Hormone-Mediated Adjustment of Sex Ratio in Vertebrates

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Synopsis

The ability to adjust sex ratios at the individual level exists among all vertebrate groups studied to date. In many cases, there is evidence for facultative adjustment of sex ratios in response to environmental and/or social cues. Because environmental and social information must be first transduced into a physiological signal to influence sex ratios, hormones likely play a role in the adjustment of sex ratio in vertebrates, because the endocrine system acts as a prime communicator that directs physiological activities in response to changing external conditions. This symposium was developed to bring together investigators whose work on adjustment of sex ratio represents a variety of vertebrate groups in an effort to draw comparisons between species in which the sex-determination process is well-established and those in which more work is needed to understand how adjustments in sex ratio are occurring. This review summarizes potential hormone targets that may underlie the mechanisms of adjustment of sex ratio in humans, non-human mammals, birds, reptiles, and fishes.

Introduction

The ratio of males to females in a population exerts important influences on the overall reproductive success of individuals in that population. As a result, the ability to alter sex ratios at either the individual or population level would be a powerful control in an adaptive sense. There is extensive evidence suggesting that vertebrates in all classes have the ability to control sex ratios at either early or late life-history stages. Further, while we often think of hormones as being influenced by the sexes of animals, a plethora of studies suggest that hormones are influences of the sexes of individuals. The purpose of this review and of the symposium is to discuss the evidence in a variety of vertebrate groups that hormones are mechanistic mediators of adjustments in sex ratios at multiple ages of animals.

Potential levels of adjustments in sex ratio

When we discuss sex ratios, it is important to clarify at which level we are focusing. First, adjustment of sex ratio can occur at either the individual or population level. For the purposes of this review and the symposium, we are primarily focusing on individual variation in sex ratios, although these adjustments most certainly have the potential to subsequently influence population sex ratios as well. Second, we see adjustments in sex ratio at multiple age levels, and the current definitions of when adjustments in sex ratio occur can be confusing. Primary adjustment of sex ratio, in most cases, is limited to the developmental window prior to fertilization, influencing the number of individuals of a particular sex that are initially produced. However, in some species that exhibit environmental sex-determination (ESD), the number of individuals of a particular sex is not established until well after fertilization. We propose that the term primary adjustment of sex ratio should include all adjustments that happen before the initial sex of an individual is determined. Secondary adjustment of sex ratio is limited to the time-window after fertilization and is accomplished by the loss of one sex. Finally, adjustment of sex ratio can occur during adulthood and result in the transition from one sex to another, and thus a loss of one sex but a gain of the other.
For an in-depth discussion on the multiple levels at which sex ratios can be analyzed and manipulated, see Crews (2013, this issue).

Evidence for hormone-mediation of sex ratios

Skews in sex ratios either of offspring or of adults commonly are observed in response to environmental or social changes. For adjustments in phenotypic or genetic sex to occur, responses to these variables must first be transduced into a physiological signal that ultimately influences the process of sex-determination. Hormones are excellent candidates for this transduction because the endocrine system as a whole regulates physiological activities in ways that maximize survival in a constantly changing environment. Indeed, there is evidence from every vertebrate group in which mechanisms of adjustment or reversal of sex ratio have been studied, that hormones are involved in the adjustment of sex ratio at all levels.

Hormonal mediation of sex ratios in humans

Over the past century, a huge number of studies have documented sex ratios skewed in response to a variety of environmental and social changes, including, for example, marital status (Norberg 2004), social class (Lazarus 2002), natural disasters (Fukuda et al. 1998), and other stressful events such as wars (James 2009) and psychological stress (Obel et al. 2007). Because the sex ratios for human offspring are most often collected at birth, it is difficult to pinpoint when the influences take place. There is evidence for manipulation at both the primary and secondary levels.

