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1 **The adaptive sex in stressful environments**

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3

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10

11 **Keywords**

12 Sex determination; Sex ratio; Maternal effect; Adaptive response; Glucocorticoids

13

14 **Abstract**

15

16 The impact of early stress on juvenile development has intrigued scientists for decades, but
17 the adaptive significance of such effects remains an ongoing debate. This debate has largely
18 ignored some characteristics of the offspring such as their sex, despite strong evolutionary and
19 demographic implications of sex ratio variation. We review recent studies that examine
20 associations between glucocorticoids, the main class of stress hormones, and offspring sex.
21 While exposure to glucocorticoids around the time of sex determination in fish consistently
22 produces males, the extent and direction of sex ratio bias in response to stress vary in reptiles,
23 birds and mammals. We propose proximate and ultimate explanations for most of these
24 trends.

25

26

27 **Sex-stress interaction**

28 Among vertebrates, sex can be determined by **environmental sex determination (ESD**, see
29 Glossary), **genetic sex determination (GSD)** or the interaction of both. The phylogenetic
30 distribution of GSD and ESD indicates that transitions between these types of sex
31 determination have occurred many times [1]. While mammals and birds have only GSD, sex-
32 determining mechanisms have high evolutionary plasticity in fish, reptiles and amphibians.
33 Nevertheless, the sensitivity of the undifferentiated gonad to circulating sex hormones is a
34 common feature shared by many vertebrates, including those with GSD [1]. Over the years,
35 the hypothesis that glucocorticoids (GCs), the main class of stress hormones (Box 1), would
36 interact with sex hormones to influence the sexual fate of the gonad has emerged following
37 numerous laboratory studies. In parallel, recent technological advances in stress
38 measurements (Box 1) have allowed the quantification of GCs in free-ranging animals,
39 enabling the discovery of a link between GC levels and sex ratio biases in natural
40 environments. Given GCs act to convert stressful stimuli into physiological signals, they are
41 promising candidates for the translation of environmental, social and state-dependent factors
42 into adaptive sex allocation.

43 Here we review the body of evidence that GCs influence **sex determination** and bias
44 the **offspring sex ratio** across vertebrates (Figure 1). This can happen directly in species with
45 ESD or indirectly through maternal effects. We then explore the potential evolutionary causes
46 explaining why, in a stressful environment, one sex would be more adaptive than the other in
47 animals with either ESD or GSD.

48

49 **Direct influence of glucocorticoids on sex determination or sex differentiation**

50

51 In fish, studies conducted to date have tended to show that at sub-optimal temperatures (*i.e.*
52 very high or very low), low pH, continuous lighting or high density – each of which can be
53 considered to be a stressful condition [2] – more males are produced (Figure 1). In fish, sex
54 determination can occur at different stages of the life cycle: at fertilization or later in
55 development (egg or larval stage). The undifferentiated gonad of fish is extremely labile and
56 sensitive to external factors [1]. In recent years, the link between stressful environmental
57 conditions and GCs has been established as being a major factor influencing sex
58 determination in fish. For instance, high **cortisol** levels at the egg stage can override genetic
59 sex in species with GSD such as the medaka (*Oryzias latipes*) [3] and rainbow trout
60 (*Oncorhynchus mykiss*) [4]. High temperatures during the larval stage can trigger higher
61 cortisol production that directly influence the sex of individuals in the Pejerrey (*Odontesthes*
62 *bonariensis*) [5] and Japanese flounder (*Paralichthys olivaceus*) [6]. Other stressful
63 conditions applied at the larval stage such as constant light exposure [7], stressful tank color
64 [8] and high fish density [9] activate the cortisol synthesis pathway and lead to a higher
65 production of males in various fish species (Figure 1). Although this has not been formally
66 tested in natural conditions with controlled quantities of cortisol, administering cortisol to
67 three-spotted wrasse (*Halichoeres trimaculatus*) induced a female-to-male sex change [10]. In
68 all these examples, more males are produced when stress is applied during the critical period
69 of sex determination or **sex differentiation**, affecting the **primary sex ratio** (PSR) (Box 2).

70 In crocodylians, low temperatures over the egg laying period produce an all-female
71 population, but in lepidosauria (lizards and snakes) and testudines (turtles) temperature sex-
72 determination is species-specific [11]. When stressed by suboptimal temperatures,
73 **corticosterone** production rises, even with a relatively small temperature increase, as
74 demonstrated in alligator lizards (*Elgaria*) [12]. However, the effects of corticosterone
75 exposure on the sex of reptilian offspring has rarely been studied and the few results

76 available are not as clear as for fish (Figure 1). Although, investigation of gene expression of
77 the key genes (*POMC* and *CRH-BP*) involved in the GC production cascade and regulation
78 showed an association between high temperature, stress and feminization in the Australian
79 central bearded dragon (*Pogona vitticeps*) [13], another recent study reported a direct link
80 between epigenetic mechanisms and temperature-dependent sex determination in the red-
81 eared slider turtle (*Trachemys scripta*) [14], ruling out GCs as an essential factor, at least in
82 this species. Experimentally introducing corticosterone to developing eggs affected the sex
83 ratio of two lizard species (*Amphibolurus muricatus* and *Bassiana duperreyi*) in an opposite
84 pattern [15] (Figure 1), but did not affect sex ratio of the mallee dragon, *Ctenophorus fordi*
85 [16]. A recent study on the broad-snouted caiman, *Caiman latirostris*, exposed eggs to a
86 synthetic GC in an attempt to influence the sex ratio of embryos already exposed to high
87 temperature (that produce 100% males), the authors were unsuccessful at deviating sex-ratio
88 in favour of females [17]. This suggests either that GCs cannot override the temperature effect
89 or that, in caiman, high levels of GCs would produce male-biased clutches anyway as
90 observed in fish (an hypothesis not evaluated by the authors). More studies are needed to
91 understand if temperature can trigger endogenous production of GCs at the period of reptile
92 sex determination.

