3
OF MOLECULES AND SEX

Variety is the Spice of Life

Scholars have written tomes on the subject of variation in animal approaches to sex/reproduction (see, for example, Bagemihl, 1999; Bell, 2008). There are lizards (and lots of insects) that don't use males at all (Crews & Fitzgerald, 1980). Instead females kick-start embryonic development without fertilization, using a process called parthenogenesis (literally "virgin birth"). There are insects that have three sexes,[1] and there are fish that have four different types of sexual beings.

Take, for example, the bluegill sunfish, a common resident of northern freshwater lakes and ponds. In the spring males build shallow, circular nests and circle repeatedly around the nest rim in order to attract a female. When she is enticed, both circle the nest at right angles to one another before resting and each depositing his (sperm) or her (eggs) contribution to the next generation of bluegills. Fertilization is external—females lay eggs in a watery nest and males swim over the eggs, layer sperm on top and hope for the best. In the afterglow, the females swim off while the males stay to aerate the nests with their tails, guard the eggs and fry, and possibly attract other females. Or at least that is the Mother Goose version of the story.

In the grown up version there are three types of males: so-called parental or bourgeois males that mature late in the game (after age 7 years), are relatively large, and have display colors. These parental males build nests, encourage passing females to lay eggs in the nests, fertilize the eggs, and then take care of the resulting offspring. A second type—bluegill sneaker males—develop young. They are the smallest males, and manage to fertilize eggs by darting in when the bourgeois males and females are going at it. All they need do is mix in some of their own sperm and, since there are lots of eggs, they manage to gain some fertilizations. Finally, satellite males mimic females in order to join spawning pairs. When sneaker males grow they can turn into satellite males using a developmental pathway that differs from that of the large bourgeois male (Godwin, 2010; Gross & Charnov, 1980).

While some fish species have several types of males, others can change completely from male to female or vice versa. In itself, this fact is astonishing, but even more amazingly, the fishes' social context controls the transformation. Consider the brightly colored reef fish called a cleaner wrasse. These smaller fish hang out in designated spots on coral reefs forming little cleaning stations. Larger fish stop by and the wrasses clean off and eat the parasites hanging onto the big fish. A commensal time is had by all. The wrasse groups consist of one or a small number of males with a dominance hierarchy and a large group of females of different sizes. If the dominant male dies or a mad scientist removes it from the group, the largest female senses the change in context and in a matter of days it transforms into a reproductively active male. This fishy ability to change sex extends to 7 taxonomic families, 27 orders, and many more species (Godwin, 2010).

Although scientists have studied socially determined sex change in fish since the early 1970s, there is still a lot we don't know about how it works. We *do* know that brain signals influence the hypothalamus and the pituitary gland which in turn notify the gonads that they should shift gears. When female wrasses sense the loss of the male their ovaries stop functioning, and as the future eggs degenerate, the gonad begins making testosterone and other hormones needed to produce a testis, and ultimately sperm. But what we don't know is pretty essential: how

do events in the social sphere become events taking place in an individual body? Are the social clues that reorient the nervous system visual, auditory, mechanical, or something we haven't yet conceptualized? These questions can certainly be investigated experimentally, and answers will provide clues as to how information crosses that border from the outside to the inside of an organism.

Social interactions determine sex in certain fish; O.K. that probably seems strange. But in many reptiles it is the temperature at which fertilized eggs incubate that selects for either male or female development. Just as surprising is how this story differs from one species to the next. For example, grow the eggs of a red-eared slider turtle at 26°C and all the resultant hatchlings are male; grow the eggs at 31°C and they all come out female. In order to obtain an all female clutch of American alligators, (very carefully) obtain eggs and grow them either at 30° or at 35°C. Or—still being very careful—grab a handful and grow them at 32.5° to 33°C and voilà—you will get all males. Finally, consider the leopard gecko. For these lizards, males develop at the higher temperatures (31–33°C) and females at the lower temperatures (23–28°C).