For primary adjustment of sex ratio to occur in humans or non-human mammals, there need to be either an excess of X-bearing or Y-bearing sperm, or differential abilities of those sperm to fertilize as a result either of sperm-function or egg-receptivity that differs on the basis of the sex chromosome carried by the sperm. James (1996, 2004) suggested that variation in testosterone:gonadotropin ratios in men and women at the time of conception underlie many of the skewed sex ratios seen in human populations. The potential relationship of the testosterone:gonadotropin ratio in men with the sex-ratio of their offspring are supported by the findings that endocrine-disrupting compounds known to depress testosterone concentrations and induce testicular dysfunction also cause significant skews in the offsprings’ gender. For example, men exposed to dibromochloropropane (DBCP), a pesticide that has estrogenic effects and lowers the testosterone:gonadotropin ratio in men (reviewed by Whorton et al. 1979; Sikka and Wang 2008), produced significantly more daughters (Potashnik and Porath 1995). Some studies have even demonstrated changes in the ratios of X-bearing sperm to Y-bearing sperm after exposure to endocrine-disrupting chemicals; persistent organochlorine pollutants increased the proportion of Y-bearing sperm in ejaculates (Ttiido et al. 2005). On the other hand, occupational exposure of men to stress, which elevates glucocorticoid concentrations and depresses levels of reproductive hormones generally, results in more female offspring (reviewed by Navara 2010). The influences of paternal stress could be mediated by elevation in levels of glucocorticoid, reduction in levels of sex steroids, or changes in other downstream mediators. Regardless, these studies indicate that hormones may act on men and play a role in sex ratio at the primary level.

While the studies listed above suggest paternal influences on adjustment of sex ratio in humans, Grant and Metcalfe (2003) agree that the levels of sex steroids at conception underlie the mechanism of humans’ adjustment of sex ratio, but suggest that the mechanism more likely lies with the female and that paternal influences may in fact result from assortative mating. Women who are more dominant tend to produce more sons (Grant 1996), and dominant women also tend to have higher concentrations of testosterone in circulation (Grant and France 2001). This trend supports the James (1996) theory. It has also been suggested that hormonal control of viscosity of cervical mucus underlies variation in humans’ sex ratios; Y-bearing sperm traverse the cervical os more efficiently. Increased production of estrogen increases cervical viscosity, which would facilitate the passage of X-bearing sperm through the cervical os and increase the number of female offspring (reviewed by Martin 1995); however, evidence for this is conflicting (Grant and Martin 1995). While there is evidence from non-human mammals that hormones within the female can influence sex ratios via the fertilization process itself (discussed later), similar effects have not been directly demonstrated yet in humans. However, women who receive gonadotropins or clomiphene to induce ovulation produce significantly more daughters, supporting the idea that gonadotropins and/or sex steroids can influence the human sex ratio early on (James 1995). It is unclear whether this is due to influences at fertilization or influences on the maternal environment during embryonic development.

There is abundant evidence suggesting that humans’ sex ratios can be controlled at the secondary
level, through sex-specific losses of embryos or fetuses. First, it was determined using cytogenic analysis of 342 spontaneous abortions that early embryonic failures are more likely to be female, and that males have a developmental advantage early on (Evdokimova et al. 2000; reviewed by Boklage 2004). Boklage (2004) suggested that adjustments of sex ratio are likely due to changes in the epigenetic environment during embryonic development. Indeed, environmental and social changes during embryonic and fetal development influence secondary sex ratios. For example, sub-optimal gains in weight during pregnancy result in the production of more daughters (Cagnacci et al. 2004). Exposure of Chilean women to an earthquake during month 3 of gestation significantly reduced the number of sons (Torche and Kleinhaus 2012). Similarly, other severe events and psychological disturbances experienced during early pregnancy also significantly influence the secondary sex ratio (Hansen et al. 1999; Obel et al. 2007). Work by Catalano et al. (2005, 2006) indicates that even major events experienced during mid-to-late pregnancy can induce sex-specific fetal loss: women in their second trimesters during the September 11, 2001, attack on New York City produced significantly more daughters. Currently, the hormonal influences on sex-specific fetal loss during pregnancy have not been tested in humans. However, given that a substantial number of studies demonstrate an influence of maternal stress on secondary sex ratios, it is possible that glucocorticoids act either directly or via downstream hormonal or non-hormonal mediators to influence secondary sex ratios (reviewed by Navara 2010). More work on this topic is needed.

