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HOMOSEXUALITY AS A CONSEQUENCE OF EPIGENETICALLY CANALIZED SEXUAL DEVELOPMENT

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ABSTRACT

Male and female homosexuality have substantial prevalence in humans. Pedigree and twin studies indicate that homosexuality has substantial heritability in both sexes, yet concordance between identical twins is low and molecular studies have failed to find associated DNA markers. This paradoxical pattern calls for an explanation. We use published data on fetal androgen signaling and gene regulation via nongenetic changes in DNA packaging (epigenetics) to develop a new model for homosexuality. It is well established that fetal androgen signaling strongly influences sexual development. We show that an unappreciated feature of this process is reduced androgen sensitivity in XX fetuses and enhanced sensitivity in XY fetuses, and that this difference is most feasibly caused by numerous sex-specific epigenetic modifications ("epi-marks") originating in embryonic stem cells. These epi-marks buffer XX fetuses from masculinization due to excess fetal androgen exposure and similarly buffer XY fetuses from androgen underexposure. Extant data indicates that individual epi-marks influence some but not other sexually dimorphic traits, vary in strength across individuals, and are produced during ontogeny and erased between generations. Those that escape erasure will steer development of the sexual phenotypes they influence in a gonad-discordant direction in opposite

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sex offspring, mosaically feminizing XY offspring and masculinizing XX offspring. Such sex-specific epi-marks are sexually antagonistic (SA-epi-marks) because they canalize sexual development in the parent that produced them, but contribute to gonad-trait discordances in opposite-sex offspring when unerased. In this model, homosexuality occurs when stronger-than-average SA-epi-marks (influencing sexual preference) from an opposite-sex parent escape erasure and are then paired with a weaker-than-average de novo sex-specific epi-marks produced in opposite-sex offspring. Our model predicts that homosexuality is part of a wider phenomenon in which recently evolved androgen-influenced traits commonly display gonad-trait discordances at substantial frequency, and that the molecular feature underlying most homosexuality is not DNA polymorphism(s), but epi-marks that evolved to canalize sexual dimorphic development that sometimes carryover across generations and contribute to gonad-trait discordances in opposite-sex descendants.

INTRODUCTION

THE COMMON occurrence of homosexuality is perplexing from an evolutionary perspective. Simple logic suggests that a fitness-reducing phenotype should be selected against, but homosexuality is nonetheless surprisingly common in human populations—e.g., a prevalence of about 8% in both sexes was reported in a large and systematic sample in Australia (Bailey et al. 2000). Existing genetic models for the evolution of human homosexuality can be separated into two major classes: one based on kin selection (Wilson 1975) and another based on sexually antagonistic alleles and/or overdominance (Camperio-Ciani et al. 2004, 2008; Gavrilets and Rice 2006; Bailey and Zuk 2009; Iemmola and Camperio-Ciani 2009). These models are all based on special cases of selection that directly, or indirectly, maintain genetic variation at loci contributing to the homosexual phenotype. However, despite numerous studies over the last decade searching for polymorphisms associated with homosexuality, no convincing molecular genetic evidence has been found despite the fact that pedigree and twin studies clearly show that homosexuality is familial (reviewed in Ngun et al. 2011). Homosexuality has also been hypothesized to be caused by nongenetic factors such as maternal antibodies against male-specific antigens (reviewed in Bogaert and Skorska 2011). This hypothesis may indeed explain some cases of homosexuality, but cannot account for most cases in men and none in women (Cantor et al. 2002). The poor correspondence between current models and data calls for a new conceptual framework to understand the evolution of homosexuality.

Here we integrate theory from evolutionary genetics with recent developments in the regulation of gene expression and 50 years of research on androgen-dependent sexual development. We first find that the existing paradigm of mammalian sexual development is incomplete, with the missing component being a system to canalize androgen signaling during fetal development such that the response to circulating testosterone is boosted in XY fetuses and blunted in XX fetuses. We integrate these data with recent findings from the epigenetic control of gene expression, especially in embryonic stem cells, to develop and empirically support a mathematical model of epigenetic-based canalization of sexual development. The model predicts the evolution of homosexuality in both sexes when canalizing epi-marks carryover across generations with nonzero probability.

We will use the term epi-marks to denote changes in chromatin structure that influence the transcription rate of genes (coding and noncoding, such as miRNAs), including nucleosome repositioning, DNA methylation, and/or modification of histone tails, but not including changes in DNA sequence. It is now well established that a parent's epi-marks sometimes carryover across generations and influence the phenotypes of offspring (reviewed in Morgan and Whitelaw 2008). Epigenetics is a relatively new subdiscipline in genetics and its importance in evolution, especially as a major contributor to realized heritability, is currently being developed and debated (e.g., Slatkin 2009; Furrow et al. 2011). Nonetheless, there

is now clear evidence that environmentally induced epigenetic modifications of genes expressed in male mice (e.g., DNA methylation; Franklin et al. 2010) that feminize their brains and behavior can be transgenerationally inherited by their offspring (Morgan and Bale 2011). Our study examines the ramifications of transgenerational epigenetic inheritance to the phenomenon of human homosexuality.

The first half of our analysis is general and applied to all sexually dimorphic traits in mammals that are strongly influenced by fetal/neonatal androgen exposure. The second half focuses on homosexuality and its similarity with other common gonad-trait discordances that have important medical significance. By homosexuality we mean any same-sex partner preference, spanning all Kinsey scores >0 (e.g., including bisexuality). Our model of homosexuality may also apply to transsexualism, but we do not develop this application here.

Classical View: Sex Hormone Differences Fully Determine Sexual Dimorphism

Beginning with Phoenix et al. (1959), a long succession of studies have consistently and unambiguously demonstrated that sexual dimorphisms of the genitalia and brain of mammals are strongly influenced by androgen exposure during fetal development. The foundation for this conclusion is that XY fetuses experimentally exposed to androgen antagonists during gestation develop feminized genitalia, brains, and behavior, whereas XX fetuses exposed to elevated androgens develop masculinized phenotypes for these same traits. Studies of untreated fetuses demonstrated that circulating androgen levels differ between XX and XY genotypes, with significantly higher average androgen levels in XY fetuses at a time at or before the genitalia, brain, and behavior become sexually dimorphic.

The logic of the "prenatal androgen paradigm" (also known as the Jost paradigm) begins with the observation that only XY embryos express the Y-linked gene SRY (Figure 1A). This gene product induces development of the testes in XY embryos, which in turn produce androgens that influence later sexual development. The absence of elevated circulating androgens during fetal development leads to the female phenotype. Although many aspects of sexual dimorphism are a response to the "organizational" effects of sex-specific differences in circulating androgens during fetal and neonatal development, full manifestation of sexual dimorphism sometimes depends on the "activational" effects of androgens and estrogens at and after puberty. In humans, the fact that XY individuals (with fully formed testicles and normal levels of circulating androgens) that are homozygous for a null allele at the androgen receptor locus (and therefore cannot respond to circulating androgens) develop fully female-typical genitalia and reproductive behavior (reviewed in Wisniewski et al. 2008) provides strong support for the prenatal androgen paradigm.

Sex Hormone Differences are not Sufficient to Produce Sexual Dimorphism

Although prenatal androgen levels play a fundamental role in sexual development, there is also evidence that the prenatal androgen paradigm is at least partially incomplete (reviewed in Davies and Wilkinson 2006). Studies in the mouse "four core" model system (in which a the male-determining Sry gene has been translocated to an autosome, enabling gender and sex chromosome karyotype to be experimentally manipulated independently) clearly demonstrate that some aspects of sexually dimorphic behavior and brain anatomy are strongly influenced by the sex chromosome karyotype rather than the level of fetal androgen exposure alone (reviewed in Arnold and Chen 2009). These studies are, however, consistent with the conclusion that androgen signaling is the predominant factor controlling sexual dimorphism in this model system.

Here we provide evidence that the prenatal androgen paradigm is missing a major component. This conclusion is based on our reanalysis of studies of circulating prenatal androgens in human and rat fetuses. In humans, the testes begin to secrete testosterone (T) in XY male fetuses beginning around the eighth week of gestation (Wilson et al. 1981).

