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Sex Determination

The single biggest difference among people is sex—traits related somehow to the size of the gametes they make. Yet the overall difference between human males and females is moderate compared to other vertebrates. Mice males and females are nearly identical, except for gamete size and related genital plumbing, whereas lions have conspicuous male/female differences. Other than gamete size, our statistically valid sex differences are few and small, and our distributions overlap extensively. How do such differences between males and females develop?

WHEN SEX IS DETERMINED

Accounts of how male and female differences develop in mammals usually begin with gonadal differentiation—the genes that determine whether gonads mature as testes or as ovaries. Yet the gonad is merely a nursery for the germ cells. The germ cells are a different tissue from the gonad in which they reside and are descended from the primordial germ cells, which differentiated in the very early embryo before the tissue that gives rise to the gonads differentiated. The gonad is the site where hormones like testosterone and estrogen are synthesized, and once the gonads form, many other aspects of the body develop a gendered morphology. The hierarchical bowling-a-strike view of sexual differentiation is that a master gene starts the gonad, and then the gonad propels the rest of the body into a male or female template. However, this controlling narrative is simply not accurate, even though widely believed and often taught.

The sex of an embryo—whether its primordial germ cells mature as eggs or as sperm—is evident before the gonads start transforming into testes or ovary.[1] In mice, male embryos grow faster than female embryos even before the gonads differentiate into ovary or testis.[2] In marsupials, both male and female external genitals start to develop before the gonads form.[3]

In humans, the Y chromosome has a version of the gene for ribosomes, the cell's protein-making module, that is not found on the X chromosome. The cells of XX and XY embryos thus differ in their ribosomes, because females have one type of ribosome and males two types, long before any gonads develop.[4] Therefore, protein synthesis takes place slightly differently in male and female embryos from the time the sperm contribution to the embryo's genome is first expressed, a few divisions after fertilization. This sex difference is manifest even before the first primordial germ cells differentiate and long before the gonads differentiate.

Thus gonadal differentiation, while important in gendered development, is not the stage at which sex differentiation occurs, because sex differences are already manifest before gonadal development starts. The genes that determine gonadal differentiation do influence an individual's ultimate bodily and behavioral presentation. I will call the key genes determining gonadal differentiation the gender-determining genes, or "gender genes" for short.

WHEN GENDER IS DETERMINED

Even though a primordial germ cell may already have a good idea, so to speak, of whether to mature as a sperm or an egg, a germ cell that reaches the gonad is influenced by whether the gonad there becomes a testis or an ovary. Sex (whether the primordial germ cells mature as a sperm or egg) and bodily gender (starting with whether the gonad differentiates as a testis or an ovary) are subject to biochemical negotiation.

In mammals, a key player in this negotiation is a gene called SRY on the Y chromosome. SRY redirects and accelerates gonadal differentiation in the direction of a testis; in its absence, the gonad differentiates more slowly into an ovary.[5] Biologists often describe SRY as the master gene controlling sexual differentiation, the essence of maleness. When present, SRY is said to take over an embryo, commanding it to develop into a male; without it, an embryo develops "by default" into a female.

Well, not so fast. As we've seen, the gonads develop after some sex differences are already determined, so SRY doesn't fully control sex differentiation; it can only influence gendered presentation to some degree. Moreover, SRY doesn't act alone. That's not to say SRY isn't important. SRY produces a protein that binds to DNA, causing sharp bends, which in turn affect whether the genes in the bent area of DNA can be expressed. SRY censors the DNA, determining which genes get their messages published throughout the cell.

In one experiment, an SRY gene was introduced into XX mice.[6] About 30 percent of these mice went on to develop testes, as well as male external genitalia and some male mating behavior. The germ cells in these male XX mice, which would have otherwise become eggs, started to develop as sperm but couldn't finish without the right accessories. Key wardrobe instructions are needed from the full Y chromosome—SRY is not sufficient. However, SRY can direct at least some female embryos to develop a masculine presentation, and can coax the testis into convincing some primordial germ cells to mature as sperm rather than eggs.

In another experiment, an SRY gene was deleted from the Y chromosome of XY mice. These mice went on to develop ovaries, as well as other feminine traits. The germ cells in many of these female XY mice, which would otherwise have become sperm, developed as eggs, even resulting in some litters.[7] Thus, the absence of SRY leads male embryos to develop a feminine presentation and can coax the ovary into convincing some primordial germ cells to mature as eggs rather than sperm.

