METHODS

Contradictory results in sex ratio studies: populations do not necessarily differ

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Abstract In avian sex ratio studies, results often differ between species and between populations within species. Some researchers argued that positive results were simply statistical artefacts and that sex ratio adjustment did not exist. However, many of the proposed mechanisms of sex ratio adjustment result in costly laying gaps. In these cases, females laying large clutches may restrict the sex manipulation to the first egg of the clutch. Consequently, detectability of sex ratio adjustment on the level of the clutch can be low. Though obvious, this fact is often neglected in the literature. Using simulations, I show that the proportion of undetected sex manipulation can be surprisingly high when the manipulation is restricted to the first egg. If the sample size is 50 broods, there is 47% and 71% chance in 6- and 12-egg clutches, respectively, that sex manipulation is undetected. Even with large samples ($n=100$), the figures are 15% and 46%. These data suggest that nonsignificant results for clutch sex ratios do not necessarily mean that sex is not manipulated in a portion of the brood, e.g. in first-laid eggs. Hence, whenever possible, data on laying order-specific sex manipulation should also be collected. Without such data, contradictory results on brood sex ratios should be interpreted cautiously.

Keywords Population differences · Sex ratio adjustment · Sex ratio variance · Simulation

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Introduction

After molecular methods for sexing birds had been developed, the number of studies published on avian sex allocation increased rapidly. Still, sex ratio adjustment is a controversial issue. Results often differ between populations of the same species (Table 1) and even two recent studies using a meta-analytic approach came to opposite conclusions. West and Sheldon (2002) performed their analyses on studies with clear predictions (i.e. females that mated to more attractive males should produce more sons and in cooperatively breeding species females without helper should bias their sex ratio towards the helping sex). They found that the observed brood sex ratio patterns from various taxa were in agreement with these predictions. They argued that 3% to 18% of the variance in sex ratio among families may be explained by facultative sex ratio adjustment. On the contrary, Ewen et al. (2004) claimed that, excluding some exceptional cases, avian sex ratio adjustment is rather a statistical artefact. They found that biological (e.g. parental traits, territory quality) and temporal traits (e.g. season) had a strong effect on sex ratios mainly in studies with moderate sample sizes, while in very large datasets the effect size was close to zero. They argued that the disappearance of significant patterns with increasing sample size suggests that the significances were due to type I error rather than real biological phenomena.

However, if large sample sizes are achieved using data from multiple years (which is often the case), the overall small effect size might be the consequence of different optima of sex allocation in different years. This is because females face different environments across years and populations and the costs and benefits of sex ratio adjustment might therefore also change. Changes in the sex allocation rules are expected especially when multiple

Table 1 Some examples of contradictory results in studies of sex ratio adjustment

Species	Study	Sample size/year	Number of years	Male attractiveness	Male quality	Female quality	Laying date	Male age	Female age	Clutch size
Parus caeruleus	Svensson and Nilsson 1996	41	1		+a	0a	0	0	0	
	Sheldon et al. 1999	41, 57	1	+	+a	0a	?	?	?	
	Leech et al. 2001	51	3		0abcd	0abcd	0	0	0	0
	Griffith et al. 2003	57	3	+	+a		0	0	0(+1)	0(-1)
Parus major	Lessells et al. 1996	103	1		0b	0b	+	0	0	0
	Kölliker et al. 1999	57	1	{+}	+c	0c	0	0	0	0
	Radford and Blakey 2000	24	5	0	0c(-1) 0e	0c(+1–1) 0e(+1)	0(+1)	0(-1)	0	
	Oddie and Reim 2002	79[23]	3		0ce	0ce[+e]	0	0	0	0
Ficedula albicollis	Ellegren et al. 1996	79	1	+	0c		0	0	0	0
	Rosivall et al. 2004	57	1	0	0c	0c	+	0	0	0

Empty cells indicate traits which were not examined in the studies. In the case of parental quality, see codes a to e below. In multiple year studies the overall result is shown. If traits were significant in certain years only, then the direction of the effect and the number of years in which it was found is indicated in parentheses. Marginally significant result is indicated in curly brackets. Between-year comparisons for the same individuals are indicated in square brackets. In Sheldon et al. (1999), first sample size refers to male attractiveness, while the second refers to all the other traits.

