (Arroyo 2002, Hipkiss et al. 2002). To estimate whether early mortality of either sex could have affected our conclusions, we simulated data under a range of conditions. Assuming that all missing nestlings were male or female did not result in a significant relationship between rainfall and sex ratio. In cases where early nestling mortality was strongly sex-biased depending on rainfall, 40% of our simulations found a significant effect of rainfall on sex ratio. These simulations suggest there are scenarios in which our missing data created a type II error, i.e. a false negative result. However, we do not believe these possibilities are very likely. First, among nestlings that we did sex, there was no difference in fledging success between sexes, with or without considering rainfall conditions. Consequently, we have no reason to predict that early mortality was strongly sex-biased. Second, most simulations still did not find a relationship between rainfall and overall sex ratio, even in those extreme cases of sex-biased mortality. Thus, we think it unlikely that missing data qualitatively affected our results.

In advance of doing the study we did not know what size effect rainfall could have on sex ratios. Our sample size (complete broods = 53, total broods = 116) is comparable to or higher than other recent studies of sex ratio bias in relation to environmental factors (Table 3). Although our power to detect effects of rainfall on sex ratio is low, we had sufficient power to detect larger differences (Supplementary material). Sample sizes of avian field studies are often limited for practical reasons. However, our power analysis suggests that much larger sample sizes may be necessary to detect sex ratio adjustments in natural population.

We do not know for certain that mockingbirds have the capacity to manipulate sex ratios; however, physiological and comparative evidence suggest the mechanism to do so could exist in this species. Stress hormones such as glucocorticoids are thought to have an important role in determining avian sex before eggs are laid (Bonier et al. 2007, Navara 2013). Maternal transfer of corticosterone to eggs may be a mechanism allowing females to adjust reproductive investment during stressful conditions and better “match” their own quality to offspring demand (Love and Williams 2008). Sex ratio manipulation has been documented across avian orders, including Bucerotiformes, Ciconiiformes, Columbiformes, Psittaciformes, Strigiformes, and

within various Old and New World passerine families, such as Acrocephalidae, Estrildidae, Icteridae, Mimidae, Muscivoridae, Paridae, Sturnidae, Troglodytidae, and Tyrannidae (Sheldon 1998, Hasselquist and Kempenaers 2002, Komdeur and Pen 2002, Benito and González-Solís 2007, Goerlich-Jansson et al. 2013, Kamiński et al. 2015, Kus et al. 2017). This pattern, coupled with a conserved physiological mechanism linked to sex determination (stress hormones), suggests that the ability to manipulate sex ratios exists broadly across avian lineages. Still, future comparative studies may reveal particular ecological factors or life history traits that constrain or promote the emergence of this trait in different taxa.

Confirming the absence of sex ratio manipulation is more challenging than verifying its presence because sample sizes limit our ability to be sure that there are no small differences, and it can be difficult to sample the extremes of selective conditions. Nevertheless, our data are most consistent with the conclusion that Galápagos mockingbirds do not bias sex ratios in response to variation in rainfall. Healthy mockingbirds may still be able to adequately provision male-biased broods even when resources are more limited. Future work could directly investigate parental condition and corresponding brood sex ratios to test whether mockingbirds in poor condition invest more in females, the less costly sex. Mockingbirds may also simply adjust reproductive investment to the environment by altering brood size without manipulating sex ratios (Curry and Grant 1990).

Our results are consistent with other studies that find no sex ratio bias even under conditions in which it would seem adaptive (Koenig and Dickinson 1996, Radford and Blakey 2000, Postma et al. 2011, Saalfeld et al. 2013, Benvenuti et al. 2018). Some species may have lost the ability to bias sex ratios (Postma et al. 2011). In others, the costs of sex ratio manipulation may outweigh its benefits (Hasselquist and Kempenaers 2002, West and Sheldon 2002). Future experimental and comparative work is needed to reconcile variation in the results of sex ratio studies and provide a unifying framework for understanding this trait in birds.

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Data accessibility statement

Files used for analysis will be available on Github, pending article acceptance.

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Table Legends

Table 1. Summary of environmental conditions, sample sizes and sex ratios across years.

	Year			
	2012	2013	2015	2016
Mean (\pm SE) cumulative rainfall at hatching 1 st				
egg (mm)	182.3 \pm 7.3	142.7 \pm 4.4	69.5 \pm 1.4	79.9 \pm 1.8
Total nests (nestlings) sexed	25 (60)	31 (73)	35 (81)	25 (64)
Sex ratio (mean \pm SE proportion male per nest)	0.42 \pm 0.08	0.56 \pm 0.07	0.47 \pm 0.07	0.48 \pm 0.07

Table 2. Model (GLMM) estimates of sex ratios (in logits) for each year of the study.

Year	Estimate	95% CI	Group ^a
2012	-0.20	-0.71 – 0.31	A
2013	0.25	-0.21 – 0.71	A
2015	-0.07	-0.51 – 0.36	A
2016	-0.06	-0.55 – 0.43	A

^aGroup assignment based on Tukey post-hoc tests between years. The same group assignment for all years indicates that no pairwise comparisons were significantly different.

Table 3: Summary of effect sizes of other recent studies of avian sex ratio manipulation in response to environmental or parental factors.

Study	Name	Nests	Chicks	Predictor variable	Effect size ^a
Abroe et al. 2007	Common yellowthroat (<i>Geothlypis trichas</i>)	138	486	Male size	0.51 (0.25)*
Benito et al. 2013	Common tern (<i>Sterna hirundo</i>)	62	258	Year quality	0.71 (0.409)*
Bouland et al. 2012	Belted kingfisher (<i>Megaceryle alcyon</i>)	23	146	Mercury contamination	1.77
Bouland et al. 2012	Eastern bluebird (<i>Sialia sialis</i>)	46	188	Mercury contamination	1.22
Bouland et al. 2012	Tree swallow (<i>Tachycineta bicolor</i>)	41	216	Mercury contamination	1.90
Bradbury and Blakey 1998	Zebra finch (<i>Poephila (Taenopygia) guttata</i>)	43	96	Diet	2.09
Clout et al. 2002	Kakapo (<i>Strigops habroptilus</i>)	18	33	Diet	4.97
Doutrelant et al. 2004	Sociable weaver (<i>Philetarius socius</i>)	58	170	Presence of helpers	3.07
Göth and Booth 2005	Australian brush-turkey (<i>Alectura lathami</i>)	--	62	Incubation temperature	7.75
Korpimäki et al. 2000	Eurasian kestrel (<i>Falco tinnunculus</i>)	83	332	Parental condition	-0.07 (0.03)*
Laucht et al. 2008	Lance-tailed manakin (<i>Chiroxiphia lanceolata</i>)	101	173	Maternal age	1.23 (0.43) ^{*b}
Li et al. 2018	Isabelline wheatear (<i>Oenanthe isabellina</i>)	42	205	Grazing pressure	1.99
Saino et al. 2007	Barn swallow (<i>Hirunda rustica</i>)	553	> 2200	Temperature during laying	0.08 (0.03)*
This study	Galápagos mockingbird (<i>Mimus parvulus</i>)	116	278	Rainfall	0.00 (0.00)*

^a Effect sizes are given as odds ratios between treatments (for categorical predictors) or coefficient estimates in logits (for continuous predictors; indicated by *). Odds ratios were calculated from reported proportion of males in each treatment. Coefficient estimates were extracted from reported logistic regression models. Standard errors of coefficient estimates are given in parentheses.

^b The quadratic term, i.e. (maternal age)² was also significant in this model. Estimated coefficient (SE) = -0.12 (0.05)