The "dazzling array" (Shoemaker & Crews, 2009: 294) of approaches to sexual reproduction found in vertebrates is called *primary sex determination*. Many vertebrates—including humans—use chromosomal (sometimes called genotypic) sex determination: a heritable genetic element attached to a chromosome usually directs development down one of two pathways. In humans and many mammals this special element is a gene found on the Y chromosome and it pushes the embryo to develop in a male direction. Somewhat mistakenly, as we shall shortly see, the mammalian Y chromosome is often said to be the sex determining chromosome, as if it determined *both* maleness and femaleness. More accurately, we can say that in most mammals the Y chromosome directs male development. In contrast, in birds, which also use a chromosomal rather than environmental mode of primary sex determination, females are the ones with the "different" chromosome, and female birds are said to be ZW while males are ZZ. Here too there is a male sex determining factor—this time on the Z chromosome. But flipping the

switch to activate male development requires two doses of the Z factor thus ensuring that ZZ embryos become males and ZW embryos become females (Gilbert, 2010).

So far then, we have learned that a change in the social structure of a group of fishes or incubation temperature in reptiles can induce a sex change or produce extreme skewing of the ratio of males to females (the sex ratio). The fact that in these organisms the signal to differentiate one sex or the other does not emanate primarily from sex chromosomes does not contradict the idea that chromosomal make-up determines sex. Here the signals are physiological and to the extent that chromosomes are involved, signals might come from any chromosome, not just ones that carry specific genes for sexual development. But the environment of the mother or father can also sometimes change the ratio of male to female births in animals (e.g. birds and mammals) with chromosomal sex determination. Remember that both parents produce gametes (either a sperm or an egg) which carry autosomes plus a single sex chromosome. When sperm and egg fuse, two chromosomes (either both the same, or one of each different one) combine and the future is predictable. In theory the chances of producing either a male or a female is 50–50. In practice, "stuff happens."

If the truth be told, the number of males vs the number of females often deviates from 1:1. In rodents, some of the events leading to such deviations have been studied, but there is still a great deal of uncertainty about whether the results apply to humans (Cramer & Lumey, 2010; Kiely, Xu, McGeehin, Jackson, & Sinks, 1999; Rosenfeld & Roberts, 2004). For example, mice raised on rich diets produce litters with more males than females; fed on a poor diet, the sex ratio drops dramatically, with litters so skewed that there were three female pups for every one male born (Rivers & Crawford, 1974). In large mammals dominant females produce more males, and general stress before or during pregnancy leads to a decrease in sons (Rosenfeld & Roberts, 2004). Diet, dominance, and stress may all have similar downstream physiological effects on conception and pregnancy, so what may be at stake is a network that rebalances itself depending on environmental input thus skewing the sex ratio in one direction or the other.

While we do not know the specific mechanisms by which a mother produces more daughters or fewer sons, there are a number of logical possibilities:

1. post ejaculation the mother's physiological state could affect the motility and transport of X-bearing vs Y-bearing sperm, as they make their way towards the unfertilized egg. Or, there may be nutritional effects in the male that harm Y-bearing sperm development before ejaculation;
2. oocyte development might vary depending on the mother's physiological state, leading to the ovulation of an egg that fuses more easily with an X-bearing than a Y-bearing sperm;
3. an equal number of XX and XY embryos may start out in the uterus, but one type may grow better than the other. In the case of a low ratio of males to females, perhaps more of the XY embryos die very early in development.

No doubt there are other possible variations on the above themes. We are not close to understanding the mechanisms leading to a skewed sex ratio in animals with chromosomal sex determination. A couple of decades of dedicated research in this area would certainly bring greater clarity.

From Chromosomal Sex to Fetal Gonadal Sex

Words matter. The phrase "sex determination" suggests that one is talking about both male development *and* female development. But often in the scientific literature the term presages a discussion of male development only. For example, in many research papers the genetic factor on the mammalian Y chromosome is called the "sex determining factor" rather than the "male determining factor." When this elision occurs, the writer may say something to the effect that female development happens in the absence of a male-determining factor or may fail altogether to mention female development. Femaleness then becomes an absence, something that happens by default, something that does not merit the same level of scientific investigation as the more active male process.

There is a history here. Aristotle wrote "The female is a female by virtue of a lack of certain qualities" (Fausto-Sterling, 2000: 347). Much more recently, descriptions of the Oedipal drama (à la Freud) consider how the female psyche must accommodate to the absence of a penis, while the male must adjust to the fear of its loss and a return to some basal, default, female state. Given our rich past of conceptualizing the female as a lack or absence, it is probably more than an accident, although less than a conspiracy, that, when writing about sex determination, scientists slip without noticing into linguistic muddiness.