**Hormonal mediation of sex ratios in non-human mammals**

Much of the literature examining potential hormonal influences on adjustment of sex ratios in non-human mammals produced results that mirror those found in humans. For example, dominance status in macaque mothers (Macaca mulatta) relates to her offsprings’ sex ratios; more dominant mothers with higher levels of testosterone produced more sons (Grant et al. 2011). Female lemurs (Microcebus murinus) that were maintained in groups, and thus experienced many dominance interactions before mating, produced 67% male offspring (Perret 1990). On the other hand, female rats (Rattus norvegicus) that were stressed prior to conception produced significantly fewer males (Lane and Hyde 1973), and activation of the stress axis via administration of adrenocorticotropic hormone (ACTH) in females resulted in the production of significantly fewer male offspring (Geiringer 1961). Thus, as in humans, dominance appears to be associated with the production of more males while stress appears to be associated with the production of more female offspring. Grant (2007), in agreement with the theories of James (1996), suggested that concentrations of circulating testosterone in the female underlie the mechanism responsible for these skewed ratios both in humans and in non-human mammals. Indeed, female field voles (Microtus agrestis) treated with testosterone and glucose produced male-biased litters (Helle et al. 2008) and Nubian ibex (Capra nubiana) females that were more dominant had higher fecal levels of testosterone and also produced more male offspring (Shargal et al. 2008). Despite the fact that concentrations of testosterone in the voles and ibexes were measured prior to conception, it remains unclear whether testosterone acts in a primary or a secondary manner.

In two studies, Grant et al. (2008) demonstrated that the concentration of testosterone in ovarian follicles may adapt an ovum to preferentially receive an X-bearing or Y-bearing sperm. Bovine ova (Bos primigenius) were collected, a sample of follicular fluid was assayed for testosterone, and the ova were then fertilized via in vitro fertilization; ova with high concentrations of testosterone were more likely to be fertilized by a Y-bearing sperm. Grant and Chamley (2010) suggested that the level of follicular testosterone may influence the development of the zona pellucida, in particular the variation in carbohydrate-based sperm-binding ligands on the zona pellucida. This remains to be tested.

While the above-mentioned studies indicate a role for females’ testosterone in the influences on primary sex ratios, there is very little support for a role of paternal hormone concentrations in non-human mammals. It is known that Y-bearing sperm are more susceptible to stress-induced damage compared with X-bearing sperm (Pérez-Crespo et al. 2008), which could provide a mechanism whereby paternal stress could influence offsprings’ sex ratios, although there are few, if any, examinations of the influences of paternal stress on offsprings’ gender in non-human mammals. Gomendio et al. (2006) showed that male red deer with high fertility rates produced more male offspring; however, it is not known whether this effect results from the females with which those males mated. More work is needed to examine the impact of hormones of the male on his offsprings’ sex ratio in non-human mammals.
There are currently few experiments showing direct influences of hormones on sex-specific fetal loss in non-human mammals; however, Krackow (1995) suggested that maternal hormones may influence sex ratios of offspring through developmental asynchrony by altering the preparation of the uterus and the developmental rate of the blastocysts. He then tested this idea by timing conception either early or late in the estrous cycle in a strain of mice (Mus musculus) that either exhibited faster development of male embryos versus female embryos and a strain with no difference in developmental timing. Matings that occurred late in the estrous cycle resulted in litters that were female-biased in the strain in which males grew faster, but not in the strain exhibiting similar development rates between the sexes (Krackow and Burgoyne 1997). This work provides support for the idea that the rate of development of the blastocyst can influence offsprings’ sex ratios. It is also known that male blastocysts are more sensitive to oxidative stress than are female blastocysts (Pérez-Crespo et al. 2005). However, it is unknown, and untested, whether hormones are involved in these processes. Krackow (1997) suggested that, in mammals that produce litters, hormone concentrations in utero may vary with the timing of insemination and ultimately influence developmental rates or survival of blastocysts in a sex-specific manner. This has not yet been tested. Krackow (1997) also suggested that litter size could influence hormone concentrations in utero and ultimately influence rates of sex-specific fetal loss. Indeed, mice with larger litters showed higher rates of sex-specific fetal reabsorption (Krackow 1992). It has also been shown in Mongolian gerbils (Meriones unguiculatus) and house mice that mothers who developed between two male sibling in utero produced significantly more male offspring (Vanderbergh and Huggett 1994; Clark and Galef 1995), and these authors suggested that programming of maternal reproductive physiology may explain these skewed sex ratios. However, more work is needed to determine the mechanism responsible.

