

### Sex determination: Are two mechanisms better than one?

For just over a quarter of a century, it has been known that the scaly groups of vertebrates collectively known as reptiles – lizards and snakes, crocodilians, turtles, and the tuatara – have some species with sex chromosomal sex determination and other species in which embryo temperature determines sex. Until recently, it was thought that the two systems were mutually exclusive. The last 11 months has seen publications challenging that perception, based on two Australian lizards. Both papers report that, in the lab, extreme incubation temperatures can override sex chromosomes in the development of gonadal sex. Following earlier hints based on hatchling sex ratios in a skink with sex chromosomes (Shine *et al* 2002), a paper by Radder *et al* (2008) applies DNA methods to this species to show that cool incubation temperatures produce males in some individuals with a female (XX) genotype. Another recent study showed that high temperatures caused male genotypes (ZZ) to become females in an agamid lizard with female heterogamety (Quinn *et al* 2007). (The notation (XX /XY) is usually reserved for male heterogamety, in which the sperm genotype determines the sex of the zygote; ZZ/ZW is reserved for female heterogamety.) The Radder *et al* study used lab incubation regimes that mimic temperature profiles of cool natural nests, so temperature probably determines sex at least occasionally in nature.

The discovery in reptiles that incubation temperature of the embryo affects its adult sex is about 40 years old (Charnier 1966 reported it in an African agamid lizard), although it was mostly the 1970s turtle work of Claude Pieau in France that gained acceptance for temperature dependent sex determination (TSD) in reptiles (e.g. Pieau 1971, 1972). TSD is widespread, found in many turtles, possibly all crocodilians, the tuatara (the sole representative of the Order Rhynchocephalia), and many lizards (Janzen and Paukstis 1991; Ewert *et al* 2004). TSD is often extreme, with one temperature range producing only one sex and other temperatures producing only the other sex. The discovery of sex chromosomes in reptiles, first observed in snakes, is not much older than the discovery of TSD, and was brought to most people's attention in Susumu Ohno's now famous book on vertebrate sex chromosomes (Ohno 1967). Heteromorphic sex chromosomes are found in most 'higher' snakes, many lizards, and a few turtles.

Despite widespread interest in reptilian sex determining mechanisms, the previous 25 years of observations have failed to identify a species with both sex chromosomes and TSD in nature. If environmental effects determine sex in some individuals, then those environmentally-influenced individuals will have the wrong gonad type for their genotype: they will be XX males or XY females. This discordance is easy to observe when a species has morphological sex chromosomes, or when genetic markers are available (e.g. PCR markers, as with the Radder *et al* study). When it was first proposed that TSD and sex chromosomes were exclusive systems, mismatches of gonad and sex chromosome type had not been observed (Bull 1980). In fact, discordance between gonad type and genotype still has never been observed in reptiles born in nature, but because Radder *et al* based their incubation profiles on temperatures from natural nests, it should occur naturally. One should also appreciate that the historical 'evidence' against coexistence is limited: herpetologists don't make a point of analyzing large samples of karyotypes merely to see whether gonadal sex matches sex genotype. We thus don't have a terribly good idea how often any reptile with sex chromosomes actually does deviate from the expected pattern of gonadal sex.

Should we therefore be surprised at the coexistence of both systems in a species? Probably. Given that the historical evidence on coexistence is largely uninformative, one's reaction to these reports depends on what seems plausible. Perhaps the major issue is whether the coexistence is an accident or adaptive. If the coexistence is an accident – that the temperature effect is merely an infrequent relict from an ancestry before sex chromosomes – then coexistence is not surprising and may have little evolutionary significance. If the coexistence is adaptive, then we should be surprised.

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There are two levels at which the adaptive basis of a sex determining mechanism may apply. One concerns the integrity of the developmental program producing males and females. If sexual development is sloppy, such that intersexes and phenotypic incongruences frequently arise under one mechanism, selection may favor another mechanism to reduce developmental mistakes. Sloppy development is not seen in reptiles with TSD, however. Gonad types seem to be consistently one type or the other, as if development follows either of two alternative pathways and the environment merely flips a switch to determine which one is followed.