93

94 **Influence of maternal glucocorticoids on offspring sex ratio**

95

96 Mother-to-offspring GCs transmission is an evolutionarily conserved mechanism found
97 across vertebrate species [18] and is one of the most studied factors in the maternal effect
98 literature [19,20]. However, studies on the relationship between maternal GCs and sex ratio in
99 fish are, to date, lacking. Three different studies investigated the link between maternal stress
100 and sex of reptiles' species with different degree of sex-sensitivity to temperature. Two

101 studies on the common lizard (*Lacerta vivipara*) and the eastern fence lizards (*Sceloporus*
102 *undulatus*) artificially exposed gravid females to high corticosterone concentration, with no
103 reported sex-ratio bias [21,22]. In *Amphibolurus muricatus*, a slightly higher percentage of
104 females was detected in clutches of mothers exposed to high corticosterone concentration
105 [23].

106 In birds, GCs play an important role in sex-ratio biases (Figure 1). Initial studies in
107 captive and free-ranging species showed that elevated levels of maternal GCs are associated
108 with female-biased sex ratios [24–27]. These studies used implants to increase corticosterone
109 levels before nesting and suggested that maternal corticosterone influences PSR. In addition,
110 elevated maternal corticosterone levels can induce higher mortality in male offspring later in
111 development, affecting **secondary sex ratio** (SSR) [28]. However, recent studies show that
112 the picture is more complex than initially thought: females with elevated corticosterone levels
113 produce more male offspring in Gouldian finches (*Erythrura gouldiae*), zebra finches
114 (*Taeniopygia guttata*), chickens (*Gallus gallus*), and ovenbirds (*Seiurus aurocapilla*) [29–32].
115 Corticosterone injections to female chickens 5 h prior to the expected time of ovulation biased
116 sex ratio towards males [31], whereas injections one hour later biased sex ratios towards
117 females [33]. More work is needed to determine whether this unexpected difference is
118 biologically significant. Most avian studies have been performed in controlled conditions and,
119 the three-way association between environmental stressors, maternal corticosterone, and PSR
120 remains to be investigated.

121 In many species of mammals, food deprivation and other environmental perturbations
122 during gestation are typically associated with female-biased sex-ratios at birth [34]. However,
123 results from studies that have measured maternal physiological stress are variable (Figure 1).
124 While some studies have shown a positive relationship between maternal GC levels and
125 female bias in the sex ratio [35–39], others have shown a negative relationship [40–42], or no

126 relationship at all [43–48]. Over half of all those studies have been conducted in rodent
127 species (Figure 1). In this group, results are still mixed, but the direction of the pattern, when
128 present, is rather homogeneous: four out of five studies show a decrease in the proportion of
129 male offspring at birth as maternal GCs increased (Figure 1). In humans, a variety of *a priori*
130 stressful events, ranging from terrorist attacks [49] to death of a close family member [50]
131 were associated with decreases in the proportion of male births, but only two studies
132 measured mother’s cortisol with different results (Figure 1). One recent study investigating
133 the link between GC levels of twenty mother howler monkeys (*Alouatta pigra*) and forest
134 fragmentation [41] showed that females always gave birth to females when their fecal GCs
135 were below a threshold of 200 ng. g⁻¹ around the time of conception (n = 16), while, above
136 this threshold, they consistently produced males (n = 19). If future work with a much larger
137 sample size confirms this extraordinary pattern, we should worry about the possible impact of
138 anthropogenic pressures on sex ratios of animals.

139

140 **Timing of sex ratio and glucocorticoid measurements**

141 The sex ratio can vary in response to stress at several developmental time points. Sex-ratio
142 bias can occur around the time of conception and sexual differentiation (affecting the PSR), or
143 during late stages of development through differential mortality (affecting the SSR) (Box 2).
144 This differential mortality is likely to have higher fitness costs, especially in monotocous
145 species (producing only a single offspring at a time), since it implies loss of offspring after
146 maternal resources have already been invested. Thus, low-cost mechanisms of offspring sex-
147 ratio adjustment around the time of sex determination and differentiation are more likely to
148 evolve (Box 2).

149 The relationship between stress at the time of sex determination or differentiation and
150 PSR has been mainly studied in fish and birds (Figure 1). In fish, high GCs at this

151 developmental point led to a PSR biased toward males. Avian meiosis can be influenced by
152 maternal GCs in response to environmental stress, but the timing of the increase in GCs
153 appears critical in determining the sense of the sex-ratio bias [31,33].