**Hormonal mediation of sex ratios in birds**

While the mechanisms of sex-determination in birds differ from that in mammals, there are parallels regarding the influences of hormones, particularly corticosterone and testosterone, on offsprings’ sex ratios. First, as in mammals, stressful situations, such as food shortages (Kilner 1998) and low quality of mates (Pike and Petrie 2006), appear to result in the production of more female offspring in birds. Male-biased sex ratios are produced by females of some avian species when mated to an attractive male (Burley 1986; Svensson and Nilsson 1996; Loyau et al. 2007). Mating with an attractive male also stimulates females of some avian species to produce and deposit higher concentrations of testosterone in egg yolks (Gil et al. 1999, 2004). Thus, as in mammals, when skewed sex ratios are observed in birds, situations that stimulate glucocorticoid elevation generally appear to result in the production of more female offspring, while situations that elevate testosterone concentrations generally appear to stimulate the production of more male offspring.

The potential mechanisms by which hormones may influence primary adjustment of sex ratio in birds are discussed in detail by Navara (2013, this issue) and Goerlich-Jansson et al. (2013, this issue); however, we will summarize the current findings briefly. Female birds determine the sex of an offspring by contributing either a Z or a W chromosome to it. Oocytes contain both sex chromosomes until just hours prior to ovulation when meiosis resumes and one sex chromosome remains in the oocyte while the other passes into the polar body with no further developmental potential. Thus, primary adjustments in sex ratio would occur prior to, or during, this meiotic segregation, while secondary adjustments would occur afterward. Several studies have tested the idea that corticosterone mediates female-biased sex ratios by providing females with implants containing corticosterone during egg production; in three different species, corticosterone implants stimulated females to produce more female offspring (Pike and Petrie 2006; Bonier et al. 2007; Goerlich 2009). However, additional studies in which corticosterone was provided at the time when sex chromosomes segregated within the female and when gender is officially determined suggest that corticosterone is not the direct modulator of adjustment of sex ratio in birds; injection of corticosterone into zebra finches (Taeniopygia guttata) and chickens (Gallus domesticus) at pharmacological levels just prior to meiotic segregation caused a male-skew in sex ratios of offspring (Gam et al. 2011; Pinson et al. 2011a), the opposite of what has been seen with long-term physiological elevations. While this indicated that corticosterone can act to skew segregation of sex chromosomes and thus primary sex ratios, additional studies in which corticosterone was administered at the same time-point, but at physiological doses, produced no skew in sex ratio in the same two avian species. This indicates that either corticosterone influences sex ratios via changes in growth or in yolk content of follicles earlier in development, or
that another downstream factor directly influenced primary sex ratios in offspring in cases in which corticosterone concentrations were elevated in the physiological range over the long-term.

