Behavioral Ecology

The official journal of the
ISBE
International Society for Behavioral Ecology

doi:10.1093/beheco/art007
Advance Access publication 21 February 2013

Invited Ideas

Chimeric embryos—potential mechanism of avian offspring sex manipulation

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Environmental and social effects on offspring sex ratio bias in birds are among the most studied topics in evolutionary biology. Although it is established that offspring sex is determined at the stage of the first meiotic division, there is no direct evidence for the common belief that the bias happens via nonrandom sex chromosome segregation. In this paper, we suggest an alternative mechanism based on the failure of polar body emission, followed by fertilization of multiple haploid nuclei through polyspermy and subsequent competition of Z and W bearing cells within chimeric embryos. Although the occurrence of failure of polar body extrusion is obvious from the observations of chimeric birds, the idea that it could be the first step of offspring sex ratio bias has been entirely overlooked. We review the evidence in support of that idea, demonstrate that it is consistent with the observations of biased offspring sex ratio reported so far, and suggest a way to verify it.

Key words: **gynandromorph, meiosis, oogenesis, polar body extrusion, polyspermy, primary sex ratio.** *[Behav Ecol]*

INTRODUCTION

Relative costs and benefits of producing sons and daughters differ across ecological and social environments. Therefore, the potential ability to control offspring sex could have strong fitness consequences. This issue has attracted attention both from theoretical (Trivers and Willard 1973; Charnov 1982; Krackow 2002; Kokko and Jennions 2008) and from empirical (West and Sheldon 2002; Alonso-Alvarez 2006) perspectives. Birds have become an especially popular model to study sex ratio (SR) manipulation. The avian female is a heterogametic sex bearing 2 sex chromosomes, Z and W, and, therefore, producing 2 types of gametes, each of them with 1 sex chromosome. Thus, the opportunity of offspring primary SR bias in this group could be relatively high. A variety of life-history traits in this group leads to clear predictions on the direction of expected sex allocation pattern, and a number of studies reported adaptive primary SR adjustments (e.g., Komdeur et al. 1997; Badyaev et al. 2002; Griffin et al. 2005; Pryke and Griffith 2009). Some remarkably strong sex biases, such as sequential production of 20 male offspring in eggs laid by an individual female (Heinsohn et al. 1997), provide strong evidence that this process might be nonrandom. Yet, the proximate mechanism behind these observations still remains highly speculative.

Several potential mechanisms could occur at different stages of egg production (Alonso-Alvarez 2006). Some of the mechanisms that were historically considered as potential explanations of SR bias, such as sex-specific fertilization and sex-specific embryo mortality, would involve a waste of time and resources devoted to egg production. They would also not explain patterns of fine-tuned primary SR bias occurring from day to day (Rutkowska and Cichoń 2002, 2006). Currently, it is a commonly accepted view that offspring sex in birds is decided after the follicular development has finished. Specifically, it is believed to be determined at the first meiotic division (MI), during which the sex chromosomes segregate (Rutkowska and Badyaev 2008). Recent evidence suggests that offspring SR bias via segregation distortion might be induced by acute hormonal treatment (Gam et al. 2011; Pinson, Parr, et al. 2011; Pinson, Wilson, et al. 2011). This is in line with previously reported effects of hormonal manipulation on offspring primary SR (e.g., Veiga et al. 2004; Correa et al. 2005; Pike and Petrie 2005; Rutkowska and Cichoń 2006; Bonier et al. 2007; Goerlich et al. 2009), but does not bring us much closer to pinpointing the specific proximate mechanism by which hormones affect sex chromosome segregation distortion. Importantly, the observed SR biases could be likewise explained by an alternative mechanism, which is also molded by hormones and acts during MI.

NEW MECHANISM OF OFFSPRING SRBIAS

The main idea of our hypothesis (Figure 1) is that hormonal fluctuations caused by external factors encountered by the female alter the normal process of MI by blocking segregation of the first polar body (PB)—a phenomenon responsible for the occurrence of mixed-sex chimeras in birds (Hollander 1975; Zhao et al. 2010). As a result,

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Received 1 October 2012; revised 12 January 2013; accepted 15 January 2013.