An alternative adaptive perspective is to consider the impact of the sex determining mechanism on sex ratio and its interaction with genetic and environmental factors that affect fitness for other reasons (Bull 1980). This perspective has received the most attention. Naively, it might seem like a good strategy to have a mix of both systems, allowing versatility of sex ratio production at the population level. Yet such population-based selection arguments fail to survive scrutiny. Instead, it has been shown that a stable sex ratio will evolve and be maintained regardless of whether sex chromosomes alone determine sex or there is an environmental influence on top of sex chromosomes – the environmental effect does not lead to a change in the equilibrium population sex ratio. Indeed, if we ignore the many nuances of biological details discussed below, population genetics models show that the population is largely indifferent to whether sex is determined environmentally or by sex chromosomes, and there is a neutral pathway by which a sex chromosome system can evolve into pure environmental sex determination, with both sexes XX or ZZ (Bull 1981). Why, then, should we be surprised at adaptive coexistence?

Our understanding of sex chromosome evolution and genetics argues against coexistence: a mechanism with both sex chromosomes and TSD should sit in an adaptive valley. For convenience, I will develop arguments assuming that sex chromosomes operate with male heterogamety, but it makes no difference to assume female heterogamety. The difficulty specifically applies to differentiated sex chromosomes. If the X-Y difference is merely due to a single gene or a mutation in a single gene, coexistence is easy, as in the sex ratio model discussed above. The problem arises when X and Y are large linkage groups and have accumulated many genetic differences, as applies to heteromorphic sex chromosomes. Nearly all of those X-Y sex chromosome differences are thought to evolve because of linkage to the sex determining locus/loci (Charlesworth *et al* 2005). Most X-Y differences not involved in sex determination *per se*, but they can have major genetic consequences when XX and XY do not develop into the right sex.

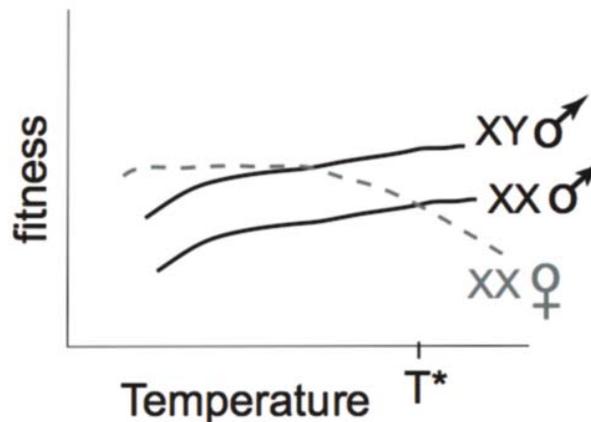
In principle, two evolutionary pathways can lead to coexisting sex chromosomes and TSD. One is that TSD evolves first, then sex chromosomes arise and evolve in a background of TSD. The other pathway is the reverse, sex chromosomes evolve first and TSD follows. Both are problematic in theory, but the difficulties posed by the latter transition – TSD evolving in a background with sex chromosomes – is perhaps easiest to understand. In general, prior to the introduction of environmental effects on sex determination, XX has a history of always being female, XY always male, and many aspects of gene expression during sex development may penalize deviations from this pattern. As noted above, environmental effects on sex lead to XX males or XY females. XY females will be at a disadvantage in many systems, because they will usually mate with XY males and thus produce 1/4 of their offspring as YY; in most sex chromosome systems, the YY genotype is inviable because of the accumulation of non-functional genes on the Y (a 'degenerate' Y). XY females may also be at a disadvantage because of any male-specific genes carried on the Y and from the single dose of X-linked genes, which has evolved in a male context. Likewise, XX males may be at a disadvantage both because the X tends to accumulate genes beneficial to females and because XX will lack fertility factors found only on the Y, as in many *Drosophila* species (Carvalho *et al* 2001).

Now consider the other direction of evolution: sex chromosomes gradually evolving in a species with TSD. The process from this perspective also poses difficulties. The evolutionary processes that convert a one-locus control of sex determination into one in which entire chromosomes are sex linked and morphologically distinct is thwarted when the inheritance of the sex alleles is softened by environmental overrides. The buildup of male-specific genes on the Y, the evolution of cross-over suppression, and the ultimate Y genetic decay, all depend on strict male-only transmission of the Y, hence in the absence of environmental effects on sex. Likewise, the build-up of X genes benefiting females will be slowed if XX is sometimes male. So there is no easy way to apply existing adaptive theory to paint an expectation of coexisting sex chromosomes and TSD.