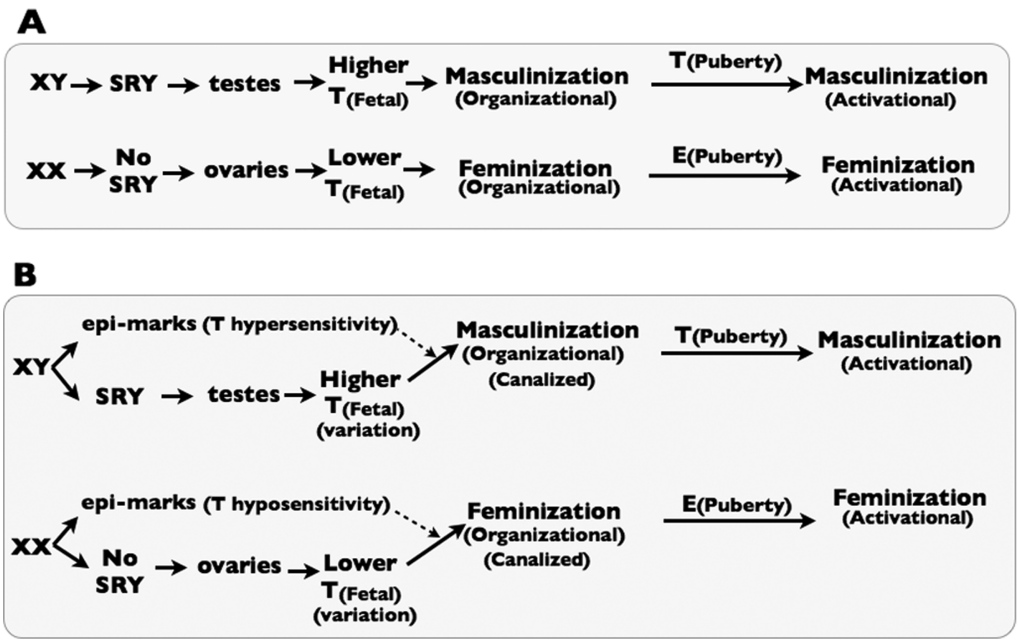

FIGURE 1. THE SEXUAL DIMORPHISM SIGNALING PATHWAY

The classical view of sexually dimorphic development (A) is that higher androgen levels in XY fetuses and adults masculinize sexually dimorphic traits and lower androgen levels in XX fetuses and high estrogen in adults feminizes development. Our analysis (B) indicates that androgen signaling includes an additional component: it is canalized by epi-marks that are produced during the embryonic stem cell stage of development.

However, T is also present in XX female fetuses in substantial amounts, originating from the fetal adrenals and from placental/maternal sources. Secretion of T by the testes increases its concentration in the blood of XY fetuses. The maximum average difference in T concentration between male (XY) and female (XX) fetuses occurs between weeks 11–17 (Reyes et al. 1974). After this time, fetal secretion of T by the testes declines markedly, causing average T values in males to become indistinguishable from levels in females (Reyes et al. 1974). Although male fetuses have higher average T than females starting around week 11, overlap in T levels between the sexes (i.e., some XX fetuses having higher T than some XY fetuses) was observed at all times except between weeks 15–19 (Reyes et al. 1974). This transient lack of overlap (and hence an unambiguous signal of fetal gender) may be genuine or an artifact due to small sample size. The latter explanation is supported by a much larger study (166 female and 185 male fetuses) of amniotic fluid collected between weeks 15–19 (T diffuses from the fetal circulation into the amniotic fluid via the skin at this stage of development), in which there was about 5% overlap (i.e., 5% of XX fetuses had higher T than some XY fetuses) in T concentration between male and female fetuses (Perera et al. 1987). In a large study of rats, significantly higher circulating T in male compared to female fetuses occurred only between days 17–21 of gestation (Weisz and Ward 1980). Despite this window of significantly elevated T in male fetuses, T levels overlapped between the sexes throughout all time points during gestation (Weisz and Ward 1980). Collectively, these studies indicate that the level of circulating T alone is not an unambiguous indicator of gonadal sex at any time during fetal development because T levels overlap between the sexes at nontrivial frequencies at all developmental time points.

Overlap in T concentrations between the sexes (despite highly significant differences in average T between XX and XY fetuses) would make the prenatal androgen paradigm incomplete (i.e., missing an important component of androgen-induced sexual dimorphism) unless discordance between the gonad and sex-specific traits is observed to be correspondingly common. This is, however, not the case. To illustrate this point, we can focus on the ontogeny of the genitalia. The human male phallus and female vulva are formed during weeks 9–15 of gestation, although the phallus requires T to continue to grow during later fetal development (summarized in Wilson et al. 1981). During this time of genital differentiation, data on fetal T collected by Reyes et al. (1974) show high overlap in T concentrations between the sexes. The same relationship is found in the rat, in which T concentrations strongly overlap between the sexes during the time (and all points previous) when the phallus and vulva are differentiating (Weisz and Ward 1980). Yet discordance between the gonad and the genitalia (including ambiguous genitalia) is rare in both humans (Sax 2002) and rats (Ostby et al. 1999; Hotchkiss et al. 2007). Therefore, the available data do not fully support the prenatal androgen paradigm because there is too much overlap in circulating androgens to be consistent with the observed low discordance between the gonad and the genitalia observed in both humans and the rat model system.

Differential Sensitivity of XY and XX Fetuses to Androgens

One can fully rescue the prenatal androgen paradigm if XY fetuses have higher sensitivity to circulating androgens compared to XX fetuses. In this case, XX and XY fetuses would respond differently even when T levels overlap to a limited degree between the sexes. Many lines of evidence indicate that this is the case.

First, in humans the expression of the 5-α-reductase-2 gene, which converts T into the more potent androgen dihydrotestosterone (DHT), is three times higher in XY fetuses than XX fetuses within the urogenital swellings and tubercles (structures that develop into the phallus or vulva; Wilson et al. 1993). Boehmer et al. (2001) review evidence that strongly supports the conclusion that this sex-specific difference in gene expression is not androgen-induced via a feed-forward process (i.e., due to changes induced by higher T in XY fetuses during earlier development). Higher conversion of T to DHT would permit XY fetuses to develop male traits even when T levels overlap (to a limited degree) with XX female fetuses, thereby promoting phallus development despite low circulating T. Similarly, lower 5-α-reductase production in XX females would prevent or reduce masculinization of the vulva when T levels overlapped (to a limited degree) with those of XY males.

Second, sex hormone binding globulin (SHBG) binds circulating T and makes it unavailable for uptake by cells. In human fetuses in which T levels overlap between genetic males and females, SHBG is markedly higher (approximately 50%) in female fetuses compared to male fetuses (Hammond et al. 1983). This elevated SHBG in XX fetuses would reduce sensitivity to circulating T when it overlaps with XY fetuses.

Third, in rhesus monkeys (but not humans), levels of circulating progesterone are markedly higher (three times) in female fetuses compared to male fetuses (Hagemenas and Kittinger 1972). Progesterone acts as an anti-androgen because it has a high binding affinity for the androgen receptor (AR), which it inactivates. Its higher concentration in female fetuses is expected to lower their sensitivity to androgen levels that overlap with males.

Fourth, human XX female fetuses homozygous for loss of function alleles at the CYP21 locus cannot produce the steroid hormone cortisol due to a block in its synthetic pathway (a form of Congenital Adrenal Hyperplasia, CAH). Buildup of intermediate products leads to their conversion to T, and consequently highly elevated circulating T in affected XX CAH fetuses. This elevated level of T begins in the seventh week of gestation (Speiser and White 2003; Trakakis et al. 2009) and "the developing fetus is exposed to the excessive adrenal androgens, equivalent to the male fetal level, secreted by the hyperplastic adrenal cortex" (New 2004),

including the period of maximal average T excess in XY fetuses (Forest 1985). Despite a male-typical level of T throughout fetal development, the genitalia of XX newborns with CAH are usually only partially masculinized—about halfway between a typical male and female genital (Hall et al. 2004), as is childhood sexually dimorphic behavior (Hines 2011). Although rates of homosexuality and transsexuality are elevated in CAH patients, the vast majority have female-typical sexual behavior (reviewed in Hines 2011). These data provide strong evidence that androgen-induced masculinization is blunted in the XX fetuses.

Fifth, human XY male fetuses with acute 17β-HSD-3 deficiency are homozygous for loss-of-function alleles at the 17β-HSD-3 locus and cannot produce T in the testes due to a block in its synthetic pathway (reviewed in Rey and Grinspon 2011). Buildup of the precursor androstenedione occurs in affected individuals, but this steroid is a much weaker androgen than T (about a hundredfold lower binding affinity for the androgen receptor; Fang et al. 2003). These males experience highly reduced circulating T throughout fetal development, although T is somewhat elevated later in fetal development due to allozymes expressed outside the testes that convert circulating androstenedione to T. Androgen-induced Wolffian duct structures (epididymis, vas deferens, seminal vesicles, ejaculatory ducts) that are in close proximity to the testes developed normally despite the low level of T they experience during their ontogeny. The more distant genitals of affected individuals, however, are highly feminized and most affected newborns are reared as females. At puberty, these individuals experience a surge in T due to the nontesticular conversion of circulating androstenedione to T (via allozymes of 17β-HSD-3) and about one-half of these individuals, reared as girls, change their sex to male (reviewed in Wisniewski et al. 2008). This is the same rate of sex change as XY individuals with normal levels of T throughout life that were raised as girls because they were born without a penis due to cloacal exstrophy (Reiner and Gearhart 2004). Only a few reports on the sexual orientation of males with acute 17β-HSD-3 deficiency are available, but they suggest a predominance of male heterosexual orientation (Imperato-McGinley et al. 1979; Meyer-Bahlburg 1993). The high level of masculinization (of the Wolffian duct structures, gender identity, and sexual orientation) indicate that, despite low fetal levels of T, the XY genotype leads to increased sensitivity of the fetuses to the action of T.