In mammals, then, gonadal gender is determined in large part by the presence or absence of the gene SRY. For this reason, SRY has assumed its legendary status as *the* sex-determining gene—if the Y chromosome is the marker of maleness, its power depends on SRY's presence on it. But is SRY really in a one-way controlling position at the top of the bowling-a-strike hierarchy? In fact, the gonads can develop at least partially into

testes on their own, even without coaxing by SRY. In XX female wallabies, genital ridges develop partially as testes in the absence of germ cells.[8] Thus, in the absence of germ cells that would turn into eggs within the ovary, the gonad moves on its own toward becoming a testis, even without SRY present.

The narrative of hierarchical control by a master gene, SRY, is thus an oversimplification. SRY is only one player in the germ-cell-to-gonad negotiation, with the role, as a genetic lobbyist, of coaxing the gonad in a male direction. SRY doesn't unilaterally control sex determination, because sex is already determined before SRY is expressed.

BEHIND THE POWER STRUGGLE

Okay, so SRY has a loud voice, but does it really bring to the table important information about how to be male? No. SRY turns out to be heavy on influence, light on substance. Here's how SRY has wormed its way onto the genetic committee that determines bodily gender.

Everyone has the genes to make both ovaries and testes, but which we make depends on some network of intergene negotiation. One key gene at the conference table is SOX9, which is located on a nonsex chromosome: it holds the basic testis recipe for all vertebrates. SOX9 is expressed in the developing gonads of male mammals, male birds, and male alligators.[9] Other genes at the conference table, in addition to SOX9 and SRY, are WT1, SF-1, and DAX-1 (or DSS) on the X chromosome.[10] Here's how the interaction goes:

1. WT1 prepares the genital ridge and adjacent kidney area. Then SF-1 and WT1 together urge SOX9 to make a testis.
2. But DAX-1 intervenes, preventing SF-1 and WT1 from activating SOX9, so an ovary forms instead.
3. In males, SRY inhibits DAX-1, permitting SF-1 plus WT1 to activate SOX9, which in turn produces a testis.

Thus SRY stops a gene, DAX-1, which itself was stopping testis development according to SOX9's recipe. Wow, not simple. Notice that SRY

and DAX-1 don't contribute materially to the recipe for making a testis. They are at the conference table just to argue, like genetic lawyers.

Nothing is universal about SRY or DAX-1; these genes don't appear in other vertebrates, including some other mammals. Species without SRY or DAX-1 have testes and ovaries, implying that different types of genetic negotiation can also produce gonadal differentiation. SOX9 is the only one of these genes with any claim to universality, at least among vertebrates. Even in species whose gonadal differentiation does emerge from the SRY-DAX-1-SOX9 committee, multiple alternative forms, or alleles, of SRY, DAX-1, and SOX9 exist, so the genetic narrative leading to bodily gendering in each individual differs depending on the precise alleles an individual has at these three genetic loci. The genes at the gonad-determination conference turn up at other conferences as well, and their expression is noted in many other tissues. These genes are a basic source of diversity in bodily aspects of gender.

SRY has pulled a coup d'état in our genetic palace, acquiring the power to preempt the differentiation of gonad into testis. But SRY is a loud-mouthed bandit living on a puny chromosome, Y, which itself recently degenerated during evolution from the X chromosome.[11] The how-to-do-it capability for testis construction resides in chromosomes other than Y.

Thus genes, including even the noisy SRY, work together during the body's development. The selfish gene is a sound byte, not science. Genes occupy a common body, their lifeboat. A selfish gene had better know how to swim. Survival for a gene means being genial—the genial gene. Not only do genes work together to jointly construct a pathway of consecutive steps in biochemical pathways, but they also collaborate in the synthesis of single enzymes. Some enzymes have multiple subunits that come from distinct genes. Furthermore, an enzyme called cytochrome *c* oxidase even has some subunits coded by genes in the nucleus and others by genes in mitochondria, so that making this enzyme involves the cooperation of nuclear and mitochondrial genes.[12]

A subversive philosophical shift is occurring in how biologists think about genes. As a student, I was taught that genes come first and the phenotype second. We live our lives with whatever traits our genes stick us with. A new view, from evolutionary developmental biology (fondly known as evo-devo), states that the traits come first. The need for a trait

appears in the world, like the ability to make a testis to contain germ cells maturing into sperm. Then genes, like SRY and DAX-1, compete to deliver that trait during development. A takeover artist like SRY with no information of its own can evolve by promising to deliver the trait faster. Ecology writes the specifications and places an order for a trait. Genes compete to deliver the trait—ecology the consumer, genetics the producer, in a client-server relationship. This new view empowers the context in which phenotype is meaningful.