Sex chromosomes offer several possible advantages in sex determination, such as developmental stability and the ‘right genes’ with the right sex, leading to superior males and females. (They can cause problems too, however.) If they do enhance male and female fitness, sex chromosomes would then be expected to take over completely, all else equal. Yet all else need not be equal: there can be an advantage of environmental sex determination over sex chromosomes. If an offspring develops in an environment that impacts its fitness as an adult, differently so for its fitness as a male than as a female, then selection favours it developing into the sex that benefits the most (Charnov and Bull 1977). Thus, environmental sex determination is favoured as a type of optimal developmental plasticity. For this model to apply to the lizards, incubation temperature must have a lasting effect on fitness, with some nest temperatures better for males (or less harmful to males) and other temperatures better for females. It is timely that the first compelling support for this model has just been published, based on an Australian agamid lizard (Warner and Shine 2008; but not the species with sex chromosomes studied by Quinn *et al* 2007).

To explain sex chromosomes and TSD being maintained together, we combine the advantages of both systems. If the lizard inhabits the appropriate type of environment for TSD to be adaptive, then genotypic sex determination should disappear because it gets in the way of optimal plasticity. However, if sex chromosomes carry genes good for males on the Y (genes good for females on the X), then an XX male will be at a disadvantage compared to an XY male at all temperatures (for example). In this case, sex chromosomes have an intrinsic advantage that will favor their retention. If, in addition, some embryo environments are so extreme that an individual will have much higher fitness as male than as female, even the XX genotype may be better off as male than as female when developing in those extremes. At any temperature, the fitness of XY males may always be better than that of XX males, but given that an embryo is XX, the environment may be extreme enough that it will do relatively better as male than as female (figure 1). TSD is then adaptive at the extremes but not at intermediates. This partitioning of mechanisms to operate in different temperature ranges appears to be how both lizard species operate: sex chromosomes control at middle temperatures, TSD at an extreme. It is also likely significant that both systems show the same pattern of sex-reversing only the homogametic (XX, ZZ) genotype, not the heterogametic (XY, ZW) genotype. This prevents the production of YY (WW) progeny, which may well be dead.

To the extent that either of these lizards are tractable for extensive field studies and lab colonies, the new systems should invigorate research on reptilian sex determination across a broad spectrum of biological approaches. At a developmental level, does temperature work through the same pathway as a sex chromosome? What characterizes the molecular pathway in an XY male at different temperatures,



**Figure 1.** A system in which sex chromosomes and temperature dependent sex determination are favored to coexist. Fitness is measured within a sex, relative to the average fitness for that sex. XY males have an advantage over XX males at all temperatures. However, if XX can become either sex, then an XX individual has higher fitness as male than as female at temperatures above  $T^*$ , even though he will have lower fitness than an XY male from the same temperature. Selection would favor a developmental response to override the female tendency of XX at high temperatures, above  $T^*$  but not below  $T^*$ .

versus an XX male at extreme temperatures? The pathways of sex development must converge to the same genes at some point; the two mechanisms could be so similar as to use the same trigger (e.g. a locus whose expression is modified at extreme temperatures; Quinn et al 2007), but such economy of mechanisms is not assured. The most basic question to answer in the field is how commonly temperature overrides sex chromosomes, a question that can now be answered merely by a combination of external examination and PCR of minute tissue samples from wild-caught animals. And the possible adaptive significance is still unknown. The adaptive-coexistence model above reveals that a species with mixed mechanisms should exhibit the most extreme sex-specific fitness effects in the temperature range that overrides sex chromosomes. As there is already evidence of sex-specific fitness effects due to incubation temperature (Shine et al. 2002), it may be feasible to test the model. At the very least, the fertility of the XX males and ZZ females should be verified. These combined sex determining mechanisms may not have been anticipated, but their discovery should lead toward a new understanding at several levels that will not be feasible in other systems.

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