Sixth, as described in the previous section, the low prevalence of gonad/genital discordance in both XX and XY fetuses, despite substantial overlap in concentrations of circulating T when the genitals develop, indicates that the sex chromosome karyotype somehow modulates sensitivity to T prior to the onset of sex-specific androgen signaling.

The examples discussed above strongly support the conclusion that XX and XY fetuses have different sensitivities to circulating androgens. Because most of the genes responsible for this asymmetric response to androgens are autosomal (see next section), they must be transregulated in response to the XX versus XY sex chromosome karyotype. Transregulation can occur in many ways, but recent studies demonstrate that the sex chromosome karyotype alone, independent of sex hormones, epigenetically regulates many autosomal genes (reviewed in Wijchers and Festenstein 2011). Epigenetic modification (i.e., methylation, histone tail modifications, and nucleosome repositioning) is emerging as a pivotal factor controlling gene expression. For example, variation in the level of a single histone modification (trimethylation of lysine residue-4 on histone-3; the H3K4me3 epi-mark) of gene promoters can account for almost 50% of the variation in genome-wide gene expression levels in the early mouse embryo (Mikkelsen et al. 2007). From these studies and others (see below), we conclude that XX- and XY-specific epi-marks almost certainly contribute to the differential sensitivity to androgens of XX and XY fetuses (Figure 1B). The remainder of this article explores the potential for sex-specific epi-marks to contribute to the canalization of sexually dimorphic phenotypes and, as a side effect of pleiotropy and transgenerational inheritance, contribute to the evolution of homosexuality and other gonad-trait discordance

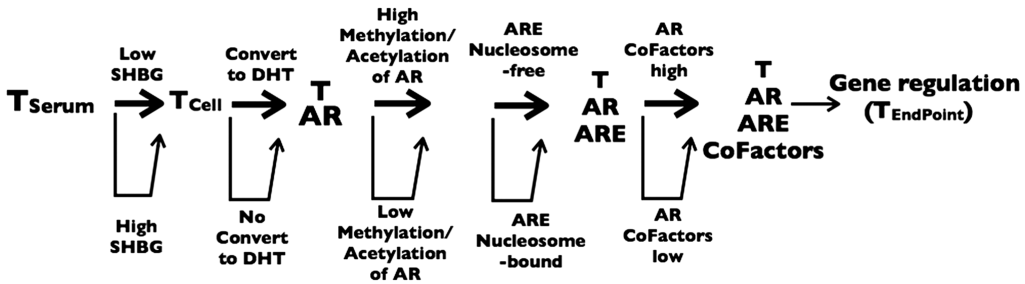

Figure 2. Androgen Signal Transduction

Steps in the androgen signaling pathway that can boost or blunt signal transduction. T = testosterone; AR = androgen receptor; ARE = androgen response element (DNA); CoFacts = androgen receptor cofactors.

such as hypospadias, cryptorchidism, and idiopathic hirsutism.

Mechanisms by which Sex-Specific Epi-Marks can Canalize Androgen Sensitivity

Canalization occurs when a developmental endpoint is reached despite environmental interference that can potentially disrupt it (Waddington 1942). The androgen signaling pathway can be disrupted by natural variation in androgen levels about their mean value as well as environmentally introduced androgen agonists and antagonists. Studies demonstrating high natural variation in fetal T (e.g., Reyes et al. 1974; Weisz and Ward 1980; Perera et al. 1987), as well as the common occurrence of environmental androgen agonists and antagonists (Fang et al. 2003), demonstrate that there is strong selection to canalize the androgen signaling pathway.

We can represent the androgen signaling pathway as flux (i.e., rate of flow) through a series of steps, each capable of being augmented or depressed, ultimately leading to androgen influence on a gene's expression (Figure 2). There is a surprisingly large number of mechanisms by which the androgen signal can be strengthened or weakened. For example, varying the SHBG concentration in the blood, converting T to the more potent DHT, posttranslational modification of the AR (phosphorylation, ubiquitylation, and acetylation) that change its activity, nucleosome placements that influence access to androgen response elements (AREs) in the DNA, and especially the concentration of the numerous and diverse array of AR cofactors. All of the steps in Figure 2 could also be influenced by sex-specific regulation of miRNA levels that are known to influence sexually dimorphism of mRNA concentrations in the brains of mice, and to be influenced by epigenetic control that is heritable across at least one generation (Morgan and Bale 2011). To canalize the impact of natural variation in T, the XY karyotype must lead to one or more epigenetic modifications that boost signal transduction through the pathway, and XX karyotypes must do the reverse.

To illustrate canalization of the androgen signaling pathway, suppose that the average fetal concentration of circulating T = 10 in males and T = 5 in females at a time when a sexually dimorphic trait develops. Further suppose that boosting epi-marks by the XY karyotype convert the actual T in the blood to a doubled endpoint signal ($T_{EndPoint} = 20$) affecting gene expression, and blunting epi-marks in XX fetuses lead to a halving of the endpoint value ($T_{EndPoint} = 2.5$). If natural variation in T causes overlap between the sexes (e.g., T in males varies between 4–16 and T in females between 2–8), then epigenetic modifications by the XX versus XY karyotype would cause the functional $T_{EndPoint}$ values to be nonoverlapping (male T = 8–32 and female T = 1–4).

XY epi-marks that boost androgen signaling, and XX epi-marks that blunt androgen signaling, can also protect against androgen antagonists. For example, rats fed daily on a naturally occurring anti-androgen found in licorice root (that blocks the action of 17β-

HSD in the T synthesis pathway) developed significantly reduced circulating T (Zamansoltani et al. 2009). Epi-marks that boost androgen signaling in XY fetuses (such as higher conversion of T to DHT or lower SHBG concentrations) would protect them from the action of anti-androgens in the same way that they protect them from endogenous variation in T, as described in the above paragraph. In the same way, epi-marks that blunt androgen signaling in XX female fetuses would protect them from environmental agents that elevate the level of circulating androgens.

An androgen mimic (e.g., as found in the Indian medicinal herb *Tinospora cordifolia*; Kapur et al. 2009) can be canalized in a different manner. It is well established that T is modified by the enzyme 5-α-reductase-2 to the more potent DHT in some tissues and this modification is necessary to achieve sufficient androgen signal to induce a male-specific phenotype, e.g., in the external genitals and the internal prostate. Work on the prostate indicates that T and DHT are interchangeable (qualitatively identical) in promoting prostate growth, but DHT is two-and-a-half times more potent (Wright et al. 1996). The conversion of T to the more potent DHT will canalize androgen signaling in the presence of an androgen mimic whenever the 5-α-reductase-2 enzyme does not catalyze the conversion of the androgen mimic to DHT. For example, again suppose that the normal concentration of circulating T = 10 in males and T = 5 in females at a time when a sexually dimorphic trait differentiates. Next suppose that a T-mimic (T_{Mimic}) is present in the fetal blood at a concentration emulating T = 5. In males, the androgen mimic poses no problem with respect to feminization, but in females the mimic would produce $T_{Signal} = T + T_{Mimic} = 5 + 5 = 10$ and a female fetus would be expected to incorrectly develop the male-specific trait. However, if the target tissue converted T to DHT, with a threshold of $T_{EndPoint} = 25$ (i.e., two-and-a-half times average T in males) to induce the male trait, then the female would be "canalized" against the androgen mimic assuming the androgen mimic was not converted to the more potent DHT (because in females $T_{EndPoint} = 2.5 * 5 + 1 * 5 = 17.5 < 25$).

Interestingly, this last mode of canalization may provide an explanation for the enigmatic within-cell conversion of T to estradiol (E) by the enzyme aromatase in the androgen signaling that occurs in the brain of rodents. Our review of many published studies of levels of circulating T and E indicates that, at its peak during the estrus cycle, unbound E is at least tenfold less common than peak unbound T in males (e.g., Bao et al. 2003; Travison et al. 2007). E is therefore a steroid hormone that is at least tenfold more potent than T (i.e., it functions at a concentration tenfold lower). By converting T to E, and assuming aromatase does not convert the androgen mimic to E, canalization will occur by the same logic as the conversion of T to the more potent DHT. A more formal model of canalized androgen signaling is provided in Appendix 1.