0 No effect, +/- positive/negative correlation between the trait and sex ratio, ? reports of significant effects without indicating the direction of the correlation, *a* overwinter survival, *b* body mass, *c* tarsus length, *d* parasite load, *e* condition (residual from body mass–tarsus length regression)

selection pressures act on sex ratio in the same population (for some examples, see Cockburn et al. 2002). Conflicting sex allocation patterns between populations may also be explained by methodological differences. Such differences may involve e.g. different breeding conditions like in the case of captive zebra finch (*Taeniopygia guttata*) studies (Burley 1986; Rutstein et al. 2005; Zann and Runcimen 2003). Furthermore, as I discuss below, some of the potential mechanisms of sex ratio adjustment may make the investigation of sex allocation difficult.

Potential mechanisms of sex ratio adjustment (for a review, see Pike and Petrie 2003) can be divided into two major groups, depending on whether sex is manipulated with or without gaps in the egg laying sequence. Because follicles are growing in a hierarchical way and the secondary follicle is in a developmental delay compared to the primary follicle, mechanisms acting after meiosis and requiring the resorption or elimination of the primary follicle or ova are expected to result in laying gaps (Krackow 1999). On the other hand, mechanisms affecting the outcome of the chromosomal segregation can lead to biased sex ratios without laying gaps. Our expectations are substantially different in these two cases. If the mechanism results in laying gaps, birds with large clutches may have to pay severe costs, such as increased predation risk and decreased viability of eggs, due to delayed clutch completion (Emlen 1997). Thus, sex ratio manipulation would be expected to be restricted to the first egg(s) in such species. In species laying small clutches (two to three eggs), there is probably no egg viability cost, so they are expected to adjust the sex of each egg. On the other hand, if sex ratio adjustment acts via mechanisms which do not lead to laying gaps, females might manipulate the sex of their offspring throughout the laying sequence independent of clutch size. So, for large clutches, the effect sizes at the clutch level are expected to differ under the two scenarios. Because we have no clear evidence so far for any of the possible mechanisms of sex ratio manipulation and not necessarily the same mechanism has evolved in different species, it is important to adjust the methods of analysis so that sex ratio patterns can be reliably detected.

Using simulations here, I report the detectability of sex ratio adjustment at the clutch level when the manipulation is restricted to the first egg in large clutches. For simplicity, I assume that only one trait is related to clutch sex ratios. I create 500 simulated populations (or studies) and report the proportion in which sex ratio manipulation in relation to this trait is undetected on the clutch level. I then compare

this clutch-level detectability to "studies" investigating specifically the sex of the first egg in relation to the given trait.

Materials and methods

Data generation

First, I created a theoretical population with 100 individuals. I assumed that only one phenotypic trait plays a role in the sex ratio manipulation of this species, and this trait ranges from 30 to 100 and is normally distributed. In the resulting model population, the mean (±SD) trait size was 64.79 ± 16.32. I assumed that females could manipulate the sex of the first-laid egg but not that of the later-laid eggs and that sex manipulation had some cost. Because benefits need to overcome the costs for a behaviour to be adaptive, I also assumed that females manipulated the sex of the first egg only in cases when the trait value was particularly large or small; otherwise, the sex of the first egg would result from random segregation. I therefore sorted the females by the trait values. The first eggs of females with high values (one third of the population) were always male; the first eggs of females with low values (one third of the population) were always female, while the sex of the first egg of females with medium trait values was decided using a random number generator (in Microsoft Excel 2000). For all eggs laid later in the sequence, sex was assigned randomly.

Keeping the trait size constant, I repeated the process 500 times. As a consequence, I had 500 "populations" with 100 individuals, in each of which females used the same sex allocation rules. The only difference was generated by the random chromosomal segregation in later-laid eggs.

To simulate the effect of different sample sizes, I took random samples of 50, 25 and 13 individuals from my original population and assigned sex to the eggs as above. I had 500 repeats for all sample sizes. Note that individuals (trait values) did not change between repeats but the sex of the eggs did.

To explore the effect of clutch size on the probability of detecting sex ratio manipulation at the clutch level, I repeated the above procedures twice. I first used medium-sized clutches with six eggs (typical of many popular study species such as collared flycatcher (Cramp 1993) and house wren (Albrecht and Johnson 2002)) and then large clutches with 12 eggs (typical of e.g. blue tit; Cramp 1993).