Corticosterone, in addition to exerting direct effects at ovulation, depresses steroidogenic functions of the theca and the granulosa layer surrounding the oocyte via central regulation of the release of LH (Etches et al. 1984). Thus, the influence of corticosterone on sex ratios could be mediated by the resulting changes in sex steroids. Treatment of females with implants containing testosterone resulted in more male offspring in two species (Veiga et al. 2004; Goerlich et al. 2009). Additionally, a single injection of testosterone administered to female zebra finches at the initiation of their clutch resulted in more male offspring in later clutches (Rutkowska and Cichón 2006). These studies indicate that testosterone may play a role in the adjustment of sex ratio, but do not allow a precise estimation of when this may occur. One additional study of chickens showed that a single injection of testosterone at a physiological dose immediately prior to segregation of the sex chromosomes also results in the production of more male offspring (Pinson et al. 2011b). Thus, a direct role for testosterone seems promising, especially since corticosterone inhibits the production of testosterone and since sex ratios resulting from treatment of the two hormones were skewed in opposite directions. Additionally, the roles of both estradiol and progesterone in primary adjustment of sex ratios have also been investigated. Estradiol did not exert an influence on offsprings' sex ratios either in quail (Coturnix japonica) or chickens. Injections of progesterone prior to segregation of the sex chromosomes resulted in significantly more female offspring (Correa et al. 2005); however, others were not able to reproduce this effect using the same methods (Pinson et al. 2011a). Overall, testosterone appears to be the most promising steroid hormone in the mediation of primary adjustment of sex ratios in birds, but other non-steroid hormones and/or non-hormonal factors may interact with testosterone to control primary sex ratios (Goerlich et al. 2010; Badyaev 2013, this issue; Navara 2013, this issue).

Birds differ from other vertebrate species in that the window during which a female can directly influence physiology ends at oviposition, because the embryo is separated from the mother by the eggshell through the remainder of development. Thus, hormonal influences on the secondary sex ratio would likely have to occur through hormones deposited in the yolk. These yolk hormones can influence sex ratios either by stimulating sex-specific embryonic mortality or by stimulating sex-specific differences in size either during the embryonic period or post-hatching. We focus here on hormonal influences on sex-specific embryonic mortality. Treatment of female European starlings (Sturnus vulgaris) with corticosterone resulted in male-biased embryonic mortality, and thus a female-biased sex ratio at hatching (Love et al. 2005); however, in the same species, no differences were found in sex ratio at hatching following direct injection of eggs with corticosterone (Chin et al. 2009). Additionally, high levels of androgens in the yolk can cause sex-specific mortality of embryos; testosterone-treatment of yellow-legged gulls’ (Larus michahellis) eggs resulted in female-biased mortality and thus male-biases at day 4 post-hatching. It was unclear in this case whether these influences occurred during embryonic development or during the first four days post-hatching (Rubolini et al. 2006). To the contrary, injecting female zebra finches with testosterone resulted in higher hatching success for females than for males (Rutkowska and Cichón 2006). Thus, the direction in which yolk-androgens influence sex biases in embryonic survival may be species-specific. Estradiol may also influence sex ratios in embryonic stages. Injections of female zebra finches with 17β-estradiol resulted in male-biased embryonic mortality, and thus more females at hatching (von Engelhardt et al. 2004). We currently need more studies testing the influences of yolk-hormones as well as hormone-mimics to determine whether secondary adjustments of sex ratio can be facultatively manipulated using hormones.

**Hormonal mediation of sex ratios in reptiles**

Unlike mammals and birds, reptiles have been relatively neglected in studies of allocation of sex and facultative manipulation of sex ratios (but see a few examples addressed below). In particular, influences of maternal stress and social interactions on reptilian sex ratios have not yet been studied to my knowledge. However, the mechanisms of sex determination and the influences of hormones on this process have been extensively studied in reptiles. From this work, we can detect one main similarity; in cases in which testosterone or chemicals with androgenic effects influence the process of sex determination, in general, more male offspring are produced (see below), as both in mammals and birds. The influences of corticosterone, on the other hand, are mixed. Corticosterone is deposited by female reptiles into yolk (Painter et al. 2002) and treatment of eggs with corticosterone influences sex ratios in two lizard.
species, but in opposing directions (Warner et al. 2009), while in another study with mallee dragons (*Ctenophorus fordi storr*), there was no effect of corticosterone treatment on sex ratios (Uller et al. 2009).

