Selfish Z chromosomes and the origin of sex reversed ZW males in tongue sole

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Abstract:

Tongue sole, *Cynoglossus semilaevis*, shows complex sex determination, in which all females are ZW while males can be either ZZ or ZW. ZW males can be produced in three ways: genetically, due to the presence of a segregating masculinizing mutation in the Z-linked sex-determining Z chromosome DMRT1; environmentally, due to elevated rearing temperature; or epigenetically, due to inheritance of the Z chromosome from an environmentally or possibly epigenetically sex reversed ZW male. Here I consider the origins and evolution of this system. I show that sex reversal of ZW individuals is likely to benefit the Z chromosome due to exclusive transmission of Z chromosomes during spermatogenesis, despite the fact that sex reversal causes male-biased sex ratios and thus reduced ZW male fitness. That is, ZW sex reversal reduces the individual's fitness but can increase the Z chromosome's fitness through that individual, implying intragenomic conflict over sex determination between selfish Z chromosomes and the rest of the genome. I derive expected equilibrium conditions for masculinizing Z alleles, invasion conditions, and expected sex ratios. I note the clear similarities as well as the differences between these masculinizing Z chromosomes and the feminizing X chromosomes that have independently evolved in several rodent lineages.

Complex sex determination in Cynoglossus semilaevis

The economically important tongue sole, *Cynoglossus semilaevis*, shows a remarkable pattern of sex determination and transmission (Figure 1). All females are ZW and all ZZ individuals are males. However, many ZW individuals develop as males. Remarkably, such sex reversed (SR) ZW males fall into at least three categories: (i) temperature SR (TSR) males that are masculinized due to development at high temperature (Chen et al. 2014; Shao et al. 2014); (ii) epigenetically SR (ESR) males, which inherit their apparently epigenetically modified Z chromosome from a temperature SR male or potentially from another epigenetically SR male (Chen et al. 2014; Shao et al 2014); and (iii) genetically SR males, which are masculinized due to their Z chromosome carrying a common mutation in the DMRT1 gene (referred to here as Z*) that causes masculinization of Z*W individuals (Cui et al. 2018). Further complicating matters, ZW males of each type appear to make few or no viable W sperm, and thus transmit their single Z

chromosome to (nearly) all of their offspring (Cui et al. 2018; Holleley et al. 2015). SR males have often been called pseudomales, however here I do not adopt that convention since a major goal is to place the *C. semilaevis* system in broader comparative context and that convention is not adopted in the other discussed sex reversal systems.

These peculiarities of sex determination have important economic implications, since masculinization of ZW individuals leads to populations being biased towards males, which are smaller and thus less economically desirable. A wealth of recent studies has increased our understanding of the molecular biology and genetics of this remarkable system. One set of studies has taken candidate gene approaches, probing the expression and regulatory impacts of known sex determination genes (Wang et al. 2021, 2022a, 2022b; Feng et al. 2021; Sun et al. 2021, 2022; Zhang et al. 2023; Liu et al. 2023; Cui et al. 2022; Cheng et al. 2022; Qi et al. 2022; Zhao et al. 2022; Li et al. 2016). Other studies have performed bulk or single-cell transcriptomic studies to identify genome-wide differences between sexes or karyotypes (Dong et al. 2021a,b; Xu et al. 2021; Liu et al. 2022; Sun et al. 2022; Wang et al. 2021a,b,c, 2023; Lin et al. 2021; Ye et al. 2020), or used GWAS to search for the genetics for sex-associated traits (Zhang et al. 2020b; Wang et al. 2022). Yet other studies have probed regulatory function and differential expression of non-coding RNAs (Dong et al. 2021a,b; Zhao et al. 2021,2022; Tang et al. 2022; Liu et al. 2021, 2022; Gong et al. 2022; Zhang et al. 2020a). Yet others have probed the effects of heat stress (Wang et al. 2019, 2020).

In contrast to this progress on our understanding of molecular and developmental biology, the evolutionary dynamics of the system have not been considered at length. Here, I first consider the system in evolutionary context, trying to understand the set of steps by which the various peculiarities may have evolved and the selective pressures driving them. I then consider the system in comparative context, comparing aspects of the system to other known atypical systems in rodents.