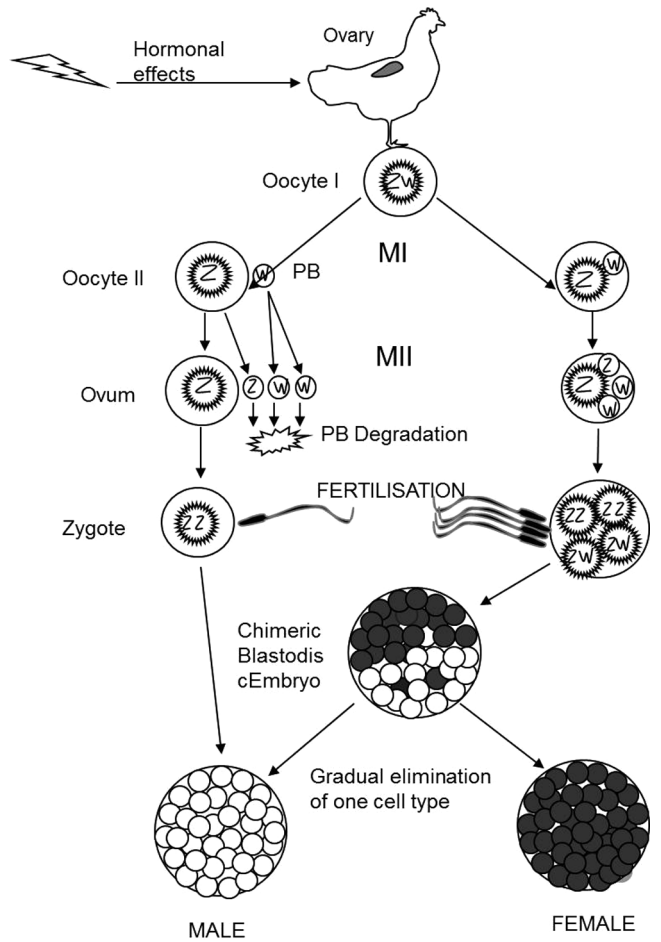

Figure 1
Model of PB exclusion failure as a possible mechanism explaining the primary SR bias in birds. Left downstream—normal meiotic division resulting in the formation of an ovum and extrusion of 3 PBs away from the cytoplasm of the ovum. PBs in this process undergo developmental suppression and degenerate. Right downstream—as a result of hormonal effects, PB extrusion is disrupted during MI and PB with its full set of chromosomes remains in the active cytoplasm. MII results in the formation of 4 female pronuclei. Polyspermy ensures fertilization of all maternal pronulei and formation of 2 different types of cells: male (ZZ) and female (ZW). During the subsequent stages of development, gradual elimination of 1 cell type (ZZ or ZW) takes place according to the hormonal/chemical milieu in the vicinity of the embryo. This leads to biased offspring SR at the egg-laying stage.

the first PB with its full set of chromosomes remains in the active cytoplasm of the ovum. Thus, at that moment, there are 2 different haploid sets of chromosomes (A + W and A + Z) in the ovum. The second meiotic division (MII) usually follows shortly after the first and results in the doubling of the chromosome content (2 sets of A + W and 2 sets of A + Z). Ovulation occurs about an hour after MI, and the oocyte is fertilized soon after the ovulation.

Physiological polyspermy in birds involves 20–60 spermatozoa undergoing acrosomal reaction and penetrating perivitelline membrane (e.g., Birkhead et al. 1993; Birkhead and Fletcher 1994). Because there are 4 different female pronuclei in the ovum, polyspermy might ensure fertilization of those pronuclei by different sperms. This process would result in formation of up to 4 diploid blastomeres (two of them will be 2A + WZ and two 2A + ZZ). Therefore, both ZW and ZZ cells coexist in the chimeric embryo after fertilization (Zhao et al. 2010). We expect that the chemical (hormonal) environment in the egg differentially affects the proliferation of Z- and W-bearing cells (Figure 2). Eventually, the growth of one cell type (ZZ or ZW) is ceased, whereas the other determines the sex of the embryo. We suggest that the latter scenario could be responsible for the SR bias observed at the egg-laying stage. Below, we review the premises supporting the suggested mechanism.