154 In most reptiles, the undifferentiated gonads are sensitive to environmental factors
155 during the middle third of the incubation period and it has been reported that maternal steroids
156 in the eggs can decline by more than 90% before the sex is determined [11,51]. It is thus
157 possible that most GCs from maternal origin would be metabolized before sex differentiation
158 and that the remaining amount (as little as 10% of the original present) would be insufficient
159 to influence sexual steroid production, explaining why most studies did not report an effect.
160 The two studies that reported an effect experimentally introduced corticosterone into the egg,
161 probably affecting PSR and led to male sex differentiation [15,17]. On the contrary, when
162 more females were produced in *Amphibolurus muricatus*, the authors proposed that it was
163 through differential mortality of the sexes, suggesting two distinct mechanisms [15]. An
164 experiment evaluating endogenous GCs production at the time of sex determination is now
165 badly needed.

166 In mammals, the relationship between maternal GC levels and PSR remains
167 unexplored since most studies measure the sex ratio at birth (Figure 1). Several studies,
168 however, support the idea that stress occurring around the time of conception plays a key role
169 in sex determination and even sex differentiation (Box 2). Maternal GCs are generally
170 measured around conception in mammals (Figure 1), but this does not provide insight into
171 when and how GCs act. We cannot discard the possibility that the effects of stress on sex ratio
172 may be delayed rather than immediate. Male fetuses are generally more susceptible to food
173 shortages or other forms of environmental harshness during the last stages of gestation [52].
174 At the moment, it is still unclear whether such sex differences in mortality are the product of
175 sexual selection (faster growth and larger energetic requirements in males) or the result of

176 adaptive manipulation of the sex ratio by mothers, or a combination of the two. Thus, studies
177 that measure sex ratio at birth can confound two different process: an adaptive effect of
178 maternal stress near conception on offspring sex ratio and passive consequence of maternal
179 stress on sex-specific fetus mortality. Orzark et al. [53] recently showed that 3- to 6-day-old
180 embryos produced by assisted reproduction in the USA were equally likely to be male and
181 female and while natural abortions during the first trimester were biased towards females,
182 second- and third trimester abortions show a strong bias towards males. This suggests that the
183 direction of sex ratio skews in response to maternal stress could change during gestation.

184

185 **Adaptive significance**

186 Some cases discussed above may be adaptive, meaning that an animal's tendency to develop
187 as a given sex, after experiencing stress directly or indirectly (through mother's influence), is
188 or has been, under positive selection. If stress-related changes in sex ratio are under positive
189 selection, this implies that one sex would have a higher fitness in stressful conditions (Figure
190 2) or that maternal stress modifies the relative costs of producing male and female offspring.
191 Indeed, in the absence of any relationship between maternal stress and the relative fitness of
192 male offspring and female offspring, potential costs of reproduction may favor the production
193 of the less costly sex by stressed females. In practice, demonstrating adaptive significance is
194 not easy because fitness is often difficult to measure, especially in the wild. Furthermore, GCs
195 and maternal GCs may be correlated with other physiological traits that are directly involved
196 in adaptive manipulations of sex ratios [54]. The crossover patterns shown in Figure 2 have
197 not yet been tested in studies investigating direct links between GCs and sex ratio. It is more
198 surprising that potential adaptive explanations are often ignored (e.g. [33,35]). We discuss
199 here potential adaptive explanations for most trends observed in Figure 1.

200

201 *Environmental predictability*

202 GCs around sex determination or differentiation may provide a ‘weather forecast’ of the
203 environmental conditions where individuals are likely to live in the future. Related to this idea
204 is the concept of external **predictive adaptive response** (PAR) [55]. Theoretical work shows
205 that external PAR should only be favored by selection where the association between early-
206 and later-life environmental conditions is strong [55]. This is most likely to be true for
207 animals with a fast life-history strategy (short lifespan, early maturity, high fecundity and low
208 per capita investment per offspring)[19]. There is increasing evidence that environmental
209 predictability can determine the strength of selection for sex ratio adjustment [56]. For
210 example, greater sex ratio adjustment is seen in ungulates with shorter gestation periods [57]
211 and primates with shorter maturation times [58], possibly because it is easier for parents to
212 predict the amount of energy they would have available for rearing offspring at the time of
213 conception. Fish and birds have, on average, shorter incubation period than reptiles (Figure
214 3), which could explain why GCs play a more important role in the process of sex ratio
215 adjustment in the former than in the latter. While the absolute proportion of studies reporting
216 stress-related biases in the sex ratio may over-estimate the true frequency of GCs effects,
217 since positive results are more likely to be published than negative ones [59], the relative
218 proportion within groups of vertebrates should not be affected. The influence of GCs on the
219 sex ratio appears to be correlated with the length of the prenatal period across the groups of
220 vertebrates (Figures 1 and 3), but this is not always the case within groups. For example,
221 marsupials and rodents have very short gestation periods compared with primates, but the
222 absence of relationship between maternal GCs and sex-ratio has been reported in each group
223 (Figure 1). It is possible that the adaptive potential of maternal stress extends well beyond
224 simply a match between maternal and offspring environment [60].