Timing of XX/XY-Induced Epi-Marks that Canalize Sexual Development

Epi-marks that boost androgen signaling in XY male fetuses and blunt it in XX female fetuses could, in principle, be produced any time prior to the onset of androgen signaling (when the fetal testes begins to secrete T). However, it is already established that epi-marks that are dimorphic between XX and XY embryos are produced during the nearly genome-wide episode of epigenetic reprogramming that occurs at the embryonic stem cell stage of early development (reviewed in Bermejo-Alvarez et al. 2011). Epi-marks produced during this early embryonic stage are known to strongly influence gene expression later in development (Mikkelsen et al. 2007). In addition, epi-marks produced so early in development would be transmitted to cell lineages leading to both the soma and the germline and would therefore have the potential to be heritable across generations. Below we develop these recent findings in more detail.

During early development, there is a nearly global erasure of epi-marks (DNA methylation and histone tail modification) that originated in the sperm and egg stages. As reviewed in Hemberger et al. (2009), the erasures occur:

when protamines are replaced by histones on the paternal genome prior to fusion of pronuclei; and during the first few cell divisions of embryonic development due to low availability of enzymes that methylate DNA (erasure on both the maternally and paternally inherited DNA). Although the majority of genes lose their gameticly inherited epi-marks at this time, some—such as imprinted genes and active transposons—somehow escape epi-mark erasure (Hemberger et al. 2009). The expansive epigenetic erasure that occurs over the first few cell divisions is immediately followed by nearly genome-wide de novo epi-marking (gene promoters remodeled by histone modification and DNA methylation; reviewed in Hemberger et al. 2009). Genes essential to stem cell functioning (including housekeeping genes) are marked by an activating epi-mark on their promoters (trimethylation of the lysine-4 residue of histone H3, H3K4me3). There is a strong genome-wide correlation (0.67) between the level of this histone modification and the level of gene expression (Mikkelsen et al. 2007). Genes used only in later development are marked by a silencing epi-mark on their promoter's histones (trimethylation of the lysine-27 residue of histone H3, hereafter H3K27me3; Mikkelsen et al. 2007), or via methylation of their promoter's DNA (Fouse et al. 2008). Interestingly, thousands of repressed genes (that are expressed later in development) are bivalently marked with both a repressing (H3K27me3) and an activating (H3K4me3) epi-mark (Mikkelsen et al. 2007).

The nearly genome-wide epi-marking that occurs in early development provides a potentially simple and efficient way for epi-marks that influence androgen signaling to be manifest across all androgen-sensitive tissues. Consider the abundant bivalent epi-marks—containing both a silencing (H3K27me3) and an activating (H3K4me3) epi-mark—discovered by Mikkelsen et al. (2007). The overriding repressive effect of the silencing epi-mark turns off these genes early on in development while the activating epi-marks enable immediate strong gene expression when the silencing part of the epi-mark is removed later in development. By increasing the size of the activating epi-mark (e.g., more CpGs of the promoter histones methylated) in genes that boost androgen signaling (e.g., those whose gene products acetylate the AR) and decreasing the size of these same epi-marks in genes that blunt androgen signaling (e.g., those coding for SHBG and/or its up-regulators), XY fetuses would be protected (canalized) from low circulating T in the fetus. The reverse pattern would protect (canalize) XX fetuses when T was atypically high.

There is clear evidence that XX and XY embryos differ epigenetically at the earliest stages of mammalian development, i.e., in the preimplantation embryo (blastocyst stage). At this time, XY embryos are physiologically distinct from XX embryos, having a higher metabolic rate, faster growth rate, and increased resistance to some stress agents (reviewed in Gardner et al. 2010). Correspondingly, by the preimplantation blastula stage, the two sexes are reported to have widespread differences in gene expression levels at many hundreds of genes, most of which are autosomal (see Bermejo-Alvarez et al. 2010 and reference therein). Regulation of gene expression in complex eukaryotes is usually accomplished via epigenetic modifications (methylation of CpGs on the DNA or modification of histone tails; see Gordon et al. 2011). Recent studies have demonstrated that, at this early embryonic stage, there are also sex-specific differences in DNA methylation on the promoters of specific loci (reviewed in Bermejo-Alvarez et al. 2011). It has also been established that the Y-linked transcription-regulating genes SRY and ZFY are expressed in the preimplantation human embryo (Fiddler et al. 1995).

There is also evidence for XX/XY-induced differences in gene expression later in development, but prior to secretion of T by the fetal testes in XY males. At this time in the mouse model system there is differential expression of 51 genes in the brains of XX versus XY embryos, most of which are autosomal (Dewing et al. 2003). These data indicate that the XX/XY karyotype somehow influences (in trans) the expression of many genes during later embryo development (but before the testes start secreting T) in a manner that is independent of androgen signaling. Such XX/XY karyotype-specific transregulation is known to occur in adult humans (Wijchers

and Festenstein 2011). For example, a gene on the human Y chromosome (TSPY) transregulates the level of expression of the X-linked androgen receptor in the adult germline (Akimoto et al. 2010).

Collectively, these studies indicate that XX and XY embryos are epigenetically differentiated by the stem cell stage of the blastocyst—far in advance of androgen production by the testes. Epi-marks produced at this time are therefore strong candidates for the causative agents underlying the canalization of sexually dimorphic development later in development in response to circulating androgens. The recent finding that environmentally induced epi-marks that reverse sexually dimorphic brain development (i.e., feminize male development) can carryover and produce the same reversal in the following generation (Franklin et al. 2010; Morgan and Bale 2011) demonstrates the potential for epi-marks laid down during very early development to influence androgen signaling later in development.

Heritable Epi-Marks

A consequence of epi-marks being laid down at the stem cell stage of development, before the division between soma and germline, is that such epi-marks have the potential to be transmitted across generations, but only when the cycle of epi-mark erasure and renewal, within and between generations, is somehow circumvented. Studies in both mice and humans clearly demonstrate that transgenerational inheritance of epi-marks occurs at nontrivial rates (reviewed in Morgan and Whitelaw 2008). When the epi-marks are sexually dimorphic, their transgenerational inheritance would be expected to influence the sexual development of opposite-sex offspring, as described in the next section.

A Model for Heritable Sexually Antagonistic Epi-Marks

Any physiological mechanism that protects XX fetuses from atypically high T and/or environmental androgen agonists during development would be favored by natural selection, assuming no counterbalancing harmful side effects. The same logic applies to mechanisms that protect XY fetus from atypically low T and or environmental androgen antagonists. As described above, sex-specific epi-marks (i.e., XX- or XY-specific) laid down during early embryonic development represent one mechanism to achieve such adaptations. However, such epi-marks would be sexually antagonistic if they sometimes carryover to the next generation and redirect development in a gonad-discordant direction. We will refer to these sexually antagonistic epi-marks as SA-epi-marks.

SA-epi-marks can be favored by natural selection. In the autosomal case, an XX- and XY-dependent epi-mark always increase the fitness of the individual in which it is formed, and when there is carryover across generations, it has only a 50% chance of decreasing fitness by being expressed in the opposite sex. The situation is somewhat more complex on the sex chromosomes but, as we show more formally below, sexually antagonistic epi-marks can be favored across the entire genome under feasible selective parameters.

autosomal mutation

We next more formally solve for the parameter space that supports the evolution of mutations that produce SA-epi-marks. Throughout, we assume that the mutation has some expression in the heterozygous state and our selection coefficients apply to heterozygotes. First consider an autosomal mutation that produces an XX- or XY-dependent epi-mark (in *cis*, at its own location) that increases fitness of one sex (say, females) by an increment s, but with probability q it carries over to the next generation and decreases the fitness of the opposite sex (sons) by a decrement σ. Because of the transgenerational effects, we need to consider the number of grandchildren of a mutant. The expected number of copies (w) of a mutant allele in the grandchildren's generation is (see Appendix 2):

$$\begin{aligned} w = {} & \tfrac{1}{2} * 1 * (1/2 * 1 + 1/2 * (1 + s)) \\ & + \tfrac{1}{2} * (1 + s) * [q/2 * (1 - \sigma) \\ & + \{(1 - q)/2\} * 1 \\ & + 1/2 * (1 + s)], \end{aligned}$$

where the first term on the right side represents the number of grandchildren when the mutation originates in a male and the second term when it originates in a female. The allele invades if $w > 1$, which for small s and q is equivalent to

$$s > q * \sigma/4,$$

or

$$\text{cost/benefit} = \sigma/s < 4/q.$$

Even when the rate of transgenerational carryover is 100% ($q = 1$), the mutation invades when the costs are fourfold larger than benefits. When transgenerational carryover is much smaller, there is essentially no constraint on the invasion. The above inequality is compatible with a two-generation generalization of Hamilton's rule: the benefit b goes to the carrier ($r = 1$) and its same sex offspring ($r = 1/2$), while the cost c goes to the opposite-sex offspring ($r = 1/2$).