Though sex manipulation restricted to the first egg implicitly assumed to work via recognition and subsequent elimination of the unwanted ova by the mothers (i.e. an expectedly accurate method, as I assumed above), I also tested a scenario when sex manipulation is less precise. In this scenario, the probability of producing male offspring in the first egg is continuously increasing from 0 to 1 with the trait size. The probability of producing males in later-laid eggs was 0.5 as above.

Statistical analysis

Throughout this study, I used generalised linear models with binomial errors and logit link (Crawley 1993) in the GENMOD procedure of Statistical Analysis System (SAS) 8.2 (SAS Institute Inc., Cary, NC, USA). Sex of the first egg or clutch sex ratio was the binomial response variable and trait value was the continuous predictor. Analyses were performed separately for each study "population". Because the dispersion parameter was larger than 1.0 in some of these "populations", I tested the significance of the parameters with F-tests (Crawley 1993; dscale option in GENMOD).

Results

The simulations show that even in species with medium-sized clutches (six eggs), the detection of sex ratio manipulation at the clutch level becomes extremely difficult if sex ratio manipulation is restricted to the first egg (Fig. 1a–d; Table 2). When 50 clutches were sampled (the average sample size per year was 58.9 for the studies in Table 1), the existing sex ratio manipulation was undetected in 47.4% of the cases. Even when sample size was twice as large, 15.2% of the "studies" did not find significant effect of the examined trait on clutch sex ratios.

In species with large clutch sizes (12 eggs), the situation is even worse (Fig. 1e–h; Table 2). Even with large samples ($n=100$), I did not find significant relationship between the trait and clutch sex ratio in approximately half of the cases (45.8%), though there was sex manipulation in all cases. However, when I analysed the effect of trait value on the sex of the first egg, the results were significant in all 500 "populations" independent of sample size.

Assuming a less accurate sex ratio adjustment, when the probability of producing male offspring in the first egg is continuously increasing from 0 to 1 with the trait size, I found an even lower sex ratio detectability. For a sample size of 50, sex ratio adjustment was undetected in 82.4% and 88.8% of the "populations" in six- and 12-egg clutches, respectively. In this case, testing the sex of the first egg also had lower statistical power as the sex manipulation was undetected in 16.8% of the "populations".