225

226 ***Integrating stress in the Charnov-Bull model***

227 Theoretical models developed by Charnov and Bull [61] predicted that selection should favor
228 ESD over GSD if 1) the environment differentially affects the fitness of male versus females,
229 so that the sex produced is the better adapted to a given environment, 2) individual has little
230 control on the environment it will experience, 3) the environment is patchy, so that all sexes
231 are produced. It results that in optimal conditions (*i.e.* those that allow long term growth and
232 survival) the sex that will have the greatest absolute fitness benefits will be favored and that in
233 harsh conditions, the sex that will be “less costly” to develop will thrive [62]. With fish,
234 developing in stressful conditions would lead to a sex ratio bias towards males. This
235 prediction involves intrinsic sex difference in life-history traits related to reproduction where
236 males generally mature earlier than females and where the cost of producing sperm is much
237 lower than the cost of developing eggs (review of 98 fish species, [63]). In addition, females
238 generally grow bigger than males [64], highlighting that females may require a higher energy
239 input. So, in a low quality patch with limited resources, developing as a male would be
240 adaptive as it would be physiologically less costly and would allow reproducing quicker [65].
241 At least for fish, cortisol would play an essential role as being a physiological transducer
242 providing information of the patch quality since fasting induces cortisol production [66].

243

244 ***Social factors***

245 Social factors like hierarchical rank shape GC levels in many species of all the above
246 described taxa [67–69]. In hermaphroditic fish, such as clownfish species or the bluehead
247 wrasse (*Thalassoma bifasciatum*), an abrupt change in the established hierarchy triggers sex-
248 change, and cortisol regulation likely plays a pivotal role in this process [69,70]. Social
249 complexity of many species of birds and mammals often leads to multiple selection pressures
250 on sex ratio adjustment, potentially operating in different directions [71]. The Trivers-Willard

251 hypothesis [72] states that females in good condition, including those of high social rank,
252 should produce more male offspring in species where sexual selection is strong because
253 relatively few high-quality males will monopolize most of the copulations. Consistent with
254 this, a meta-analysis of data for ungulates provided support for the Trivers-Willard hypothesis
255 [57]. Complications arise when daughters “inherit” their mother’s social rank or adult
256 daughters compete with their mothers for local resources, as is the case with some primates
257 and marsupials [45,73,74]. When this occurs, females of high rank can produce more
258 daughters whereas females of low rank can produce more sons especially when competition
259 for resources is high [58]. In primates, a meta-analysis shows that subordinate individuals
260 produce more cortisol than dominants [75], although this negative relationship between social
261 status and GC production might not be necessarily true for all mammalian species [76].
262 Hence, the diversity of associations between maternal social status and offspring sex may
263 contribute to the differences in the sense of the effect of maternal GCs on offspring sex ratio
264 in mammals.

265 There is also variability in the directional patterns of the stress-induced effects on sex
266 ratio of birds, which may partially be explained by the different types of relationships
267 between mate attractiveness and relative fitness of sons and daughters. In birds, more than
268 90% of all species are socially monogamous. Mate choice is constrained by the availability of
269 unpaired individuals in the population and having an unattractive mate can be stressful for the
270 female [77–79]. The mate attractiveness hypothesis predicts that females mated to
271 unattractive males should produce more daughters because these daughters will not inherit
272 their father’s unelaborated sexual ornaments [80]. However, the reverse prediction exists in
273 Gouldian finches and maybe other, similar species because of the higher mortality for
274 daughters than for sons of genetically incompatible pairs [81]. In both Gouldian and zebra
275 finches, females with elevated corticosterone levels produce more male offspring [29,30]. It is

276 assumed that such manipulation is under female control, because female birds are the
277 heterogametic sex (Box 2). In species in which males are the heterogametic sex, fathers may
278 play a role in controlling the sex of offspring. Recent advances in mammals shows that the
279 proportion of X- and Y-chromosome bearing sperm can vary between individuals, reinforcing
280 the need to consider paternal characteristics, including stress, in future studies ([82,83], see
281 Outstanding questions).

282

283 ***Predation and sex-biased dispersal***

284 Sex-ratio adjustments in response to stress might be especially important in situations where
285 predation is the main stressor since it is a major evolutionary force acting on prey and changes
286 in predation risk tend to occur slowly rather than varying greatly from one year to the next
287 [84]. Individuals within the same population can be exposed to varying degrees of risk. For
288 instance, young are generally more vulnerable to predation, but their probability of being
289 predated upon sometimes depends on body condition [85]. For individuals subjected to high
290 predation risk, escaping through a higher dispersal potential might constitute an optimal
291 strategy to increase the probability of survival. In this sense, offspring common lizards
292 (*Zootoca vivipara*) whose mothers were exposed to predator cues disperse three times further
293 than those of non-exposed mothers [86]. Alternatively, one could argue that philopatry would
294 instead increase in offspring of stressed females, as demonstrated in common lizards
295 artificially exposed to GCs [87]. In either case, it is possible that the sex with the highest
296 probability of survival (by staying or migrating more than the other sex) in a dangerous
297 environment would be overproduced. In lizards and fish, there is no clear general pattern of
298 sex-biased dispersal, although juvenile males have been reported to disperse more in some
299 lizards [88] and migrating fish (e.g. *Oncorhynchus mykiss* [89]). The picture is clearer for
300 mammals in which males generally (but not always) disperse more than females, while the

301 reverse is typically observed in birds [90,91]. Hence, if we consider that high dispersal
302 potential is somehow related to differential survival between sexes under high predation, then
303 more males should be produced in the majority of mammals, while more females should be
304 produced in most birds. Studies combining measurements of predation risk, sex ratio and GC
305 levels are now needed to test these predictions.