X-linked mutation

When the mutation is X-linked, it occurs in a female with probability 2/3 and in a male with probability 1/3. First assume that the mutation is dominant and canalizes development toward the female phenotype and is expressed in XX fetuses. The expected number of copies of a mutant allele in the grandchildren's generation is (see Appendix 2):

$$w = 1/3 * 1 * ((1 + s) * 1) + 2/3 * (1 + s) * [q/2 * (1 - \sigma) + (1 - q)/2 * 1 + 1/2 * (1 + s)],$$

where the first term on the right side represents grandchildren when the mutation originates in a male and the second term when it originates in a female. The allele invades if $w > 1$, which for small s and q is equivalent to:

$$s > q * \sigma/4$$

or

$$\text{cost/benefit} = \sigma/s < 4/q.$$

This is the same constraint that was found for autosomal linkage.

Next assume that the X-linked mutation is expressed in XY embryos and canalizes development toward the male phenotype. The expected number of copies of a mutant allele in the grandchildren's generation is (see Appendix 2):

$$w = 1/3 * (1 + s)(q * (1 - \sigma) + (1 - q) * 1) + 2/3 * 1 * (1/2 * (1 + s) + 1/2 * 1),$$

where the first term on the right side represents grandchildren when the mutation originates in a male and the second term when it originates in a female. The allele invades if $w > 1$, which for small s and q is equivalent to:

$$s > q * \sigma/2$$

or

$$\text{cost/benefit} = \sigma/s < 2/q.$$

In this case, the cost/benefit ratio must be half as large as the autosomal case for the mutation to invade. Nonetheless, the mutation can invade under a broad and feasible range of parameter space. For example, if transmission across generations (q) is 0.25, the mutation will invade when costs are eight-times larger than benefits. Our X-linked analysis has assumed dominance of the epi-mark producing mutation. For partial dominance, the selection coefficients s (female canalization) or σ (male canalization) must be multiplied by a dominance scaler h ($0 < h \leq 1$).

X-linked trans-effect

Our model has assumed that an X- or autosome-linked mutation producing an SA-epi-mark makes the epigenetic modification in *cis* at its own location (i.e., it epi-marks itself). When the mutation produces an SA-epi-mark in *trans* anywhere else in the genome, the same equations as described above can be applied by replacing the parameter q with q/2, since the mutation cosegregates with its SA-epi-mark with probability 1/2. When the SA-epi-mark is produced at another locus on the same chromosome, the parameter q must be replaced with $q * (1 - r)$, where r is the recombinational distance between the

mutation and the SA-epi-mark it produces, and $(1 - r)$ is the probability that the mutation and its epi-mark cosegregate.

Y-linked mutation

Lastly, when the mutation is Y-linked and canalizes development toward the male phenotype, the allele invades when $s > 0$ irrespective of the value of σ and irrespective of where the epi-mark is produced.

Generalizations

These calculations demonstrate that mutations causing sexually antagonistic epi-marks can invade even when the cost to the harmed sex far exceeds the benefit to the favored sex. This conclusion holds irrespective of linkage to the sex chromosomes or autosomes. Such invasions are expected to lead to the eventual fixation of mutations producing SA-epi-marks, unless there were some additional factors such as frequency-dependent fitness.

Although our model predicts that mutations causing SA-epi-marks will go to fixation, the androgen-induced phenotypes they affect may nonetheless be highly variable. This is expected because epi-marks can be highly variable despite genetic monomorphism. As described in the next section, monozygotic twins at birth show strong differences in methylation levels of individual promoters and large differences in gene expression levels at as many as hundreds of gene loci. These data indicate that epi-marks are intrinsically variable and that the same fixed mutation can produce variable epi-marks. More extreme epi-marks (e.g., with denser or longer tracts of histone modification and/or DNA methylation) would span more chromatin locations and hence have a higher probability of serendipitously achieving at least partial transgenerational inheritance.

Homosexuality and SA-Epi-Marks

Although pedigree studies indicate a familial association of homosexuality in both males (e.g., Hamer et al. 1993) and females (e.g., Pattatucci and Hamer 1995), more than a decade of molecular genetic studies have produced no consistent evidence for a major gene, or other genetic marker, contributing to male homosexuality (reviewed in Ngun et al. 2011). Moreover, the most recent genome-wide association study using exceptionally high marker density found no significant association between homosexuality in males and any SNPs (Ramagopalan et al. 2010). These negative/inconsistent results may reflect insufficient statistical power, but they also support another agent causing the familial association of homosexuality: epigenetic inheritance.

There is a consensus among studies comparing homosexuality in monozygotic versus dizygotic twins that one or more coinherited elements (assumed to be genes, but which could just as well be heritable epi-marks) contribute substantially to this trait—accounting for an estimated 20–50% of the phenotypic variation in sexual orientation in both sexes (Kirk et al. 2000; Alanko et al. 2010; Langstrom et al. 2010; Burri et al. 2011). However, estimates of proband concordance among monozygotic twins (i.e., the probability that a twin is homosexual given that the other twin is homosexual) are surprisingly low in both sexes (about 20%) for a trait predominantly influenced by genetic factors (Bailey et al. 2000; Langstrom et al. 2010). Correspondingly, studies of twins consistently report a high "nonshared environment" contribution to homosexuality, typically accounting for at least 50% of the phenotypic variation in both sexes (Kirk et al. 2000; Alanko et al. 2010; Langstrom et al. 2010; Burri et al. 2011). The substantial estimated heritability of homosexuality, low proband concordance between monozygotic twins, and negative results from numerous molecular genetic association studies are collectively consistent with an epigenetic causation for homosexuality that contains two independent components: monozygotic twins share inherited (transgenerational) gonad-discordant SA-epi-marks influencing androgen signaling (contributing to the observed substantial heritability estimates and negative results from genetic association studies), but do not share one or more de novo gonad-concordant epi-marks (including erasure of a coinherited SA-epi-mark) that are laid down during fetal development (independently in each twin) that also influence androgen signal-

ing (contributing to the observed low concordance between monozygotic twins).

To understand how the homosexual pattern of substantial estimated heritability and low concordance between identical twins can be feasibly caused by epigenetic inheritance, we summarize results from studies of twins. In the remainder of this section, we first focus on arbitrary phenotypic traits and how epigenetics can contribute to both phenotypic similarity and dissimilarity between monozygotic twins. Next we focus on twin studies of traits other than homosexuality that are strongly influenced by fetal androgen signaling and well characterized at birth by numerous studies. Finally, we extend these studies to homosexuality.

ARBITRARY TRAITS

Empirical studies indicate that epigenetics can contribute substantially to the similarity of identical twins. For example, Gartner and Baunack (1981) used an isogenic mouse line to created monozygotic and dizygotic "identical" twins and compared them for a variety developmental traits. They consistently found higher phenotypic similarity between monozygotic compared to dizygotic identical twins, despite the fact that all mice were isogenic and developed (in utero and postnatally) in surrogate mothers. This finding, and others summarized in Wong et al. (2005), supports the conclusion that there is a substantial contribution of shared epi-marks to the phenotypic similarity of twins.

Empirical studies also indicate that epigenetics can contribute substantially to the dissimilarity of identical twins. Bouchard et al. (1990) compared a large sample of human identical twins who were reared together or apart since birth. They found that phenotypic dissimilarity for a wide diversity of traits (with a substantial "environmental" component of variation) was commonly no higher when twins were reared apart. This finding indicates that many phenotypic differences between monozygotic twins developed prenatally, i.e., at a time of extensive de novo epigenetic programming. This finding also indicates that nonshared epi-marks laid down independently in each individual twin (or other types of relatives) during fetal development may contribute importantly to the "environmental variance" that causes heritability to be less than one for most traits. This conclusion is supported by a study that compared methylation levels at the promoters of four genes in newborn monozygotic human twins (Ollikainen et al. 2010). Median differences in methylation levels were only about 3–4% (about half the value for dizygotic twins), but values as high as 54% were seen when looking at individual CpG units. At the level of genome-wide gene expression, Gordon et al. (2011) found that some newborn monozygotic twins had over 600 genes at which expression differed by at least twofold. They also found that newborn identical twins that separated earlier in development (1–3 days compared to 4–9 days postfertilization) had larger differences in their gene expression profiles, indicating that "this short period, very early in development, represents an important window for epigenetic variability" (Gordon et al. 2011).

TWIN STUDIES OF ANDROGEN-INFLUENCED TRAITS OTHER THAN HOMOSEXUALITY

Evidence that epigenetics contributes to high levels of phenotypic variation for traits influenced by fetal androgen exposure comes from studies on two phenotypes in humans: hypospadias (subterminal opening of the urethra on the phallus) and cryptorchidism (one or both testes fail to descend into the scrotum by birth). Like homosexuality, in which the genitals are concordant with the gonad but sexual preference (and brain anatomy; e.g., Savic and Lindström 2008) is not, both traits represent a gonad-trait discordance in which one aspect of androgen signaling matches the gonad while the other does not. In hypospadias, the phallus is generally male-typical in size, shape, and internal composition, but the length of the urethra is feminized (shortened). In cryptorchidism, the phallus is usually normal in all respects, but the position of the gonad is feminized (nondescended).