Reptiles exhibit a range in their sex-determining systems, including both genotypic sex-determination (GSD) and temperature-dependent sex-determination (TSD). There are some accounts of biases in sex ratios in reptilian species that exhibit GSD (Lovern and Passak 2002; Olsson et al. 2007; Cox et al. 2010), and, as in birds and mammals, these biases must occur prior to, or during, fertilization. Lovern and Passak (2002) showed that females who had blood collected from them produced male-biased sex ratios compared with those who had not, and the authors suggested that the sampling of the blood may have influenced hormone profiles either through the stress imposed by collection of the sample or the decreases in hormone concentrations as a result of removal of the blood. Lovern and Wade (2003) then showed that concentrations of testosterone in yolk samples collected from eggs in the oviduct were higher in male-producing eggs than in female-producing eggs. They suggested that hormone concentrations in the yolk may influence the likelihood of fertilization by male-inducing versus female-inducing sperm. Olsson et al. (2007) suggested that skewed sex ratios in Australian painted dragons (*Ctenophorus pictus*) result from sex-chromosome-specific survival of sperm, and that the female may regulate this during storage of sperm. How hormones may mediate differential survival of sperm within the female is unknown.

As in mammals and birds, adjustments of sex ratio that occur in GSD reptiles after fertilization are secondary in nature. In two turtle species that have heteromorphic sex chromosomes (*Staurotypus triporcatus* and *S. salvinii*), secondary sex ratios can be adjusted through exposure to 17β-estradiol, not through sex-specific embryo mortality, as is seen in mammals and birds, but through sex reversal; male turtles treated with estradiol during embryogenesis developed functional female morphology (Freedberg et al. 2006). Secondary skews of sex ratios in reptiles can also occur through sex-specific embryonic mortality or, in viviparous species, sex-specific embryonic reabsorption (Blackburn 1988; Burger and Zappalorti 1988). However, there is not much evidence for either in reptilian species, nor have the influences of hormones on these mechanisms been examined.

In contrast to species that exhibit GSD, reptiles that exhibit TSD have the potential of controlling sex ratios at the physiological level prior to oviposition, and at the behavioral level after oviposition. These species have homomorphic sex chromosomes, and the sexes of offspring are ultimately determined by the temperature at which eggs are incubated, a phenomenon exhibited by a wide variety of reptilian species, including crocodilians, turtles, and some lizards (Bull 1980; reviewed by Nakamura 2010). Where, then, may hormones act to influence sex ratios in reptilian species that exhibit TSD?