306

307 **Concluding Remarks**

308 The sensitivity of the sex ratio to GC levels appears very variable among vertebrates. The
309 effects are more prevalent in fish and birds than in reptiles and mammals. The different
310 mechanisms of sex determination (genetic vs. environmental) are not sufficient to explain
311 these results because GSD is found in birds and ESD occurs in many species of reptiles.
312 Stress-related sex ratio biases are not necessarily more extreme in fish, but the direction of the
313 sex-ratio skew is uniform in comparison with the other taxa. While it is possible to propose an
314 adaptive explanation for most of these trends, more studies are necessary to test them in a
315 comprehensive meta-analysis. Fitness functions are essential for understanding the adaptive
316 significance of stress-related sex-ratio bias and there are obvious benefits to conducting such
317 investigations in the wild. A better knowledge of the physiological mechanisms that link
318 maternal and environmental stressors to offspring, by taking advantage of both new non-
319 invasive tools (Box 1) and useful animal models, would also be particularly helpful for an
320 integrative understanding of stress-related sex ratio variation (see Outstanding questions).

321

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- 575

Box 1: Stress and glucocorticoids

576

577 A widely used definition of “stress” is “the physiological cascade of events that occurs when
578 the organism is attempting to resist death or reestablish homeostatic norms in the face of
579 insult” [2]. The secretion and synthesis of glucocorticoids (GCs) is considered the primary
580 endocrine response (together with the release of catecholamines) to unpredictable or
581 uncontrollable stimuli (stressors) in the environment [92]. In the wild, high GC levels could
582 result from many different types of stressors including predator attack or presence [93], high
583 population density [94], habitat degradation [95], food shortages [96], social conflict [97] or
584 human activities [98]. Here we considered all GCs increase resulting from these stressful
585 cases, although it is worth noting that harsh conditions do not necessarily provoke a stress
586 response [76].

587 Stressors can be classified as **acute** or **chronic**, with acute being of short duration (minutes to
588 hours) and chronic being days to months [84]. Some acute stressors can, however, have long-
589 lasting consequences. The effects of stress may even span generations. These
590 transgenerational effects are often due to the transfer of GCs from the mother to the progeny,
591 through eggs in fish [99], birds [100] and reptiles [101] or blood and milk in mammals [102].
592 The reliability of GCs to assess chronic stress has recently been questioned [103]. Indeed,
593 animals can show unchanged or lower GC levels after exposure to chronic stress [103]. Some
594 of this ambiguity may be due techniques used to quantify GC levels and habituation to the
595 chronic stress methodology in the laboratory studies [104]. Although blood sampling has been
596 commonly used for measuring GC concentrations, it is not necessary the most pertinent
597 method [105]. Integrated and non-invasive measure of GCs, such as those from feces, hair,
598 scales, feathers, surrounding water, may more reflect the cumulative exposure of individuals
599 to GCs rather than repeated measurements of GCs in blood or saliva [104–107]. The
600 evaluation of several stress response parameters remains strongly recommended for a correct

601 interpretation of data [106].

602

603 **Box 2: How GCs interact with sex determination and sex differentiation**

604

605

606 Gonadal sex determination refers to “the decision to differentiate as a testis or an ovary” [1],
607 so that sex determination occurs chronologically before sex differentiation and GCs could
608 interfere with both processes. For instance, in avian species, corticosterone may influence
609 sex-chromosome segregation at the first meiotic division, thereby acting directly on sex
610 determination. This is possible because, in birds, females are the **heterogametic sex** (ZW
611 chromosomes) and have therefore a high degree of control over the sex ratio of the offspring
612 they produce (Figure I). In mammals, morphological differences between X-chromosome-
613 bearing (X-CBS) and Y-chromosome-bearing sperm (Y-CBS) have been found. X-CBS are
614 larger than Y-CBS because they contain more DNA and these two kinds of spermatozoa can
615 have differential survival rates both before and after ejaculation (*i.e.* in the female
616 reproductive tract) [82]. Stress might directly alter the female physiology, making sperm
617 survival sex chromosome specific [34]. There is also evidence that the oocyte susceptibility to
618 X-CBS or Y-CBS can vary according the stress of the female [108].