The prevalence of cryptorchidism is substantial and similar to that of human homosexuality (2–9%; Bay et al. 2011), while that of hypospadias is substantial but somewhat lower (prevalence of about .3–4%; Ahmed et al. 2004; Boisen et al. 2005). Animal models

indicate that exposure to androgen antagonists during a short period when the genitals differentiate (but not later in development) leads to highly elevated levels of both hypospadias and cryptorchidism, confirming that both traits are strongly influenced by fetal androgen signaling. In humans, however, the simultaneous expression of naturally occurring hypospadias and cryptorchidism is rare (i.e., they usually occur in isolation; Weidner et al. 1999). This pattern indicates that the two traits are caused, in large part, by different disruptions to androgen signaling. Like homosexuality, both traits display a familial association: when one brother in a family is affected, the prevalence is elevated in other brothers by about tenfold for hypospadias and threefold for cryptorchidism (Weidner et al. 1999). Similar to homosexuality, both traits are usually not shared among monozygotic twins (approximately 25% concordance for each trait; Fredell et al. 2002; Jensen et al. 2010). Also like homosexuality being elevated in individuals with loss of function at the CYP21 gene (but this gene not being a major cause of female homosexuality), extensive genetic studies have found that while loss of function at some candidate genes can lead to both hypospadias and cryptorchidism, the majority of cases are not associated with any known mutations (reviewed in Bay et al. 2011; Kalfa et al. 2011). Further evidence for a substantial nongenetic contribution in the case of cryptorchidism is: higher concordance (twofold) between dizygotic twins than that between singleton brothers (Jensen et al. 2010); and the high incidence of cryptorchidism (up to 70%) observed in some isolated wildlife populations despite no genetic evidence for inbreeding or a founder effect—presumably due to an environmental hormone-signaling disruptor (Latch et al. 2008).

The familial association observed in both androgen-influenced traits (cryptorchidism and hypospadias) indicates a substantial contribution of some coinherited factor (gene or epi-mark), while the low concordance for both traits observed in monozygotic twins indicates a substantial contribution of a "nonshared environment." Since the traits are measured in newborns, the nonshared environment must occur during gestation. But since monozygotic twins would be expected to share nearly all environmental effects during gestation (like exposure to androgen antagonists), something other than traditional environmental variation is almost certainly responsible for their observed low concordance. Epi-marks influencing androgen signaling that are laid down independently between monozygotic twins are the most feasible candidate to account for the strong "nonshared environment" component of both androgen-influenced traits. If these epi-marks sometimes escaped transgenerational erasure, they could also account for the familial association of both traits.

HOMOSEXUALITY

As described above, there is compelling evidence that epi-marks contribute to both the similarity and dissimilarity of family members, and can therefore feasibly contribute to the observed familial inheritance of homosexuality and its low concordance between monozygotic twins. We also showed that two other androgen-sensitive phenotypes, cryptorchidism and hypospadias, show the same pattern of high prevalence, strong familial associations, low monozygotic twin concordance, and discordance between the gonad and the trait (i.e., testes paired with undescended gonad(s) or testes paired with short urethral length). Just as epigenetics is a probable etiological agent contributing to cryptorchidism and hypospadias, so too is it a probable agent contributing to homosexuality. In this case, epi-marks that sometimes carryover across generations would contribute to the causation of homosexuality and its observed heritability while de novo epi-marks produced independently in each monozygotic twin would account for the low observed concordance for homosexuality between monozygotic twins.

An inherited gonad-discordant epi-mark causing homosexuality must not be masked by any gonad-concordant epi-marks produced during the recipient's ontogeny. This would occur most simply when an inherited epi-mark is stronger than average and is combined with a relatively weak de novo epi-mark(s) produced in the recipient. These two processes (i.e., transgenerational inheri-

tance of an epi-mark and its penetrance once inherited) are collectively subsumed in our model parameter q = probability that an SA-epi-mark carries over to the next generation and decreases the fitness of the opposite sex (see above modeling section).

An epi-mark producing homosexuality must also have a highly restricted effect, i.e., cause discordance between the gonad and sexual preference, but no discordance for other traits such as the genitalia and sexual identity. As described above, most cases of cryptorchidism and hypospadias are not associated with gonad-trait discordance for other androgen-influenced traits. This observation demonstrates that gonad-trait discordances can occur independently for different traits in the same individual. The most feasible explanation for this independence is the exceptionally wide diversity of AR cofactors that are known to occur and their high tissue specificity (Heemers and Tindall 2007)—each of which may be epigenetically regulated independently.

Although we cannot provide definitive evidence that homosexuality has a strong epigenetic underpinning, we do think that available evidence is fully consistent with this conclusion. For example, we now have clear evidence that epigenetic changes to gene promoters that influence their expression (e.g., levels of CpG methylation) can be transmitted across generations (Franklin et al. 2010) and that such heritable epigenetic changes can strongly influence, in the next generation, both sex-specific behavior and gene expression in the brain (Morgan and Bale 2011). As a consequence, we next apply our model of sexually antagonistic epi-marks to the human homosexual phenotype (as described in Table 1 and Figure 3).

Discussion

Sexually antagonistic selection is now well appreciated as a powerful factor in biological evolution (Parker 1979; Haig 1993; Rice and Holland 1997; Partridge and Hurst 1998; Chapman et al. 2003; Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009). It has been shown to significantly affect or drive a number of biological phenomena and processes, including survival and fertility (Rice 1996), mate choice (Gavrilets et al. 2001), genetic differentiation (Hayashi et al. 2007), reproductive isolation (Gavrilets 2000) and speciation (Parker and Partridge 1998; Gavrilets and Waxman 2002; Gavrilets and Hayashi 2005), sex chromosome evolution (Rice et al. 2008), sib competition (Rice et al. 2009), maternal selection (Miller et al. 2006), and grandparental care (Rice et al. 2010). This paper argues that sexually antagonistic selection can also be involved in epigenetic effects and explain the enigmatic high prevalence of several fitness-reducing human characters. As described below, our model and its predictions are consistent will the major empirical patterns associated with male and female homosexuality, and other common gonad-trait discordances.

Homosexuality is frequently considered to be an unusual phenotype because it represents an evolutionary enigma—a trait that is expected to reduce Darwinian fitness, yet it persists at substantial frequency across many different (possibly all) human populations. However, from the perspective of other traits influenced by fetal androgen signaling, and in which there is gonad-trait discordance, the high prevalence of homosexuality is not unusual. For example, the prevalence of hypospadias (gonad-trait discordance for urethral length) varies from 0.4% to 1% in newborns, and when including milder cases (ascertained in three years postpartum), its prevalence can be as high as 4% (Boisen et al. 2005). This phenotype is expected to interfere with sperm transfer during copulation, but despite this fitness cost, it persists at substantial frequency. Cryptorchidism (gonad-trait discordance for the position—abdominal versus descended—of the gonads) is associated with reduced fertility and increased rates of testicular cancer. The prevalence of this androgen-influenced trait is 2-9% (Bay et al. 2011). Examples of other androgen-influenced phenotypes with a high prevalence of gonad-trait discordance, but less obvious fitness effects, are male childhood cross-gender behavior (3.2%; van Beijsterveldt et al. 2006), female childhood cross-gender behavior (5.2%; van Beijsterveldt et al. 2006), and female idiopathic hirsutism (i.e., male-like pattern of body hair in the absence of both atypical menstrual cycles and elevated circulating androgens, 6%; Carmina

TABLE 1

Sexually antagonistic epi-mark hypothesis of homosexuality

We describe our hypothesis for an epigenetic cause of homosexuality as a series of statements (see Figure 3 for a graphical summary):

a) Empirical studies demonstrate that XX fetuses are canalized to blunt androgen signaling (lower sensitivity to T) and XY fetuses are canalized to boost androgen signaling (higher sensitivity to T).

b) Empirical studies demonstrate the production of XX- and XY-induced epi-marks in embryonic stem cells and extensive sex-specific differences in gene expression at this time. Epi-marks laid down during the embryonic stem cell stage are also established to influence gene expression later in development. This stem cell period is the most plausible candidate time point for the production of epi-marks influencing sensitivity to androgens later in development (canalization of fetal androgen signaling).

c) Epi-marks produced in embryonic stem cells are mitotically transmitted to cell lineages leading to both the soma and the germline, and hence can contribute to pseudo-heritability when they escape erasure across generations (nonerasure in the primordial germ cells and in the zygote and first few cell divisions of the next generation). Animal models as well as human data unambiguously demonstrate that such a multistep escape from erasure does occur at nontrivial frequency.