The cumulative result of this slippage has been a comparative lack of research on female development (Fausto-Sterling, 2000). Knowledge of ovarian development lags behind our understanding of the testis, a situation one group of scientists has called "amazing." Indeed, as have feminist critics since the 1980s (Fausto-Sterling, 1989), today's researchers acknowledge that one reason we still know comparatively little about the ovary is that "the prevailing view that ovarian development is the 'default' state [has] commonly led to an incorrect assumption that no active genetic steps need to be taken to specify or create an ovary," a view that is logically untenable given what we know about how genes act during development (Wilhelm, Palmer, & Koopman, 2007: 20). Slowly, but still very unevenly, scientists have begun to remedy this imbalance in knowledge about male vs female development and at the same time show that development and maintenance of the ovary does not rely on a passive molecular pathway (Veitia, 2010). A great example can be found in the work of Shoemaker and Crews (2009). Figure 3.1 is a simplified version of a diagram found on page 296 of their article.

Christina Shoemaker and David Crews, biologists at the University of Texas, Austin, divide vertebrate gonad development into two phases (Shoemaker & Crews, 2009). Initially, construction proceeds identically in chromosomally male and female embryos; the result is a pre-gonadal structure sometimes referred to as "equipotential" because it can develop in either a male or a female direction. In the older literature this undifferentiated gonad is called the "indifferent gonad."

In Figure 3.1 this point in the pathway comprises the base of the Y. A number of genetic factors act during this period, presumably as part

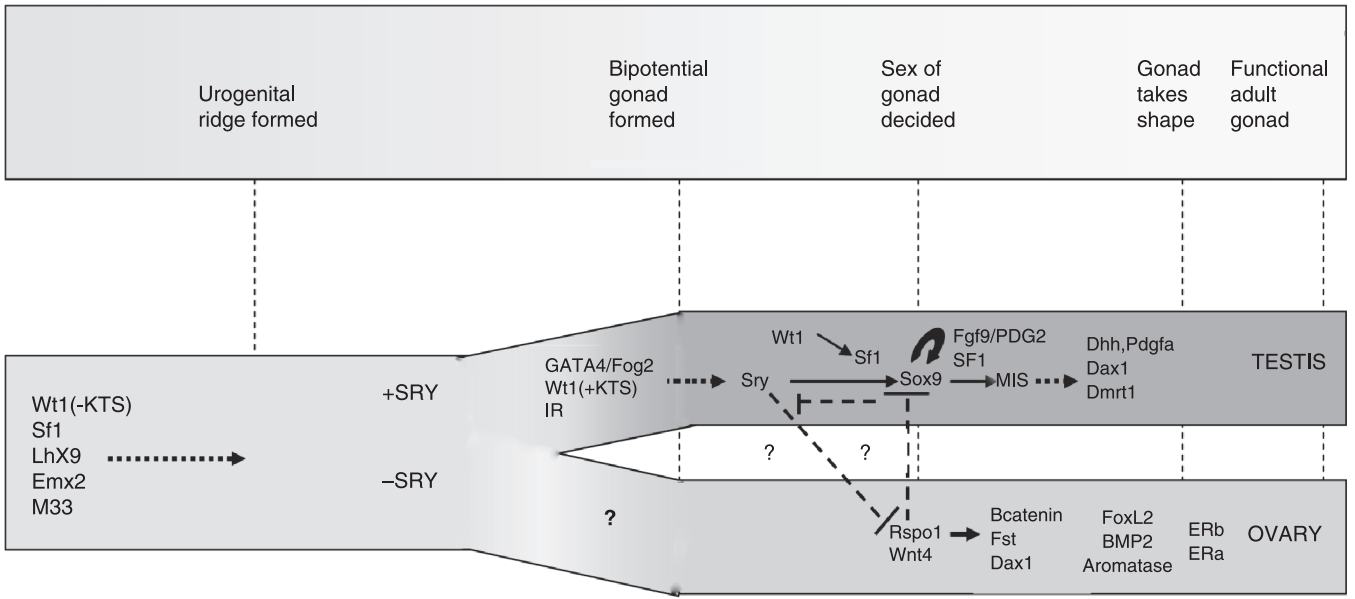

Figure 3.1 Mammalian gonad formation: tissue development and underlying genetic activities.