Female reptiles may influence their offspring's sex as early as during development of the ovarian follicles, through deposition of hormones in the yolk. Hormone concentrations in the yolk, primarily testosterone and estradiol, have been measured in >18 reptilian species to date, and of the 13 TSD species studied, concentrations differed between the sexes in seven (reviewed by Radder 2007). Janzen et al. (1998) measured concentrations of testosterone and 17β-estradiol in the yolks of freshly laid eggs collected from species that exhibit TSD (*Trachemys scripta elegans*, *Chelydra serpentina serpentine*, and *Chrysemys picta bellii*) and GSD (*Apalone spinifera hartwegi* and *A. mutica mutica*). Species that exhibited TSD had higher concentrations of testosterone in the yolk, and in one TSD species at one incubation temperature (27.6°C), high levels of testosterone in the yolk were related to male-biases in the sex ratio. Bowden et al. (2000) demonstrated dramatic differences in sex ratios of painted turtles (*C. picta*) across seasons, and concentrations of testosterone and estradiol changed seasonally as well. In addition, as estrogen levels and the estrogen:testosterone ratio in egg yolks increased, more males were produced. Ding et al. (2012) also showed that testosterone and estradiol in the yolk of a TSD gecko species (*Gecko japonicus*) were related to incubation temperatures that produced sex-biased clutches, but in that study, yolk steroids were not clearly related to the gender of the offspring. The authors suggested that maternal control of gender via hormones is secondary to control via temperature. Similarly, Elf (2003) suggested that, in alligators and snapping turtles, temperature influences concentrations of estradiol in the yolk which, in turn, controls expression of key sex-determining genes such as SF-1. However, more recent work in which natural levels of yolk steroids were measured and then compared with sex of offspring from within the same egg suggests that there is no relationship between maternally-derived yolk hormones and sex ratios in reptiles with TSD (Juliana et al. 2004; Radder et al. 2007; Warner et al. 2007; reviewed in Radder 2007). Thus the role of yolk steroids in the adjustment of sex ratio...
remains unclear in reptiles. Paitz and Bowden (2009) introduced the idea that yolk steroids may be initially inactivated via sulfonation and reactivated by sulfatases in the embryo at critical stages of development (Paitz and Bowden 2013, this issue). Thus, maternal modulation of sulfotransferases or epigenetic modulation of embryos such that embryonic sulfatase activity is altered in a sex-specific way could modulate relationships between yolk steroids and sex ratios, and potentially explain the conflicting results that are currently seen. More work is needed in this area.

Females may also skew sex ratios by facultatively controlling incubation temperatures that alter hormone concentrations in the embryo. Indeed, facultative manipulations of offprings’ sex have been documented in species with TSD in response to gender imbalances within the population (Robert et al. 2003) and also based on mating experiences during the breeding season (Olsson and Shine 2001). This can be accomplished by altering locations of nests (e.g., Doody et al. 2006) or their depths (e.g., Mrosovsky and Provancha 1989). The extensive work of Crews et al. on the red-eared slider turtle (Trachemys scripta) suggests that the role of sex steroids in TSD is still unclear, since treatment with androgenous steroids at least partially alters the sex-determination process, but inhibition of endogenous steroid hormones using antagonists does not (Matsumoto and Crews 2012). Work on a variety of systems indicates that TSD is influenced via the interaction of hormonal factors and genes, such as CYP19, FoxL2, and Dmrt1 (Matsumoto and Crews 2012), that are key to the sex-determination process. In particular, ovarian differentiation appears to be controlled by estrogens synthesized after CYP19 expression has been induced (Nakamura 2010). Discussion of these detailed mechanisms is beyond the scope of this review, so instead see Nakamura (2010) and Matsumoto and Crews (2012).

Hormonal mediation of sex ratios in fishes

Fish exhibit a large variation in sex-determining mechanisms, ranging from gonochoristic systems in which ovaries and testes are produced and gender remains static throughout life; normal hermaphrodites, in which sexes can change later in life; and finally to systems in which individuals are synchronously hermaphroditic and contain functional male and female tissue at all times (Devlin and Nagahama 2002). Additionally, sex-determining mechanisms include GSD, ESD, and environmental sex reversal (ESR), in which individuals change sex in response to environmental and/or social cues during adulthood. As a result, potential mechanisms of adjustment of sex ratio are just as diverse as in fishes, as is the potential for hormonal mediation. Here we review some examples of hormone targets for manipulation of sex ratio in fishes.