d) Epi-marks blunting (in XX fetuses) or boosting (in XY fetuses) androgen signaling will be sexually antagonistic (SA-epi-marks) when they have a nonzero probability of carryover across generations and are expressed in oppose sex descendants. Such carryover will contribute to discordance between the gonad and one or more sexually dimorphic traits.

e) Our modeling work shows that SA-epi-marks are favored by natural selection over a broad span of parameter space because there is a net benefit to the carrier (due to canalization of sexually dimorphic development) that is not offset sufficiently by transmission (and fitness reduction) to opposite sex descendants.

f) Genetic mutations causing SA-epi-marks are expected to fix in populations and are therefore not expected to be polymorphic except transiently during their initial spread within a population. Therefore, no association between genotype and homosexuality is predicted.

g) Because the androgen signaling pathways differ among organs and tissues (e.g., use of different AR cofactors), the same inherited SA-epi-mark can affect only a subset of sexually dimorphic traits, e.g., no effect on the genitalia, but a large effect on a sexually dimorphic region of the brain.

h) Shared, gonad-discordant SA-epi-marks that carryover across generations would contribute to the observed realized heritability of homosexuality, e.g., monozygotic twins share the same SA-epi-marks coinherited from a parent.

i) Unshared, gonad-concordant SA-epi-marks, produced during fetal development, would contribute to the low proband concordance of homosexuality observed between monozygotic twins, i.e., they need not share SA-epi-marks generated during development that occurs after the twins have separated.

j) Homosexuality occurs when an individual inherits one or more gonad-discordant SA-epi-marks that are not masked nor erased by the production of de novo gonad-concordant SA-epi-marks that accrue during ontogeny. The SA-epi-mark(s) influence androgen signaling in the part of the brain controlling sexual orientation, but not the genitalia nor the brain region(s) controlling gender identity.

1998). From these examples it is clear that the substantial prevalence of homosexuality (a gonad-trait discordance) is not unusual for a phenotype strongly influenced by fetal androgen exposure.

Why should phenotypes associated with fetal androgen signaling commonly have high frequencies of gonad-trait discordance? We do not know. The simplest hypothesis is that environmental stress and androgen agonists and antagonists are sufficiently common that they generate constant selection for new, more effective epi-marks that protect (canalize) each sex from their effects. Some of these newly evolved epi-marks escape the normal generational cycle of erasure/reprogramming and thereby carryover across generations (by happenstance and with moderate to low probability) and lead to gonad-trait discordance. Since it is now well established that environmentally induced epi-marks, like those from prenatal/perinatal stress, are common and can be heritable with sex-specific effects on the brain and behavior (Franklin et al. 2010; Morgan and Bale 2011), it seems inevitable that some epi-marks produced by new mutations (coding for epi-marks that canalize sex-specific development) will also sometimes carryover across generations and form SA-epi-marks. Our modeling analysis clearly

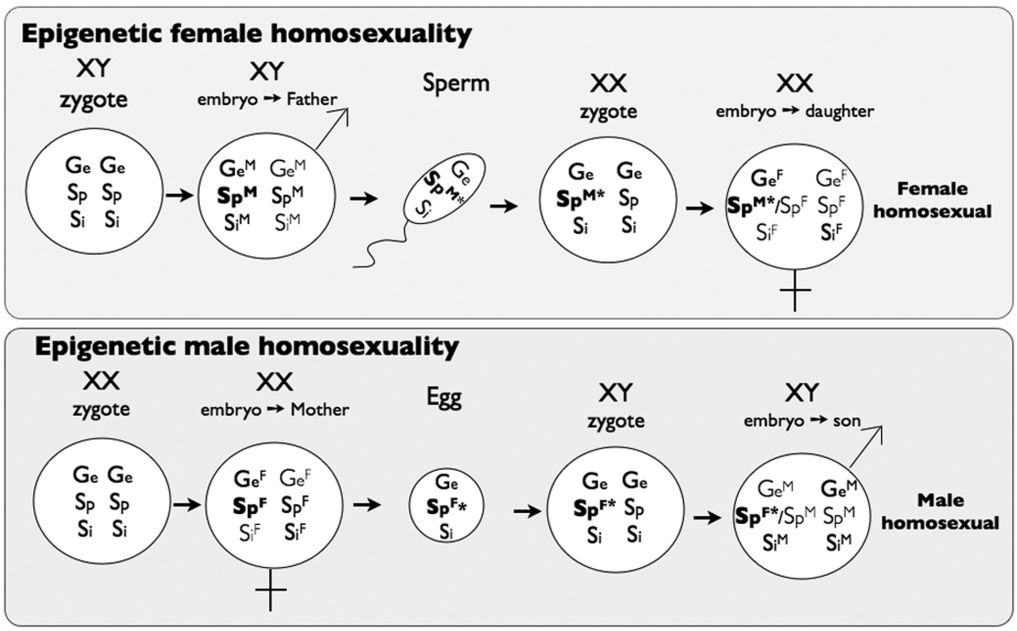

Figure 3. SA-Epi-Marks and Homosexuality

Our SA-epi-mark model predicts that homosexuality is produced by transgenerational epigenetic inheritance. As in nature, epi-marks are assumed to sometimes carryover across generations (depicted by the "*" superscript) and epi-mark strengths are assumed to be variable, irrespective of genetic polymorphism (depicted by the intensity of their letter symbols). Masculinizing (superscript "M") epimarks are produced in response to the XY genotype in the early embryo stage (stem cells) and canalize male development by increasing sensitivity to fetal T. Feminizing (superscript "F") epi-marks similarly canalize female development by reducing sensitivity to fetal T. Homosexuality occurs when one or more stronger than average epi-marks—bold, that canalize sexual preference (Sp), but not the genitals (Ge) nor sexual identity (Si)—carryover across generations into an opposite-sex descendent and cause gonad-trait discordance when combined with weaker than average (light) de novo sexually concordant epi-mark(s).

demonstrates that mutations that cause epi-marks that blunt androgen signaling in XX fetuses, or boost it in XY fetuses, can have a selective advantage even when they carryover across generations at nontrivial frequency and reduce fitness by feminizing or masculinizing opposite-sex descendants. Modifiers that restrict androgen blunting/boosting epi-marks to the appropriate sex would be expected to eventually evolve and produce nearly invariant canalization, but new mutations creating new epi-marks may continue to evolve (requiring new modifiers) if androgen agonists and antagonists are sufficiently variable across time and/or space.

Another possibility leading to persistently high levels of gonad-trait discordance is an arms race between male- and female-benefit SA-epi-marks that blunt/boost androgen signaling during fetal development. The accumulation of such SA-epi-marks favoring one sex generates selection in the other sex to evolve new epi-marks that protect them from opposite-sex SA-epi-marks that sometimes carryover across generations. Such an arms race could, in principle, lead to protracted periods of high levels of gonad-trait discordance.

Levels of gonad-trait discordance among androgen sensitive traits are highly variable. The genitalia (phallus or vulva) and core sexual identity (masculine or feminine) are highly canalized, with gonad-trait discordance levels of 1/10,000 or less (Sax 2002; Swaab 2007). However, prevalence of gonad-trait discordance for components of the genitalia and sexual identity can be orders of

magnitude higher, as we previously described for homosexuality, cross-gender behavior, hypospadias, cryptorchidism, and idiopathic hirsutism. The developmental switch to form a phallus or a vulva (or the switch to form a masculine or feminine gender identity) in response to high or low fetal T concentrations dates back to at least the origin of mammals (over 200 million years ago). This long time period would have provided substantial opportunity to evolve modifiers that reliably canalize these sex-specific developmental dichotomies.

Although major dichotomies of the genitalia (phallus/vulva) and sexual identity (male/female) have been invariant over long periods of evolutionary time, the phenotypes of the genitalia and sexual behavior evolve rapidly in all species, including mammals (reviewed in Eberhard 1996). Such continual and rapid evolutionary modification of the structural attributes of genitalia and sexual behavior would be expected to provide far less time for the evolution of reliable canalization, and hence the observed higher gonad-trait discordance for components of the genitalia and sexual behavior. Although we know of no reported data, we suspect that few if any of the sexual signals used by chimps (our closest relative, from which we have evolved independently for only six to eight million years) are sexually attractive to humans, and hence that most of the neurological networks underlying human sexual attraction are relatively newly evolved. This recent evolution may explain the observed lower canalization of human sexual orientation, and hence the higher prevalence of homosexuality (compared to the very low prevalence of gender dysphoria). The rapid and surprisingly extensive divergence of the male genitalia between humans and chimps (see Cold and McGrath 1999) may similarly be causally associated with the high prevalence of cryptorchidism and hypospadias. Similarly, the rapid divergence in body hair between humans and chimps may explain the high prevalence of idiopathic hirsutism in females.