Note: Genetic events that underlie development of the gonad in mammals. Genes known to act at specific developmental time points are listed. Solid lines indicate direct regulation of events. Dashed lines represent indirect or not yet completely defined relationships. The initials Wt1, SRY, GATA4, Fog2, KTS, IR, Sf1, Fgf9, PDG2, Mis, Sox9, Dhh, Pdgfa, Dax1, Dmrt1, Rspo1, Wnt4, Bcatenin, Fst, FoxL2, BMP2, ERb, ERa, each represent specific genes acting at the indicated location. Some of these are further explained in the text.

For a color version of this figure please go to www.routledge.com/cw/fausto-sterling

of equipotential gonad formation. And these factors act both in chromosomal male and chromosomal female embryos. (For linguistic simplicity I am writing as if everything always proceeds in the described manner. But of course any number of unexpected developments, such as a failure of one or more genes to act, or a genetic variant of some sort that acts in an unexpected manner, can alter the most frequent developmental pathways.)

Once the indifferent gonadal tissue forms, the gonad begins to develop in either a male or a female direction. For years scientists searched for the master gene that controls male development (sometimes erroneously called the master gene for sex determination). In mammals, the gene finally identified after a number of false starts is a male-determining factor on the Y chromosome called Sry (**S**ex **R**eversal on the **Y** chromosome). The product of the Sry gene binds to a control segment of a gene called Sox9 (**S**ry-related HMG b**ox**), which is located on the long arm of chromosome 17 (an autosome). Male development requires the actions of both Sry and Sox9 in the correct sequence and in the absence of either gene, potential males develop instead as females—with one exception: these women have no ovaries (Harley, Clarkson, & Argentaro, 2003). This points to the fact that a critical aspect of female sex determination—the steps that actively lead to ovary formation—is still poorly understood. As indicated in the diagram, several other genes participate in a reticulated pattern of gene activity directed at the formation of a normal testis. This is the process that sends *fetal gonadal sex* in a male direction. And it turns out the process runs more like a parliament than an autocratic director. It is time for new metaphors.

The indifferent gonad develops in a female direction under the influence of two genes (with, I suspect, more still to be discovered, since research here has lagged behind[2]). Both the gene **F**orkhead b**ox** protein **L2** (FoxL2) and Wnt4 (**W**i**n**gless **t**ype MMTV integration site family) control the activities of other genes in the ovarian differentiation gene network. FoxL2 resides on chromosome 3 and Wnt4 is on the 1st autosome. In mice missing FoxL2 and Wnt4 chromosomal females develop into males. In 2006 a new gene called **R-spo**ndin**1** (Rspo1 found

on autosome 1) emerged as a key player in female development. Both XX mice and XX humans lacking R-spondin1 activity develop testes and internal and external male genitalia even though they lack *Sry*, the so-called "master gene for male development" (Parma, Radi, Vidal, Chaboissier, Dellambra, Valentini, et al., 2006; Tomizuka, Horikoshi, Kitada, Sugawara, Iba, Kojima, et al., 2008).

Discovering the role of R-spondin1 in ovary development was a breakthrough in understanding the relationship between the developing male and female gonads. One current hypothesis is that Rspo1 and Wnt4 join forces to inhibit Sox9 activity and thus all of the testis differentiation factors that work downstream of Sox9. It now seems possible that Sry activity inhibits Rspo1, thus stopping female development. Cell biologists Leo DiNapoli and Blanche Capel note how the discovery of the role of R-spondin in ovary determination has changed how we frame our understanding of sex determination. Moving from the older representation of male development as active and female development as passive these scientists write, it seems that "the bipotential gonad is the battleground between two active and opposing signaling pathways . . ." (DiNapoli & Capel, 2008: 4).

So, with the help of these networks of clever molecules that signal cells to develop one way or the other, by the 12th week of human development human embryos have either actively developing testes or actively developing ovaries. *Fetal gonadal sex* (a.k.a. primary sex determination) is good to go. So good, in fact, that (at least the testes) take on developmental responsibilities by producing fetal gonadal sex hormones. *Fetal hormonal sex*, in turn, plays a critical role in the development of secondary sex determination—the differentiation of male or female internal organs and, in due time, the differentiation of the external genitalia.

From Fetal Hormonal Sex to Genital Sex

The theme of indifference, or bipotentiality, courses through the story of sexual development. Not only does the gonad itself begin as a bipotential structure, so too do the accessory ducts, needed to transport sperm or eggs, to sustain fetal development, and, generally, to carry out the nitty gritty of sexual reproduction. Hormones produced by the

developing testes or ovaries select which set of ducts survive early development, and influence their proper differentiation.