The selfish Z hypothesis

Perhaps the most important evolutionary insight into the system comes from recognizing that Z chromosomes systematically enjoy a transmission advantage in SR ZW males. Whereas ZW females are expected to transmit the Z chromosome to roughly one-half of their offspring, ZW males transmit the Z chromosome to all of their offspring. Therefore, in the simplest cases, in which ZW males and ZZ males have equal reproductive capacity, the Z chromosome stands to as much as double its reproductive fitness by causing masculinization of a ZW individual (at equal sex ratio). Therefore, for instance, starting from a hypothetical starting point of equal sex ratios and a 100% penetrant ZZ/ZW system, a newly arising Z chromosome haplotype that systematically or conditionally caused or allowed masculinization of ZW individuals would be expected to enjoy a very large advantage and to invade the population. Invasion of the masculinizing Z into the population is expected to bias

the sex ratio towards males, leading to decreased average fitness of males relative to females. Eventually, an equilibrium is expected to be reached when the transmission advantage of masculinizing Z chromosomes through ZW males is balanced out by the decreased average fitness of males due to skewed sex ratio. In the next two sections, I provide an explicit analysis of this verbal model.

Equilibrium dynamics of the system: simplified models

Throughout, we assume equal reproductive fitness of males of all karyotypes, and Mendelian transmission of chromosomes for ZZ males, ZZ* males and ZW females, but only Z transmission and no W transmission from ZW or Z*W males.

We first consider simplified scenarios. First, let us consider the case with no GSR males, so that the population consists of ZW females, ZZ males, and TSR/ESR ZW males, with the probability of SR of a ZW individual is independent of Z haplotype. We define p as the fraction of ZW individuals that develop as males due to either TSR or ESR. Because both ZZ and ZW males produce only Z-bearing sperm, karyotype depends only on the chromosome inherited from the ZW mother. Therefore, under Mendelian segregation we expect equal numbers of ZZ and ZW individuals (0.5 each). In total, then, we expect frequencies of 0.5(1-p) ZW females, 0.5p ZW males and 0.5 ZZ males.

Second, let us consider a simplified case with only GSR (no TSR or ESR). We can consider a Z chromosomal allele that consistently masculinizes ZW individuals. Following the convention of using X* to refer to a feminizing X chromosome (see below), I use Z* to indicate such a consistently masculinizing Z (although formally this is a new Y chromosome). Because W chromosomes are only transmitted through eggs and Z* only through sperm, each diploid karyotype can only be formed through a single type of fertilization event (Z*W by Z* sperm + W egg; Z*Z by Z* sperm + Z egg; ZW by Z sperm and W egg; ZZ by Z sperm and Z egg).

Let us define f(karyotype) as the frequency of individuals carrying that karyotype (f(ZZ), f(Z*W), etc.). As above, all eggs are produced by ZW females, thus we expect equal probabilities of Z-bearing and W-bearing eggs (0.5 each). This fact implies that ZW+Z*W individuals (produced from W-bearing eggs) are expected to account for half of the population, and ZZ+Z*Z individuals the other half:

f(ZW)+f(Z*W) = f(ZZ)+f(Z*Z) = 0.5

The same fact also implies that we expect equal frequencies of ZZ and ZW individuals (fusion of Z/W-bearing eggs with Z-bearing sperm) as well as equal frequencies of Z*Z and Z*W individuals (fusion of Z/W-bearing eggs with Z*-bearing sperm):

f(ZZ)= f(ZW)
and

f(Z*Z) = f(Z*W)

At equilibrium the fraction of Z*-bearing sperm is simply expected to be equal to the fraction of individuals produced by fertilization by Z*-bearing sperm, which is f(Z*Z)+f(Z*W). The fraction of Z*-bearing sperm is also expected to be equal to the fraction of Z*-bearing sperm produced by all males, which is all sperm from Z*W individuals plus one-half of sperm from Z*Z individuals, divided by the population frequency of males, which is 1-f(ZW). Setting these two quantities equal, we have:

f(Z*Z)+f(Z*W) = [f(Z*W)+f(Z*Z)/2] / [1-f(ZW)]

Because f(Z*Z) = f(Z*W) we have

2 x f(Z*Z) x [1-f(ZW)] = 3/2 x f(Z*Z), which yields

1-f(ZW) = 3/4,

which along with the above implies that at equilibrium,

f(ZW)=f(ZZ)=f(Z*W)=f(Z*Z) = ¼, with females accounting for a total population frequency of f(ZW) = ¼. (Note that this result follows the result obtained in the X* case by Burt and Trivers (2009)).