619 Furthermore, phenotype and genotype are not always aligned. A potential well-conserved
620 mechanism involves control of aromatase (enzyme converting androgens into estrogens). In
621 fish, as in reptiles, aromatase is central to determining the sexual fate of ambisexual gonads
622 [51,109]. This enzyme could be controlled by GCs, as demonstrated in the Japanese flounder
623 where complex cortisol/glucocorticoid receptors (GR) bind to the glucocorticoid response
624 element (GRE) on the aromatase promoter to decrease aromatase expression, resulting in an
625 overproduction of males [6] (Figure I). Surprisingly this interaction between
626 GCs/GR/aromatase appears to be well conserved in human testicular Leydig cell tumors, also
627 triggering a decrease in estrogen production [110]. In a normal functioning, the appropriate

628 intrauterine sexual steroid balance is also fundamental in humans, since female differentiation
629 (occurring from 7 to 12 weeks post conception) is vulnerable to androgen before the
630 protective placental aromatase appears [111]. During this sex differentiation period, standard
631 cortisol production reduces androgen synthesis, allowing normal female sex differentiation
632 [111]. Remarkably, in humans, mutations in the cortisol synthesis pathway, decreasing
633 cortisol production, is involved in the virilization of 46, XX girls with congenital adrenal
634 hyperplasia [112]. Cortisol thus appears to plays a key role in different species concerning the
635 balance of sexual steroids. The extent to which stress-linked cortisol production would affect
636 human and other animals sex differentiation, remains an open question. We nevertheless
637 recommend systematically investigating the genotypic sex of focal species to detect for
638 potential stress-induced sex reversal.

639

640 Figure I An overview of mechanisms by which glucocorticoids (GCs) can influence sex
641 determination and sex differentiation in vertebrates. X-CBS: X-chromosome-bearing sperm;
642 Y-CBS: Y-chromosome-bearing sperm.

643

644

645 Glossary

646 **Acute stressor:** short-term environmental challenges to the physiology of the animal.

647 **Chronic stressor:** long-term environmental challenges to the physiology of the animal.

648 **Cortisol:** The main glucocorticoid hormone produced by fish and most mammals through the
649 hypothalamic-pituitary-adrenocortical (Interrenal for fish) axis, and released in response to
650 stressors.

651 **Corticosterone:** The main glucocorticoid hormone produced by birds, reptiles and
652 amphibians and released in response to stressors.

653 **Environmental sex determination (ESD):** sex is determined by external factors, such as
654 temperature, water (pH, oxygen), or density of conspecifics.

655 **External predictive adaptive response (PAR):** a form of developmental plasticity in
656 response to environmental cues acting early in life, but where the advantages of the induced
657 phenotype manifest only later in life.

658 **Genetic sex determination (GSD):** sex is determined by the presence or absence of genes,
659 generally located on sex chromosomes.

660 **Glucocorticoids (GCs):** a class of steroid hormones present in all vertebrates, involved in
661 many physiological processes such as response to stress.

662 **Heterogametic sex:** the sex that has two different sex chromosomes (male XY in mammals;
663 female ZW in birds).

664 **Homogametic sex:** the sex that has two identical sex chromosomes (female XX in mammals;
665 male ZZ in birds).

666 **Primary sex ratio (PSR):** initial sex ratio when sexual differentiation occurs.

667 **Offspring sex ratio:** proportion of offspring that are males at different scopes (species,
668 population, individual, clutch/litter). Here, sex-ratio is generally considered at the clutch/litter
669 level.

670 **Secondary sex ratio (SSR):** sex ratio after sex differentiation that results from potential sex-
671 specific mortality before birth.

672 **Sex determination:** any of the various mechanisms in which the sex of the individual animal
673 is determined.

674 **Sex differentiation:** The process following sex determination, where the development of a
675 undifferentiated primordial gonad into testes or ovaries occurs.

676

677

678 Figure Caption.

679 Figure 1. Effects of glucocorticoids (GCs) on the sex ratio of different species of fish, reptiles,
680 birds and mammals (animal design: Pierre Lopez). Glucocorticoids were applied or measured:
681 before conception; around sex determination or differentiation; during the late phase of
682 gestation or development. High levels of stress can induce increases in the proportion of
683 males (animals in blue), reductions in the proportion of males (animals in red) or no change at
684 all (animals in grey). GCs were measured in the mother for all mammals and birds (except
685 34), while for fish and most reptiles (except 23; 24) it was measured on the animal itself.
686 Number in bracket indicates the corresponding reference. Full black circles indicate species
687 for which primary sex ratio was assessed, while empty circles indicate species for which
688 secondary sex ratio was determined. Note that for the two marsupial species (42,47) the exact
689 timing of GCs measurement relative to pouch status is unknown due to its particular
690 development.* Caimans were exposed to a temperature protocol producing 100% males and
691 exposure to GC did not change this pattern.

692

693 Figure 2. The pattern of fitness that can be observed if the sex-ratio adjustments in response to
694 stress are adaptive. The three panels represent adaptive scenarios because the lines cross. Sex
695 A can be either male or female. The sex that gains the greater fitness benefits in a hazardous
696 environment (sex A) should be preferred under high stress conditions, and the other sex (sex
697 B) should be preferred under low stress conditions.

698

699 Figure 3 Duration of incubation or gestation time (days) as a potential driver of sex-ratio
700 adjustments in response to stress in teleost fish (n=53), birds (n= 447), reptiles (n=7) and

701 mammals (n=420). Box plots show the mean represented by black diamond, the median
702 represented by lines, first and third quartiles as well as outliers represented by full circles
703 outside the box. Note that 14 outliers > 400 days were omitted in mammals for design
704 purpose. Signs and background color represent the strength of the match between the
705 environment experienced at the time of sex determination (SD) and the one experienced by
706 newly born offspring, with “+” indicating a strong match and “-” indicating a weak match.
707 The dashed line represents the average SD for most represented species (note that it could
708 occur during incubation or later for some fish and reptiles). Data were extracted from the
709 dynamic energetic budget website (<https://www.bio.vu.nl/thb/deb/>) reporting the life history
710 traits of more than 1500 species.