Discussion

Many previous studies on sex ratio adjustment found contradictory results among populations (Table 1). The
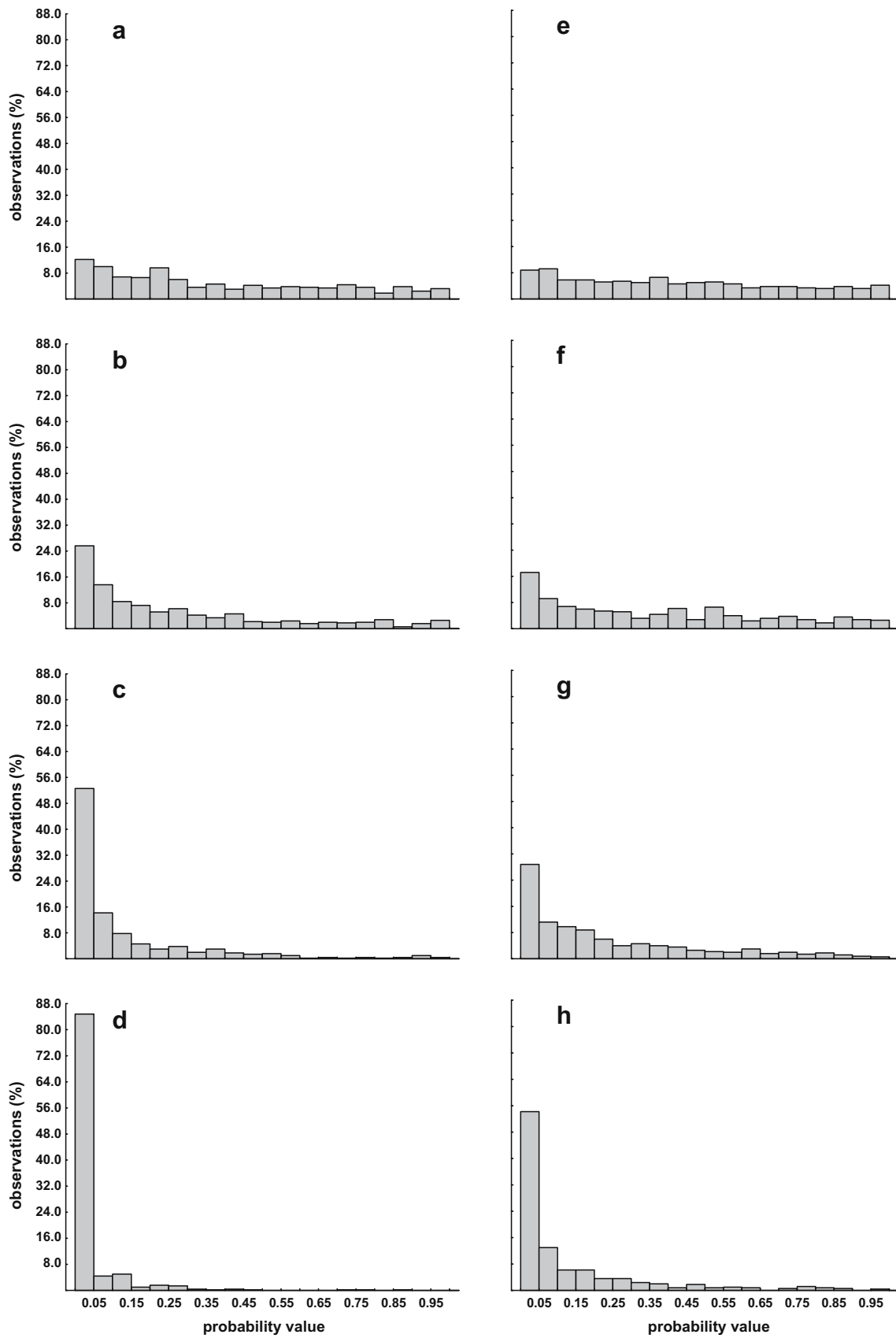
◀ **Fig. 1** Detectability of sex ratio adjustment at the clutch level, when the adjustment is restricted to the first egg. The figure shows the distribution of the p values from 500 different populations when females follow the same sex allocation rule (i.e. bias the sex of the first egg in relation to a given trait). Results are significant when $p <$ 0.05. The clutch size is six (**a**–**d**) or 12 (**e**–**h**). The sample size within each population is 13 (**a** and **e**), 25 (**b** and **f**), 50 (**c** and **g**) or 100 clutches (**d** and **h**)

figures derived from my simulations show, however, that it is easy to find contradictory patterns even if females follow the same decision rule in all populations. When females manipulate the sex in the first egg but not in later-laid eggs (e.g. due to the costly laying gaps resulting from sex ratio adjustment; Emlen 1997) and we consider clutch sex ratio as the object of our investigation, random segregation of the sex chromosomes in later-laid eggs will often mask the effect of maternal manipulation. As shown here, the probability of this mistake is remarkable (47.4% and 71.2% with a sample size of 50 broods in species with six- and 12-egg clutches, respectively; for details, see Fig. 1 and Table 2) even when the sex manipulation mechanism is accurate. When the accuracy of the mechanism is lower, the chances of mistake can be even higher (the figures in this case can be as high as 82.4% and 88.8%). One question remains, however. Does sex ratio adjustment result in laying gaps? The answer is unknown, because there is no clear evidence so far for any of the suggested mechanisms (Pike and Petrie 2003). More importantly, though mechanisms acting after meiosis (and resulting in laying gaps) are considered more costly (Pike and Petrie 2003), sex ratio adjustment may have evolved multiple times and thus multiple mechanisms may exist. Interestingly, in a couple of species, strong sex bias among the first chicks has been found, while there was no bias in younger sibs (Arnold et al. 2001; Blanco et al. 2002). Furthermore, the strongest bias in brood sex ratios was found in species with small clutches (Heinsohn et al. 1997; Komdeur et al. 2002; Pike 2005). On the other hand, biased sex ratios have also been observed in later-laid eggs in other species (Dijkstra et al. 1990; Rutkowska and Cichoń 2002; Rutstein et al. 2004).