Equilibrium dynamics of the system: full model

Let us now consider the case with both GSR Z*W males and TSR/ESR ZW males. In this case, all the arguments just above apply, except that here because the frequency of females is f(ZW) x (1-p), the frequency of males is 1-f(ZW) x (1-p), and we have:

f(Z*Z)+f(Z*W) = [f(Z*W)+f(Z*Z)/2] / [1-f(ZW) x (1-p)], which yields

f(ZW) x (1-p) = ¼, which then yields

f(ZW) = f(ZZ) = 1/(4-4p)

and

f(Z*W) = f(Z*Z) = (1-2p)/(4-4p),

with females accounting for a total population frequency of f(ZW) x (1-p) = ¼

when p ≤ 0.5, whereas

f(Z*W) = (Z*Z) =0 and f(WZ) = f(ZZ) = 0.5 at p ≥ 0.5

with females accounting for 0.5(1-p) of the population.

Invasion analysis

We next consider the conditions under which the Z* allele is expected to increase in frequency when initially rare. The Z* is found only in Z*W or Z*Z males, and Z*W and Z*Z individuals are expected to be produced at equal frequency (see above). Thus, if the frequency of Z* is q then we expect q Z*W males and q Z*Z males in the population. At constant population size and sex ratio r, males have $2/r$ descendants on average, while females have $2/(1-r)$ descendants. We thus expect the q Z*Z males to transmit the Z* chromosome to q/r descendents and the q Z*W males to transmit to the Z* chromosome to $2q/r$ descendents, leading to a frequency of Z* in the next generation of $(q/r + 2q/r) / 2 = 3q/2r$. Thus q in the next generation is greater than q in this generation so long as $3q/2r / 2 > q$, or $r < ¾$. Note that this is consistent with the 1:1:1:1 karyotypic equilibrium found above in the absence of non-genetic ZW males. When Z* is very rare, the sex ratio $r ≈ ½(1+p)$, yielding the condition that the Z* allele will invade so long as $½(1+p) < ¾$, or $p < ½$.

On the selective value of temperature sex reversal

The other major question that arises in considering this system is the selective value of temperature SR. In particular, does the propensity to undergo SR in response to elevated temperature increase or decrease a Z chromosome's fitness? This question reduces to whether the Z chromosome has greater fitness in a ZW individual if that individual is male or female. In a ZW female, the Z chromosome has expected fitness equal to one-half times the fitness of an average female, whereas in in a ZW male, the Z chromosome has expected fitness equal to one times the fitness of an average male (because all sperm contain the Z). As noted above, average female fitness is equal to $r/(1-r)$ times average male fitness. Thus, whether the Z chromosome has greater fitness in males or females depends on the ratio of $2:r/(1-r)$. This reduces to $r:2/3$, meaning that ZW chromosomes benefit from sex reversal so long as r is less than 2/3.

As noted above, at equilibrium in the presence of a Z* haplotype, r is expected to equal 3/4, which according to the above would imply that the propensity to undergo sex reversal due to temperature is likely a net cost to Z chromosomes under equilibrium conditions. However, given that 3/4 is not hugely larger than 2/3, it is possible that complications to the scenario not included in this model, in particular differences in fitness between males with different karyotypes, could change this conclusion.

What is expected in the absence of a Z* allele, i.e., in a hypothetical ancestral population before the Z* allele invaded the population? As noted above, under these circumstances, the expected sex ratio $r = (1+p)/2$. Under these circumstances, the evolutionary stable strategy for propensity to undergo SR is that p that leads to $r = 2/3$, or $p = 1/3$ assuming random mating. Notably, however, if mating is not random, then temperature differences across locations will lead to variation in r

across populations. Rates of migration will also come into play, since if migration is limited, epigenetic SR will further increase r in warmer environments (i.e., r will both be increased by temperature SR in this generation and by epigenetic inheritance of SR from temperature SR fathers from the previous generation). The potential dynamics are complex and beyond the scope of this analysis, however it is clear that the propensity to undergo SR will be beneficial under relatively low r, but costly under high r.

Comparisons to feminizing X chromosomes in rodents

The structure and dynamics of the *C. semilaevis* system show clear similarities to several cases described in rodents (Burt and Trivers 2009; see Saunders and Veyrunes 2021 for a recent review). Like nearly all mammals, nearly all rodents retain the ancestral mammalian X and Y chromosomes. However, in several species there exists two forms of the X chromosome, one of which is a standard X chromosome while the other, generally denoted X*, has the distinctive phenotype of causing XY individuals to develop as females. Because YY mammals are generally inviable, the X* chromosome benefits because in crosses between XY males and X*Y females, the X* is transmitted to two-thirds of viable progeny (XY, X*X and X*Y). Thus, insofar as lost YY embryos are at least partially replaced by viable embryos of other karyotypes (a likely scenario for viviparous species), the X* chromosome can benefit and invade the population.