711
712

713 Highlights

714

- 715 • Offspring sex ratios have been shown to correlate with environmental stressors and
716 maternal stress in many vertebrate species.
- 717 • There is an adaptive advantage for parents to produce the sex that is more likely to
718 survive and reproduce in a future hazardous environment.
- 719 • In the fastest life histories, there is more likely to be a close good match between the
720 environment around sex determination and the offspring’s future environment.
- 721 • Glucocorticoids are key messengers of environmental contexts that likely influence the
722 sex determination processes of various species.
- 723 • A combination of field and laboratory studies is required to understand the extent to
724 which stress influences offspring sex from conception to birth.

725

726 OUTSTANDING QUESTIONS:

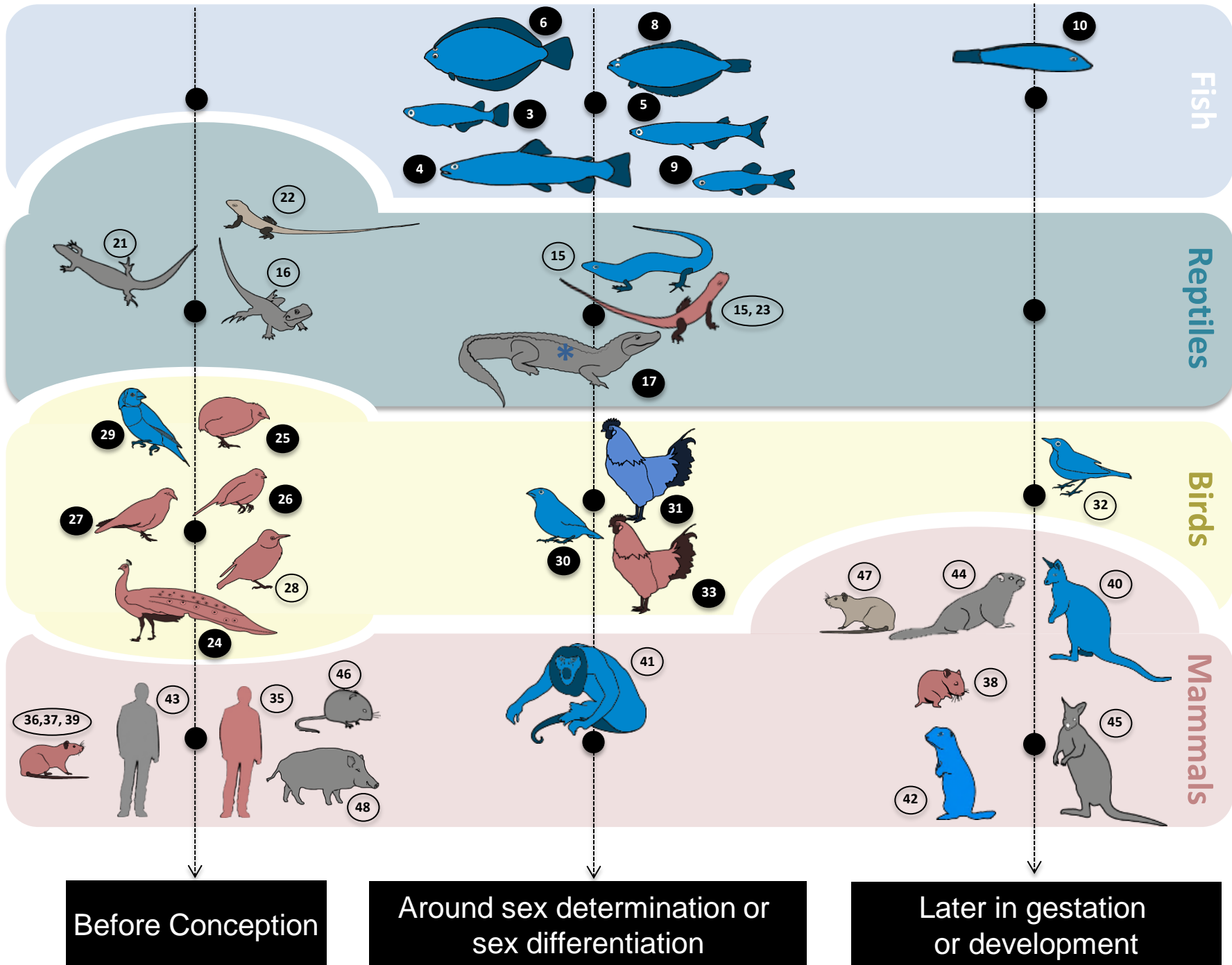
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728 Although we have demonstrated that there is a relationship between stress and sex ratio and

729 that this varies strongly between vertebrates, many areas are still little understood. Questions
730 for the future include:

- 731 • Do chronic and acute increases in GCs act on sex determination through similar
732 mechanisms?
- 733 • Do increases in GCs during the sex differentiation period in reptiles change the sex
734 ratio?
- 735 • To what extent could sexual dimorphism in other life history traits such as age at
736 maturation, growth or metabolism drive the production of a certain sex under stressful
737 conditions?
- 738 • To what extent key life history traits, allowing to distinct fast and slow selected
739 species, explain the sensitivity of offspring's sex to stress?
- 740 • Does paternal stress explain sex ratio skews that are not explained by maternal stress
741 in species, such as mammals; where males are the heterogametic sex?
- 742 • Are sex ratio skews the product of differences in the susceptibility of the two sexes to
743 environmental stressors or are they the consequence of adaptive adjustment by
744 parents?
- 745 • Does the presence of the chorion, which potentially protects the embryo during initial
746 phase of sex differentiation mechanisms from external stressors, increase selection for
747 a master sex determining genes in some fish species?
- 748 • Is the differential mortality between sexes linked with stress at the earliest stages of
749 development?
- 750 • To what extent are epigenetic mechanisms and stress-related information linked?
- 751 • Do GCs act alone to skew sex ratios or in combination with other non-sexual steroid
752 molecules such as glucose?

753



Fish

Reptiles

Birds

Mammals

Before Conception

Around sex determination or sex differentiation

Later in gestation or development

Without Stress

Gamete Level

50% 50%

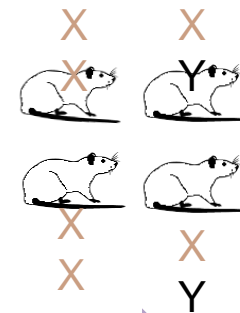
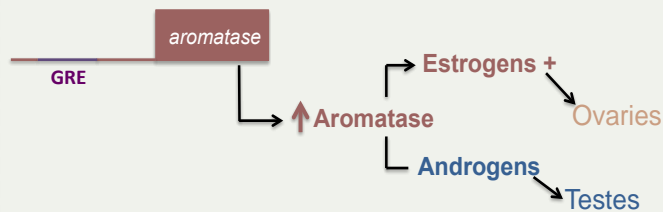
WZ ZZ

50% 50%

XY XX

Hormonal Level

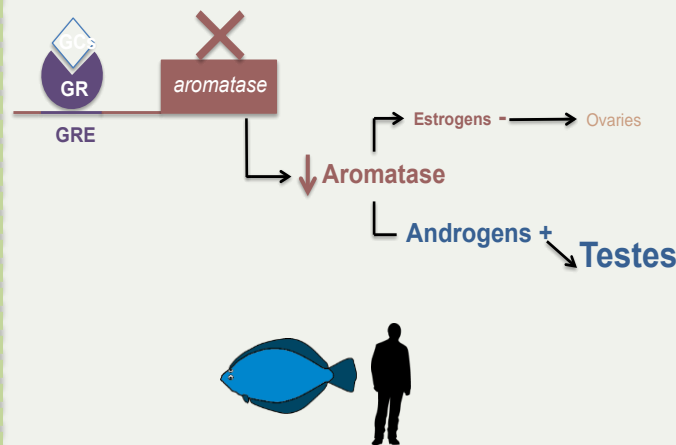
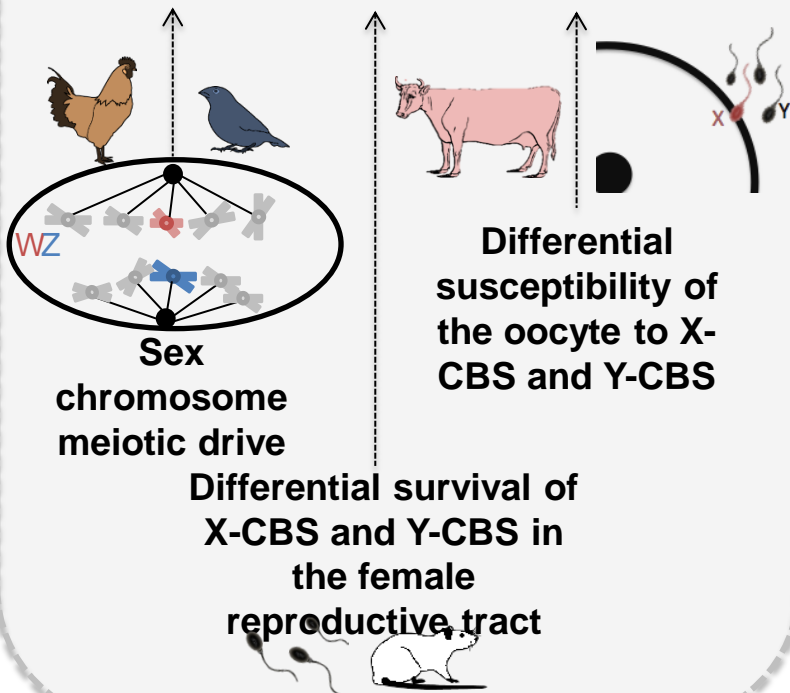
Gene Enzyme Hormones Organs



Sex determination

Sex differentiation

With Stress



Control of the aromatase by GCs