In >50 species in which the influences of exogenous hormone treatment during gonadal development have been studied, steroid hormones resulted in reversals of genetic sex. As a result, similar treatments are routinely utilized in fisheries to purposefully manipulate sex in cultured species (Piferrer 2001). In general, oral administration of estrogens induces ovarian development while administration of androgen induces testicular development (Yamamoto 1962; Nakamura et al. 1998; Nakamura 2010). Despite this knowledge that sex ratios can be manipulated relatively easily in fishes that exhibit GSD, there are few studies examining the potential for facultative adjustment of the sex of offspring in fishes. Karino et al. (2006) and Karino and Sato (2009) showed in guppies (Poecilia reticulata), a system with XY sex determination, females mated to attractive males (either with long tails or bright orange spots) produced male-biased sex ratios. To determine whether these biases were under male control or female control, they mated females with test males, but changed the females’ assessments of these males by presenting the females either with more attractive or less attractive males; females produced male-biased broods when the test male to which they were mated was perceived as more attractive (Sato and Karino 2010). To our knowledge, the guppy is the only species in which facultative manipulation of primary sex ratios has been documented in a fish. As with other GSD systems, manipulation of primary sex ratio in this species would likely have occurred either through preferential fertilization of Y-bearing sperm. However, since guppies are viviparous, the skewed sex ratios could also have been secondary in nature. Tests examining hormonal influences on fertilizations by X-bearing sperm versus Y-bearing sperm and on sex-specific mortality of embryos are needed. While facultative adjustment of sex ratio has not been reported in others species (and particularly in oviparous fish), there are mechanisms by which females could potentially manipulate sex ratios via hormones. Fishes, like birds and reptiles, deposit hormones into developing oocytes. For example, cortisone, testosterone, and estradiol of maternal origin pass into eggs and subsequently alters the offspring’s phenotype in multiple species (de Jesus and Hirano 1992; Hwang et al. 1992; McCormick 1999; Schreck et al. 2001; Auperin and Gesling 2008). While, to my
knowledge, influences of maternal hormones on sex ratios have not been reported, cortisol in eggs exerts influences on sex-determination in at least two species that exhibit TSD; eggs treated with corticosterone cause masculinization of pejerrey (Odontesthes bonariensis) and Japanese flounder (Paralichthys olivaceus) (Yamaguchi et al. 2010). Fernandino et al. (2012) showed that elevations of cortisol in pejerrey larvae increased concentrations of 11-ketotestosterone, which indicates an interaction of cortisol and reproductive hormones in the modulation of sex ratios. Thus, there is potential for facultative adjustment of sex ratio in response to stress, particularly in fish that exhibit TSD, and this needs to be tested.

Of particular interest in fishes is the ability of many species to change their gender during adulthood in response to social and environmental changes, and while this has more of an influence on an individual’s gender, rather than on sex ratio per se, hormones are intimately involved in this process. Gonadal tissues of adult fishes can morph between sexes in response to environmental change, social status and cues, endocrine hormones, and life stage. For example, in bluebanded gobies (Lythrypnus dalli), the loss of the dominant male from the social group signals the largest female to undergo a sex change into a male gonadal phenotype, and such females exhibit increases in dominance behaviors as well (Rodgers et al. 2005; Godwin 2010). It appears in this, and other, species that the down-regulation of aromatase expression is intimately involved in the sex-changing process, and aromatase inhibitors cause transitions from female to male morphologies while treatment with estradiol exerts the opposite effect. In addition, treatment of many species, including wrasses, parrotfishes, and gobies, induces sex changes from females to males. The influences of hormones on sexual changes in morphology and behavior are described in detail by Godwin et al. (submitted for publication) and Maruska and Fernald (2013, this issue).

Concluding statements

The process of adjustment of sex ratios, whether primary or secondary in nature, is sensitive to hormones in virtually all groups of vertebrates, and there is a striking pattern across groups in which androgens stimulate the production of more males; however, the mechanisms responsible are likely very different. It remains interesting that a hormone normally produced by sexually mature, phenotypic males also appears to play a role in the initial production of male offspring, at least in terms of biases in sex ratio, in all vertebrate groups studied. A detailed discussion of adjustment of sex ratios in amphibians is not included here because that taxon was not included in the symposium; however, sex ratios in amphibians also appear to be under maternal control (e.g., Sakisaka et al. 2000), and a discussion of how hormones may interact with sex ratios in amphibians would serve as a helpful addition to the comparisons made here. Overall, more work examining facultative adjustments in sex ratio, particularly patterns of adjustment in reptiles and fishes, and additional exploration of potential mechanisms in birds and mammals, is needed.

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Hormones and vertebrate sex ratios


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