In addition, in one species, the wood lemming *Myopus schisticolor*, the Y chromosome is lost during oogenesis of X*Y females, leading to all eggs containing the X* chromosome (Fredga et al. 1976, 1977). These features of the wood lemming, and the evolutionary dynamics they describe, are most similar to those of the Z* system of *C. semilaevis*, given that in both cases the Z/Z*/X* chromosome benefits primarily by biased transmission through sex reversed heterogametic individuals. Indeed, with the expectation of the complication of TSR/ESR in *C. semilaevis*, the system may be seen as a first approximation to be exactly analogous in structure. The similarities to the other feminizing X systems are less striking but still notable. In all cases, SR is driven by a chromosome that is putatively selfish: while it benefits from increased transmission through SR individuals, it reduces those same SR individuals' overall fitness by biasing sex ratios to SR individuals' offspring's detriment.

Despite these striking similarities, several important differences of the system are of note. The first obvious difference is in the existence of non-genetic SR individuals in *C. semilaevis* but not rodents. Second, whereas reduction in gamete production and/or viable embryos for XY females may not greatly reduce the number of offspring, since reproduction of mammalian females is likely not primarily dependent on the number of gamete cells produced (e.g., Bulmer 1988), likely reduction in overall sperm production of ZW males due to apparent inviability of W-bearing sperm presumably reduces male capacity to sire offspring and thus to a further substantial overall reproductive disadvantage for SR ZW males. Third, the

molecular mechanisms and karyotypic implications of the alleles responsible for sex reversal are quite different in the two cases. Whereas in *C. semilaevis*, the sex determining gene is present on the sex reversing chromosome (i.e., *DMRT1* on the Z; Shao et al. 2014), in rodents the sex determination gene is on the other sex chromosome (*SRY* on the Y), thus the straightforward mechanism of SR by alteration of expression of the sex determining gene is possible and observed in *C. semilaevis*, but not in rodents. By contrast, in rodents feminization appears to be associated with X-autosome fusion (Jarrell et al. 1995; Romenenko et al. 2020; Baudat et al. 2019), suggesting that alleles of as-yet unknown ancestrally autosomal genes may generally be responsible for feminization, and that feminization is associated with substantial changes in the karyotype. Fourth, in multiple rodent lineages with feminizing sex chromosomes, compensatory sex chromosome drive is observed, with males predominantly transmitting the Y chromosome, with the effect of producing more sons, which are expected to have higher fitness under female-biased sex ratios (Romenenko et al. 2020; Saunders et al. 2021; Gileva 1987; Hoekstra and Hoekstra 2001). Interestingly, there are now reports from multiple feminizing X chromosome systems suggestive of context-dependent sex chromosome drive, in which males adjust the ratio of X-to-Y-bearing sperm in response to female karyotype (Y bias when mated to XX or XX* females, but X bias when mated to X*Y females) in a pattern predicted by selection for increased production of sons (Romenenko et al. 2020; Saunders et al. 2021). While the same considerations could predict that W chromosome drive could benefit *C. semilaevis* females, to my knowledge such drive has not been reported. Fifth, several phenotypic differences have been reported between Y-bearing and other females in rodents, ranging from greater size to greater overall fitness to changes in sociality, however to date no analogous differences have been reported in *C. semilaevis*. Notably, under viviparity X and Y chromosomes may have evolved lesser and greater demand on maternal resources, respectively (Haig 2000), and some of these phenotypic differences are consistent with these differences (in particular, greater birth weight for Y-bearing females than non-Y-bearing female; Roy 2021). If so, ZW and SR males in *C. semilaevis* may not show as many phenotypic differences, though further study will be necessary to determine whether this is so.

Concluding remarks

I have argued that the fascinating sex determination system of *C. semilaevis* has been driven by selfish Z chromosomal alleles that either directly convert or permit conversion of ZW individuals into males, and that these selfish chromosomes benefit due to exclusive Z chromosomal transmission through spermatogenesis while reducing the overall fitness of the sex reversed ZW males due to male-biased sex ratios and potential sperm reduction. More study of this system will be necessary to understand more of the phenotypic impacts of sex reversal as well as the population genetics of Z alleles. It will be particularly interesting to learn whether alleles that resist temperature and/or epigenetic SR exist, and whether, as predicted by the considerations presented here, these non-SR alleles are rising in frequency in the population. Alternatively, if Z chromosomes turn out to show a universal propensity

for temperature and epigenetic SR, this will suggest that there exist features of the system neglected here, in particular potential additional advantages to ZW males or to Z chromosome transmission through ZW males.