In both XX and XY embryos, the bipotential gonad develops cheek by jowl with a structure called the mesonephros (literally, the middle kidney). In early mammalian development, the mesonephros functions as an embryonic kidney, but as development proceeds the emergence of the more familiar, bean-shaped kidneys (the metanephros) found at (and before) birth supplants the mesonephros' waste-elimination role. The middle kidneys connect via long ducts to a temporary embryonic structure called the cloaca.[3] While each XX and XY embryo has a pair of mesonephric kidneys and ducts to match the pair of gonads, the cloaca is a single centrally located structure (see Figure 3.2). But (as the overexcited TV ad man would yell) wait! There's more! Each XX and XY embryo, at this bipotential stage, sports another set of ducts that sit parallel to the mesonephric ducts, and like them, connect centrally to the cloaca. Because these lie right next to the mesonephric ducts, anatomists call them the paramesonephric ducts (also called the Müllerian ducts, after the anatomist who first found them).

From Fetal Gonadal Sex to Fetal Hormonal Sex and Thence (at last) to Genital Sex

Once the fetal gonads start to function, the double set of ducts begin to change. In humans, by about eight weeks, the fetal testis produces two critical hormones. The first, Anti-Müllerian Factor (AMF), eliminates the female developmental option by causing the paramesonephric ducts to degenerate. The second, fetal testosterone, repurposes the mesonephric ducts, influencing them to develop into the vas deferens, epididymis, and seminal vesicle. This early expression of *fetal hormonal sex* thus inhibits female development and encourages male differentiation. In XX embryos the fetal ovaries start to differentiate between eight and twelve weeks. Estrogen produced first by the mother and then by the fetal ovaries encourages the paramesonephric ducts to develop into the oviducts (Fallopian tubes), and where the tubes fuse along the anatomical midline, the uterus, cervix, and upper portion of the vagina form. As this female potential develops under the influence of *fetal hormonal sex*,