Further investigation of sex ratios may shed light on the possible mechanisms, but this requires more detailed analyses which take into account that the probability of sex manipulation may vary with laying order. Collecting and analysing such data requires much additional work

Table 2 The percentage of populations where sex ratio adjustment is undetected when the manipulation is restricted to the first egg

	Sample size (number of broods)			
	13	25	50	100
Six-egg clutch	87.8	74.4	47.4	15.2
Twelve-egg clutch	91.2	82.8	71.2	45.8

from field biologists; therefore, a method which could be used for testing the first egg versus the whole clutch manipulation hypotheses using brood sex ratio data would be useful. Krackow et al. (2002) suggest that the variance of brood sex ratios may depend on the mechanism of sex allocation. In case of brood sex ratio manipulation, the probability of producing sons (p) will differ among broods; thus, the resulting distribution will be a mixture of binomials and will probably be overdispersed (Krackow et al. 2002). If the manipulation is restricted to a few eggs, then the random processes in unmanipulated eggs will decrease the difference in p among broods and, as a consequence, overdispersion is also expected to decrease. The larger the proportion of unmanipulated eggs, the smaller the deviation from binomial expectations. We may therefore expect that the deviation from binomial variance will decrease with clutch size if only a small, fixed number of eggs are manipulated. However, when exactly one egg is manipulated and the population sex ratio of this one egg is similar to that of unmanipulated eggs, the manipulation of this egg will not result in increased variation of p among broods. In this case, clutch size effect on sex ratio variation cannot be expected. Actually, it will be the proportion of variation explained by the explanatory variable that will decrease with clutch size (Fig. 2). However, analysing explained variance for all the possible explanatory variables and clutch sizes is not a straightforward method, principally because this would require very large sample sizes for each clutch size. Thus, I have to conclude that first egg versus whole clutch hypothesis cannot be reliably tested using exclusively brood sex ratio data.

In any case, the mechanism of sex ratio adjustment which was tested here could be considered unrealistic if the observed effect sizes strongly differed from those in natural
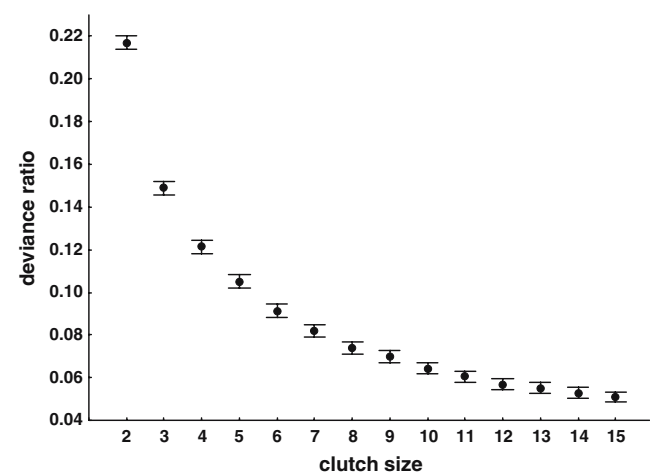
Fig. 2 The effect of clutch size on the proportion of brood sex ratio variance explained by the studied trait when this trait affected only the sex of the first egg. The mean deviance ratio ± standard error of the 500 "populations" ($n=50$ in each) is shown

populations. However, this was not the case. For instance, two studies on collared flycatchers (i.e. a species with an average brood size of six) for which effect size (r) could be calculated (as the square root of deviance ratio; Ellegren et al. 1996) reported very similar values (0.288 and 0.279; Ellegren et al. 1996 and Rosivall et al. 2004, respectively) to those found in this study for a sample size of 50 and brood size of six (mean r=0.302).

Thus, I argue that sex manipulation restricted to the first egg is not unrealistic and can easily be overlooked when analysing brood sex ratios due to the low power of statistical tests. This may explain part of the contradictory results in the literature (though non-stochastic explanations, like variation in sex allocation optima between years or populations, probably also exist). Accordingly, contradictory or negative results based on clutch sex ratios should be interpreted carefully and should not be used as an argument against the existence of sex ratio adjustment. Instead, it is necessary to test first egg and whole clutch sex patterns together to reach any reliable conclusion on the presence, direction and population or species differences of sex ratio manipulation because the general mechanism of sex ratio manipulation is unknown, and it is questionable whether a single general mechanism exists.

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