WHEN Y DOES NOT EQUAL MALE

SRY's power, as we've seen, is far from absolute—it must negotiate with other members of the gender-gene committee to effect the differentiation of a gonad into a testis. Sometimes SRY is dispensable altogether, as in the case of *Ellobius lutescens*, a mole-vole. This burrowing mammal is 10 to 15 centimeters long with a velvety cinnamon coat and lives in semi-desert areas of the Caucasus, eastern Turkey, Iraq, and Iran, where it feeds on underground plant parts. Males of this species have no Y chromosome, nor any SRY gene anywhere. Yet *E. lutescens* males are still real males: they make sperm in testes.[13] Males of another mole-vole, *Ellobius tancrei* from Uzbekistan to Sinkiang, China, also don't have Y chromosomes.[14]

In other cases, SRY and the Y chromosome may be present but be completely overridden by other genes, outvoted on the gender-gene committee. In four species of South American vole mice of the genus *Akodon*, 15 to 40 percent of the females have both SRY and a Y chromosome, yet they are still female and make eggs. These females evidently have genes that silence the noisy SRY.[15]

That SRY can be completely outvoted foreshadows discussion later on that genes on the X chromosome in humans control how much effect testosterone has on tissue development. Thus, even if SRY succeeds in obtaining legislation to produce a testis, resulting in the synthesis of testosterone, the genetic bureaucracy has a say in whether the legislation is implemented. The genetic bureaucracy may partially implement the legislation by ensuring that testosterone has only little effect, or it may fail to implement the legislation at all, as in the case of complete androgen insensitivity.

Therefore, among mammals, a Y chromosome and an SRY gene are neither necessary nor sufficient to determine male sexual identity. Admittedly, in some species, including humans, SRY is a major player on the gender-gene committee. Yet even in species where SRY is empowered to cause testis development, SRY does not control how much effect the hormones secreted by the testis have on the body's adult morphology.

Thus, the development of even as basic a difference as that between males and females does not follow a standard template either across or within species. The bowling-a-strike view of development as the unfolding of a hierarchy of successive genetically mandated decisions simply doesn't occur. Instead, every individual has his or her own unique and equally valuable narrative of how the gender-gene committee fashioned the compromise that became that individual's embodiment of gender and sexuality.

WHEN OVARIES AND TESTES COMBINE

Every aspect of the body is on the table for the gender-gene's committee to negotiate, even the structure of the gonads themselves. Most gender-gene committees, with or without the presence of SRY, pass a resolution creating only a testis in males and only an ovary in females. In some species, though, even this most elemental aspect of bodily gender has been given a different configuration.

Among *Talpa occidentalis*—another burrowing mammal, an old world mole from the Iberian peninsula—all females have ovotestes, gonads containing both ovarian and testicular tissue.[16] The ovotestes occur at the site in the body where simple ovaries are found in other species. *Talpa* XX individuals have ovotestes and make eggs in the ovarian part of their ovotestes. They don't make sperm, but they do have both sperm-related and egg-related ducts. The testicular part of these ovotestes secretes testosterone. XY individuals have testes only and make sperm.

Four species of old world moles are now known whose females have ovotestes instead of ovaries.[17] Yet when a human is born with ovotestes, bells and whistles sound in the hospital as though a law of nature had just been threatened. Old world moles would view modern medicine as primitively mistaken. Thus, even gonadal structure doesn't follow a standard template across mammals.

WHAT HAPPENS TO THE "EXTRA" X

In mammalian species where females are XX and males are XY, females have an embarrassment of riches—two X chromosomes where one suffices. Expressing both X chromosomes would presumably provide an overdose of the enzymes tweaked to work at the lower concentrations produced by a single X chromosome (as occurs in males). The workaround for females is to make one of the X chromosomes inactive. One of the Xs scrunches up, becomes unavailable for transcription into protein, and appears under the microscope as a speck in the nucleus called the Barr chromatin body.[18]

Which of the two X chromosomes is inactivated in a cell is pure chance, a flip of the coin. Hence one cell might use the X chromosome inherited from Dad, while the cell next door uses the X chromosome inherited from Mom.[19] If, however, one X chromosome contains a poorly functioning gene, females have an alternative. Cells with that chromosome can be weeded out and replaced by cells expressing the other X chromosome. The advantage of diploidy is maintained across whole cells, rather than between genes within a cell.