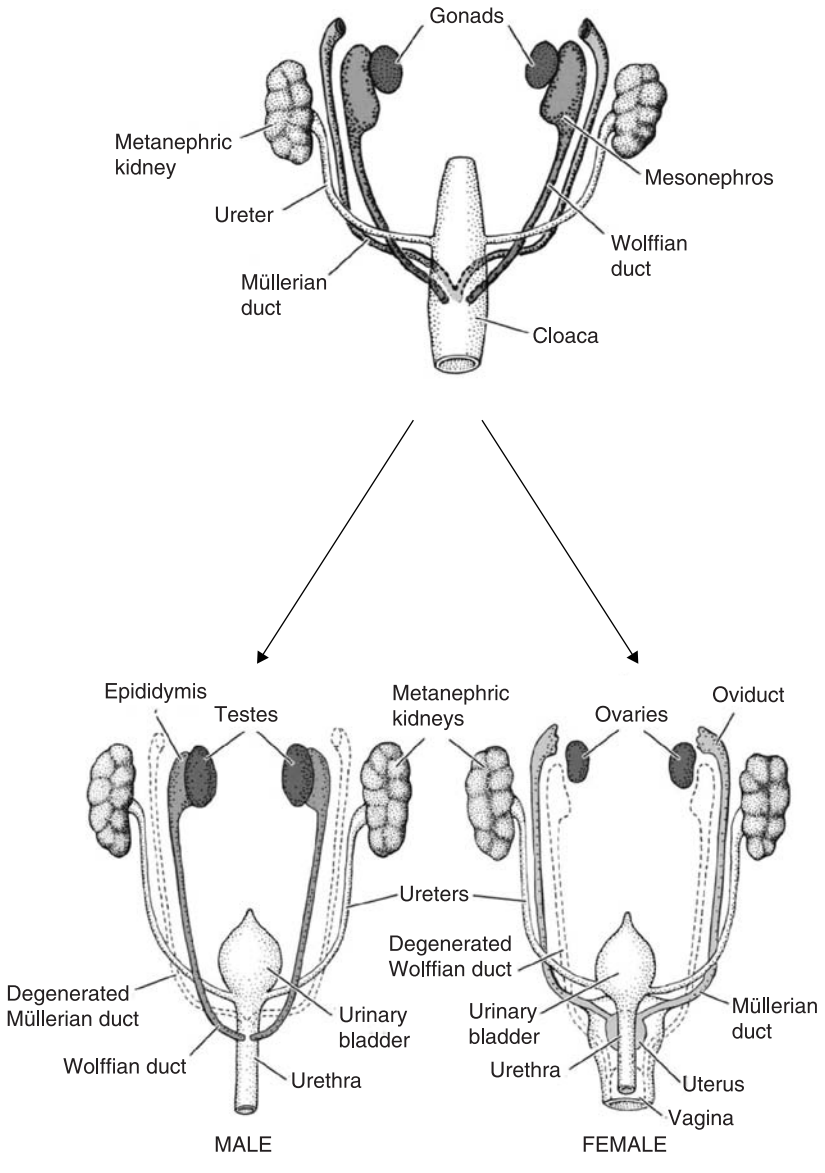

Figure 3.2 Development of male and female internal genital ducts.

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the mesonephric ducts degenerate, possibly due to the absence of testosterone.

As the embryo establishes *internal reproductive sex* (all the aforementioned ducts and tubes), it starts, in an overlapping time dimension, to build *genital sex*. Here too, fetal hormonal sex plays a critical role. And here too, the fetus starts out indifferent, or bipotential, with regard to the external bits—penis, clitoris, scrotum, and vaginal folds (see Figure 3.3). In the moment of indifference both XX and XY infants have the identical phallus. The phallus, however, responds to fetal hormonal sex. Under the influence of androgens such as testosterone or dihydrotestosterone, it grows, differentiating into a penis. Under the influence of estrogen the phallus becomes a clitoris.

In analogous fashion, the labioscrotal swellings, identical at the bipotential stage, under the influence of androgen, fuse along the central midline to become the scrotum or they remain open to become the outer lips of the vagina. The urogenital folds either (when influenced by androgens) fuse along the midline, enclosing the urethra and becoming the shaft of the penis or remain open and become the inner lips of the vagina.[4] It should be noted that knowledge about the molecular details of external genital differentiation, including the precise role(s) of estrogen, remains underdeveloped. Here again, we know less about female than male development, and here as well, if researchers choose to investigate the details more thoroughly we will learn a great deal more than we currently understand. Nonetheless, at the end of this period during which external genital sex bifurcates from a bipotential anatomy to either male or female, we can say that the fetus has developed *genital sex*.

With all this bipotentiality going around, the fog surrounding the birth of infants with mixed sex may have begun to lift. All that needs to happen is that something out of the ordinary switches or derails the process of sexual development at one of the levels from chromosomal to genital sex. For example, rarely an XY child is conceived who carries a genetic mutation that prevents the body's cells from "seeing" or binding testosterone. Even though the fetal gonad produces androgens, the cells cannot capture the androgen molecules and thus cannot use them to move development in a male direction. Such androgen insensitive XY