OCCURRENCE AND ORIGIN OF GYNANDROMORPHIC BIRDS

Gynandromorphic individuals have both male- and female-specific cells, often apparent as a bilateral asymmetry. Gynandromorphic birds comprise a wide range of species, such as black-throated blue warblers (*Dendroica caerulescens*, Patten 1993), house sparrows (*Passer domesticus*, Abella 2002), pheasants (*Phasianus* sp., Huxley and Bond 1934), white-ruffed manakins (*Corapipo altera*, DaCosta et al. 2007), and black redstarts (*Phoenicurus ochruros*, Weggler 2005). Among pigeons, 182 cases of spontaneous chimeras were described (Hollander 1975). There might be various mechanisms responsible for the occurrence of a gynandromorphic phenotype. Chromosomal bases of gynandromorphism were confirmed in zebra finches (*Taeniopygia guttata*, Agate et al. 2003) and domestic chickens (*Gallus domesticus*, Cock 1955; Zhao et al. 2010). Study by Zhao et al. (2010) on 3 hens lead to conclusions that mixed-sex chimerism arises as a result of the failure of extrusion of a PB during MI and subsequent fertilization of both a Z- and W-bearing female pronuclei. This conclusion supports the mechanism of SR bias suggested in the current review.

FAILURE OF PB EXTRUSION AND ITS DEVELOPMENTAL POTENTIAL

The failure of PB extrusion is a widespread phenomenon occurring in the animal kingdom, happening in response to many external stimuli such as physical or chemical shock (e.g., Yang and Guo 2006; Piferrer et al. 2009). In females of higher vertebrates, oocyte maturation takes place inside the female and involves meiotic cell cycle progression from prophase I to metaphase II and extrusion of the first PB. Steroid hormone fluctuations are the natural triggers of this process (Johnson and Van Tienhoven 1980). Given that PB emission is mediated by actin, the polymerization of which is known to be driven by hormones (e.g., Manavathi and Kumar 2006), any abnormal hormonal fluctuation around the time of MI might disrupt the process of PB extrusion. For instance, follicle stimulating hormone controls MI progression and PB extrusion of hamster oocytes (Plancha and Albertini 1994).

In birds, PB extrusion takes place approximately 1–2 h prior to ovulation (Yoshimura et al. 1993). Although not possible to observe in action, the failure of PB extrusion can be inferred from the occurrence of polyploid embryos. In birds, spontaneous triploids are one of the suggested reasons of embryonic mortality (Forstmeier and Ellegren 2010). Yet, the potential to form triploids is a trait achievable under artificial selection in chickens (Thorne et al. 1991). Detailed analyses performed on 147 triploid chicken embryos lead to the conclusion that 12% of them were caused by MI suppression (Fechheimer 1981). Although the polyploid embryos cannot provide the potential pathway for offspring SR bias, the above evidence offers an additional indication that PB extrusion failure is commonplace in birds.

It has been established that the chromosomes from the first PB have the same genetic potential as those remaining in the oocyte after the MI and that under the appropriate conditions, they can cause normal embryonic development. For example, the first PB from mice injected into the enucleated mature oocyte and

artificially inseminated gives rise to successful development in 57% of embryos implanted to the foster females (Wakayama and Yanagimachi 1998). Inferring from how natural gynandromorphs occur in birds (Agate et al. 2003; Zhao et al. 2010), we assume that avian PBs are also capable of giving rise to functional organisms.

DIFFERENTIAL DEVELOPMENTAL POTENTIAL OF MALE AND FEMALE BLASTOMERES IN BIRDS

For the suggested mechanism to work, elevated hormonal flux in the female should not only block the emission of the PB but should also directionally affect the competition between male and female cells in the chimeric blastodiscs. In fact, some of the triploid genotypes are more likely to survive than others during embryogenesis, suggesting the possibility of unequal developmental potential intrinsic to Z and W (Thorne et al. 1991). Triploids with ZWW survive only a few days of incubation, whereas individuals with ZZW and ZZZ can survive to hatching and maturity. Furthermore, unfertilized chicken eggs exhibit high incidence of Z chromosome bias (Klein and Grossmann 2008). Such an effect could be caused by sex differences in developmental potential and/or a segregation bias toward Z in unfertilized eggs (Klein and Grossmann 2008).