WHEN TEMPERATURE DETERMINES SEX

Although testes and ovaries are much the same across all the vertebrates,[20] the negotiations that lead to whether a gonad is furnished as testes or ovaries differ among the classes of vertebrates. Among reptiles, specifically turtles, crocodiles, and some lizards, gonadal identity is determined by the temperature at which eggs develop, not by chromosomes.[21] The eggs are usually laid in the ground and covered with sand or moist dirt from which they absorb water, swelling in size as they age. Reptile embryos start developing within their egg, and after a while primordial germ cells form. When reptile primordial germ cells move to the genital ridges of their parents, both the germ cells and the parental em-

bryo presumably experience the same environmental temperature. Both germ cells and parent therefore receive the same message about which sex to develop as, and their agendas automatically agree.[22] But could there be a difference of biochemical opinion? Might an intersexed phenotype result if the temperature were changed between the time the primordial germ cells first differentiated and the time the gonad differentiated into a testis or an ovary? Such experiments don't appear to have been tried yet.

Temperature-dependent sex determination is unavailable to warm-blooded animals, such as birds and mammals, who live at only one temperature. Instead, birds and mammals concoct genetic schemes to determine sexual identity. We've already seen the mammalian schemes, which usually involve the X and Y chromosomes. The story is reversed in birds.

ZZ MALES AND ZW FEMALES

In birds and snakes, the sex chromosomes are called Z and W instead of X and Y. Males have ZZ chromosomes, and females are ZW, the opposite of mammals, where females have two identical sex chromosomes and males two different ones.

In birds, something on the W chromosome convinces the gonad to turn into a single ovary on the bird's left side. Something else on W tells the gonadal ridge to start synthesizing estrogen, which makes the gonadal ridge continue along its path of turning into an ovary. In the absence of estrogen, the genital ridge turns "by default" into two testes, one on the right and one on the left.[23]

The avian negotiation is thus the mirror image of that in mammals. In mammals with XY males, the genital ridge heads off to become an ovary unless the noisy SRY speaks up to argue for a testis. In birds, the genital ridge heads off to become testes unless a militant counterpart of SRY on the W chromosome insists on an ovary.

Did chance create birds and mammals with mirror-image schemes for determining gonadal identity? Or are the opposite schemes somehow adaptive? Birds and mammals have fundamentally different social lives. In mammals, females carry and control the young, whereas in birds, both males and females control the young, who reside in a common nest.

Could these differences have worked their way back to the genome, determining how natural selection molds the rules for gonadal sexual identity? I don't know. My conjecture is that birds and mammals differ in whether their social systems require the male or female to develop a gendered presentation first. For mammals, intrasex competition for access to reproductive opportunity may be higher in males than females, and in birds the reverse. If so, the sex experiencing the higher intrasex competition may evolve the accelerated development of a gendered presentation.

THE COSTS OF GENETIC MYTHOLOGY

Development begins and ends with egg and sperm, one big gamete and one little gamete. Although this overall beginning and end point may be the same in many species, we see no standard templates for how female and male development are accomplished. How an animal's sex is decided, whether it will make eggs or sperm, varies among species. The decision is genetic in some species, physiological in others; even when it is genetic, various genetic criteria apply, depending on the species. And the individual's development is no more ordered or predictable than the outcome of a day's parliamentary debate. A diversity of people emerges from a cacophony of developmental histories. No one or two developmental narratives can be privileged as a standard against which to judge the rest.

Why is the bowling-a-strike metaphor, the cascade of successive downstream genetic decisions culminating in birth, so persistent in developmental biology, in spite of so much contrary evidence? I suggest that its persistence stems from a desire to own and control development. If a master gene produces some trait, then anyone who owns the patent for that gene controls the trait. But if traits emerge from a committee of genes, then what's to own? Buy out the whole committee? One can patent a gene, but not a relationship between genes. If the body emerges more from intergene relationships than from the individual genes themselves, then control of development moves beyond human ownership.

The material consequence of trying to own human development is evident in the money lost by biotechnology's efforts to patent the human genome. For the most part, the patent on a gene is worthless, because no

gene works alone. My emphasis on genetic cooperation is not an ode to natural harmony. Rather, I argue that the present emphasis on individualism in science—from the selfish gene to the selfish organism—is empirically misleading, one result being that genetic engineering investors are wasting real money.