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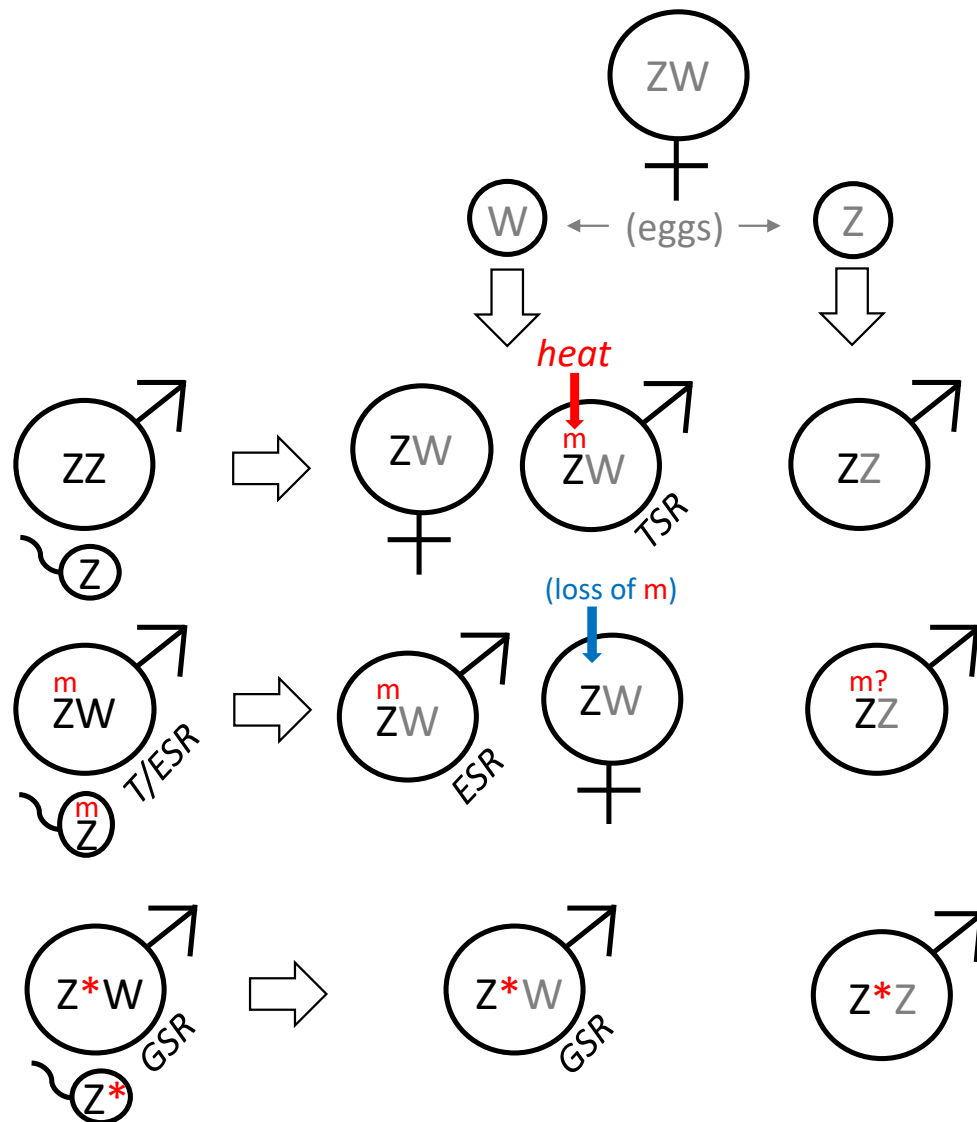
Figure 1. Schematic of the *C. semilaevis* sex determination system. Outcomes are shown for crosses between males with the three different karyotypes (left) and females (all ZW, top). Red indicates masculinizing factors, including epigenetic marks (m), a masculinizing DMRT1 mutation (*) and heat; blue indicates de-masculinizing loss of masculinizing "m" marks." Haplotype gamete karyotypes produced by each class of individuals are indicated. Black/grey indicates chromosomes inherited from father/mother. TSR/ESR/GSR indicates temperature/epigenetic/genetic sex reversal (masculinization of ZW individuals). m? indicates that the epigenetic status of epigenetically modified Z chromosomes in ZZ males is unknown.