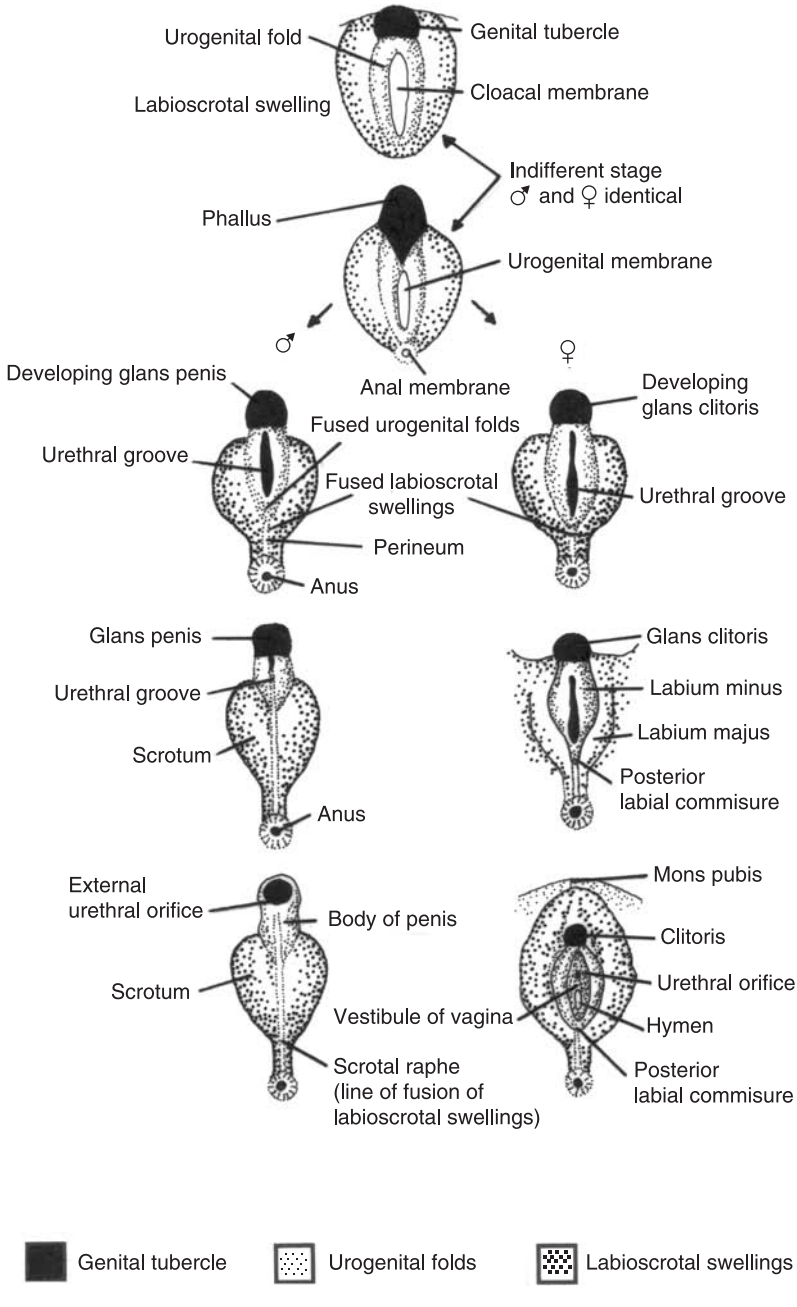

Figure 3.3 The development of the external genitalia from the indifferent (fetal) stage to full formation at birth.

Table 3.1 Some common types of intersexuality

Name	*Cause*	*Basic clinical features*
Congenital Adrenal Hyperplasia (CAH)	Genetically inherited malfunction of one or more of six enzymes involved in making steroid hormones	In XX children, can cause mild to severe masculinization of genitalia at birth or later; if untreated, can cause masculinization at puberty and early puberty. Some forms drastically disrupt salt metabolism and are life-threatening if not treated with cortisone.
Androgen Insensitivity Syndrome (AIS)	Genetically inherited change in the cell surface receptor for testosterone	XY children born with highly feminized genitalia. The body is "blind" to the presence of testosterone, since cells cannot capture it and use it to move development in a male direction. At puberty these children develop breasts and a feminine body shape.
Gonadal Dysgenesis	Various causes, not all genetic; a catch-all category	Refers to individuals (mostly XY) whose gonads do not develop properly. Clinical features are heterogeneous.
Hypospadias	Various causes, including alterations in testosterone metabolism	The urethra does not run to the tip of the penis. In mild forms, the opening is just shy of the tip; in moderate forms, it is along the shaft; and in severe forms, it may open at the base of the penis.
Turner Syndrome	Females lacking a second X chromosome (XO)	A form of gonadal dysgenesis in females. Ovaries do not develop; stature is short; lack of secondary sex characteristics; treatment includes estrogen and growth hormone.
Klinefelter Syndrome	Males with an extra X chromosome (XXY)	A form of gonadal dysgenesis causing infertility; after puberty there is often breast enlargement; treatments include testosterone therapy.

babies are born with highly feminized genitalia and are often identified as girls at birth, even though they are chromosomally and gonadally male. At puberty, still unable to respond to testosterone, they develop breasts and a feminine body shape by responding to the estrogen made by their testes. There are many other examples of intersexual development (now also called Disorders of Sexual Development, or DSDs), some of which I have listed in Table 3.1.

Finally, then, we have layered the more obvious bits and pieces of sex into a proper order of development and into their proper place. But we have yet to consider behavior and the brain—that astonishing organ

that underpins our fears, our desires, our interests in particular types of partners, our emotions, our styles of courtship, etcetera, etcetera, etcetera. Does sex reach into the heart of our brains? Do brains have a sex? Read on.

Further Reading

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