Although analysis of developmental potential of triploids (Fechheimer 1981; Thorne et al. 1991) and unfertilized blastodiscs (Klein and Grossmann 2008) suggests Z dominancy over the W in the competition environment, we argue that this does not determine the easiness of manipulating offspring sex toward one chromosome over the other. In order to predict the direction of the expected bias, one should take into account species specificity and acuteness of hormonal stimuli. For instance, chronic corticosterone elevation biases the SR toward females in the Japanese quail (*Coturnix japonica*, Pike and Petrie 2005) and in the white-crowned sparrow (*Zonotrichia leucophrys*, Bonier et al. 2007), whereas an acute dose of this hormone results in male-biased SR in the chicken (Pinson, Parr, et al. 2011). Given that the manipulation of maternal hormonal level during egg formation might result is sex-specific embryo mortality detected at the postlaying stage (Love et al. 2005; Rutkowska and Cichoń 2006), it is possible that they could also reflect the processes acting during potential competition of male and female cells at the prelaying stage. Finally, not only maternal hormones and other components of the yolk might affect the result of competition within chimeric embryos. Temperature is another factor responsible for differential embryonic mortality in birds (Göth and Booth 2005). It has to be established whether and how those factors influence the fate of different cell types.

HOW TO VERIFY THE MECHANISM?

If the suggested mechanism was entirely efficient, we would expect that all embryos at the egg-laying stage have clearly defined sex (Figure 2). At that stage, the ratio of cells with different set of the sex chromosomes would be extremely low and thus very difficult to detect. Thus, to verify if the proximate mechanism of this phenomenon suggested here is correct, one would have to demonstrate that factors known for inducing the primary SR bias cause existence of chimeric embryos at the early stages of development (Figure 2). The task achievable using currently available techniques is to detect chimerism before one type of blastodermal cells outnumbers the other.

If the mechanism is not entirely efficient and the proliferation of both cell types continues until egg laying, we could observe gynandromorphic embryos and later, adults. Another possibility is that failure of MI and subsequent fertilization of diploid egg may result in the formation of triploid embryo. Thus, a general prediction that follows from our idea is that factors that are known to cause SR distortion could also lead to increased frequency of polyploids and gynandromorphs.

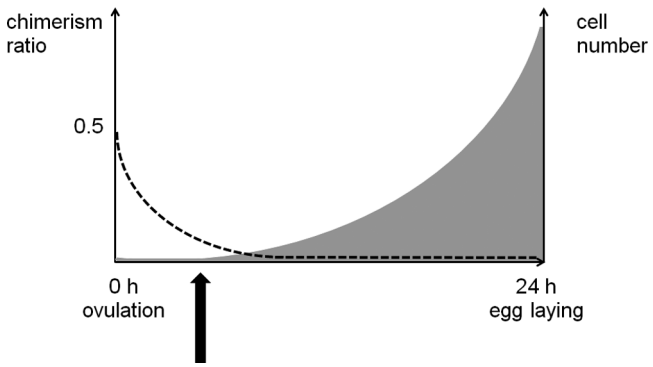

Figure 2
Schematic presentation of the prelaying embryonic growth and hypothetical elimination of 1 cell type. Under the model suggested here, the growth of the embryo occurs at the normal speed (shaded area), reaching, for example, up to 15 000 in the zebra finch (Birkhead et al. 2008) and 60 000 cells in the hen (Carsience et al. 1993). If PB extrusion failure occurs and the female pronuclei are fertilized by up to 4 sperm, at the very beginning of embryonic growth, one should observe nearly equal ratio of ZZ- and ZW-bearing cells (beginning of the dashed line). Initially, both cell types could proliferate. However, because of their differential proliferation rate at the altered hormonal milieu, one cell type might cease the growth and the other cell type will be predominant at the stage of egg laying. Verification of the mechanism is the most likely at the very early stages of embryonic development (black arrow), when there are relatively few cells and the contribution of both types is high.

CONCLUSIONS

Describing the mechanism behind SR bias in birds is crucial for understanding its evolution (e.g., Uller et al. 2007) and it might also have practical consequences for conservation studies (Heinsohn et al. 1997) and poultry breeding. Despite its importance, the proximate mechanism has not been identified yet. From an evolutionary perspective, the idea that avian offspring sex determination might be postponed beyond MI and happen gradually is a very attractive concept. Compared with sex chromosome segregation, our scenario would allow more time for the integration of the information carried in the yolk and therefore better fit between the sex and egg quality, which reflects environmental factors experienced by the female. Such concordance, often mediated by hormones, is in fact expected in oviparous species (Bowden et al. 2000; Rutkowska and Badyaev 2008). Failure of PB extrusion is generally considered as an undesired event. If that phenomenon could indeed facilitate offspring SR manipulation, it would confer some selective advantage.

We thank Mariusz Cichoń and Shinichi Nakagawa for helpful comments on the earlier versions of the paper. Dana Seaman helped to edit the manuscript.

Forum editor: Sue Healy

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