Unlike past genetic models of homosexuality based on kin selection, overdominance, or sexually antagonistic alleles (Camperio-Ciani et al. 2004, 2008; Gavrilets and Rice 2006; Iemmola and Camperio-Ciani 2009), our model of homosexuality via SA-epi-marks predicts no genetic polymorphisms will be associated with homosexuality. Polymorphic phenotypes but monomorphic genotypes are predicted to occur because of the nonzero probability of cross-generation transmission of heritable SA-epi-marks coded by mutations that are fixed except during brief and transient periods of the recruitment of new mutations. If this epi-inheritance were the sole cause of homosexuality, then we would expect high concordance between monozygotic twins—which is not observed. Low concordance of monozygotic twins indicates that homosexuality (and other common gonad-trait discordances) require the combination of an inherited stronger-than-average sexually discordant epi-mark and a weaker-than-average sexually concordant epi-mark produced during early fetal development (that does not mask or erase the inherited sexually discordant epi-mark; Figure 3).

The point estimates of an approximately 8% prevalence of homosexuality in both sexes (Bailey et al. 2000) and an approximately 20% proband concordance for homosexuality for both sexes among identical twins (Bailey et al. 2000; Langstrom et al. 2010) can be combined to estimate the transmission rate of an SA-epi-mark that causes homosexuality. We start with the general relationship,

$$\mathrm{Prev}_{\mathrm{MHS}} = \mathrm{P}_{\mathrm{strong(F)}} * \mathrm{P}_{\mathrm{unerased}} * (1 - \mathrm{P}_{\mathrm{strong(M)}}),$$

where $\mathrm{Prev}_{\mathrm{MHS}}$ is the prevalence of male homosexuality, $\mathrm{P}_{\mathrm{strong(F)}}$ is the probability of a stronger-than-average feminizing epi-mark in the mother, $\mathrm{P}_{\mathrm{unerased}}$ is the probability that this epi-mark is not erased when it is passed to the son, and $(1 - \mathrm{P}_{\mathrm{strong(M)}})$ is the probability that there is no stronger-than-average masculinizing epi-mark produced in the son that masks the inherited strong transgenerational feminizing epi-mark. In our modeling section, the scaler q equals the joint probability of nonerasure of an SA-epi-mark and its being paired with a relatively weak de novo epi-mark in the recipient—i.e., $q = \mathrm{P}_{\mathrm{unerased}} * (1 - \mathrm{P}_{\mathrm{strong(M)}})$. If we assume that strong masculinizing and feminizing epi-marks are equally common (i.e., $\mathrm{P}_{\mathrm{strong(M)}} = \mathrm{P}_{\mathrm{strong(F)}}$), then the probability of proband concordance ($\mathrm{C}_{\mathrm{proband}}$) between monozygotic twins is then $\mathrm{C}_{\mathrm{proband}} = 1 -$

$P_{strong(M)}$. Note that $Prev_{MHS} = C_{proband}(1 - C_{proband})P_{unerased}$, from which $P_{unerased} = Prev_{MHS}/[C_{proband}(1 - C_{proband})]$.

Proband concordance of monozygotic twins estimates the probability that there is no strong masculinizing epi-mark in the son that overrides the inherited feminizing epi-mark, so $C_{proband}$ is estimated to be 0.2. The prevalence of homosexuality estimates its rate of occurrence in the general population ($Prev_{MHS} = 0.08$). Substitution of these values and solving for a homosexual-inducing SA-epi-mark's transmission rate gives a value of $P_{unerased} = .08/[.2(1 - .2)] = 0.5$. Therefore, the low concordance for homosexuality among monozygotic twins (20%) when coupled with low prevalence in the general population (8%) indicates that a causative SA-epi-mark has a high transgenerational transmission rate (50%). This value may or may not be unusually high. Transgenerational epi-marks canalizing sexually dimorphic traits lead to conspicuous gonad-trait discordances in opposite sex offspring. In contrast, transgenerational epi-marks influencing most other traits would likely go unnoticed because they lead to increased parent-offspring similarity and would therefore be confounded with genetic heritability.

Although the studies are somewhat contradictory, there is evidence that relatives of male homosexuals have higher fecundity on one or both sides of their family pedigree (reviewed in Schwartz et al. 2010). Homosexuality via SA-epi-marks would predict higher fecundity of opposite-sex relatives if these epi-marks increased the level of sexual attraction to the opposite sex in the father or mother of female and male homosexuals, respectively. The finding in an Italian population of higher than average fecundity in the maternal female relatives of homosexual males (replicated in two independent studies: Camperio-Ciani et al. 2004; Iemmola and Camperio-Ciani 2009) is consistent with this prediction. Another study found the same pattern for aunts in a sample of white men in England—but the pattern was reversed in nonwhites and extended to more categories of relatives and in both sexes (Rahman et al. 2008). A second study in England found more relatives for homosexual compared to heterosexual men on the paternal side of the family lineage and the same but nearly significant pattern ($P = 0.058$) on the maternal side of the family (King et al. 2005). Another study found that homosexual men have more siblings (of both sexes) compared to a sample of heterosexual men (Blanchard and Lippa 2007). In aggregate, these studies are consistent with an SA-epi-mark causation of homosexuality because they indicate that homosexual men have more fecund mothers and/or female relatives on the maternal, paternal, or both sides of the family. The heterogeneity in these studies could arise if different ethnic groups are fixed for mutations producing different SA-epi-marks that are inherited primarily through only the matriline, the patriline, or through both lineages.

Ours is not the first nongenetic hypothesis for the evolution of human homosexuality. One highly intuitive, nongenetic hypothesis for homosexuality is that it is due to reduced androgen signaling that occurs after the first trimester of gestation (Swaab 2007), i.e., after the genitalia have formed. Many environmental agents can potentially reduce androgen signaling and these could episodically affect some periods of fetal development and not others. Studies with rhesus monkeys clearly demonstrate that sex-specific behavior and the genitals can be masculinized/feminized during different gestational time periods (reviewed in Thornton et al. 2009). Therefore, discordance between the gonad/genitals and sexual orientation could be feasibly produced by fluctuating exposure to environmental androgen agonists and antagonists. Despite its intuitive appeal, we do not think that this "timing" hypothesis is consistent with available data. The micropenis phenotype is produced in humans when there is sufficient T during the first trimester of development to induce normal phallus formation, but too little T is produced during the second and third trimesters to stimulate its continued growth (for example, due to gonadal dysgenesis after the first trimester). These individuals are usually reared as males and they show no elevation in gender dysphoria and only a small increase in same-sex partner preference (reviewed in Wisniewski et al. 2008). This pattern indicates that low androgen signaling during the second two trimesters of

fetal development is not associated with substantially elevated levels of same-sex partner preference in XY males.

Another nongenetic model for homosexuality is based on birth-order effect, in which males with more older brothers are more likely to be homosexual (reviewed in Bogaert and Skorska 2011). Because available evidence indicates that birth order can at most account for only one in seven homosexual men (Cantor et al. 2002), and because this hypothesis does not apply to female homosexuality, we think that most of the phenomenon of human homosexuality cannot be attributed to this nongenetic mechanism.

Although we think that the low concordance for monozygotic twins argues against most extant genetic hypotheses as a major cause of human homosexuality, we want to clearly state that our epigenetic hypothesis is not mutually exclusive with some influence of genetic polymorphisms contributing to homosexuality (or an important role for a birth-order influence). Future genome-wide association studies should eventually find such genetic polymorphisms if they exist and contribute substantially to human homosexuality.

PREDICTIONS

A major strength of our epigenetic model of homosexuality is that it makes two unambiguous predictions that are testable with current technology. Therefore, if our model is wrong, it can be rapidly falsified and discarded.

First, future, larger-scale genetic association studies will fail to identify genetic markers associated with most homosexuality. Our model does not preclude some mutations being associated with homosexuality because it is already established that some mutations affecting androgen signaling (e.g., those contributing to CAH or CAI) can strongly influence the level of gonad-trait discordance for sexual orientation. Our model does predict, however, that any genetic associations discovered in the future will be weak and account for little of the phenotype variation in sexual orientation.

Second, future genome-wide epigenetic profiles will find differences between homosexuals and nonhomosexuals, but only at genes associated with androgen signaling in the later parts of the pathway (e.g., AR cofactors or miRNAs that regulate them) or be restricted to brain regions controlling sexual orientation, i.e., not affecting sexually dimorphic traits like genitalia or sexual identity.

It may be feasible to readily test the second prediction with current technology in the case of female homosexuality. Our hypothesis predicts that differences will be found when comparing the genome-wide epigenetic profiles of sperm from fathers with and without homosexual daughters.

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APPENDIX 1
A Formal Model for Epigenetic Canalization of Androgen Signaling

The androgen signaling pathway can be represented as a series of n steps at each of which the signal can be augmented (in males) or depressed (in females) by the effects of epi-marks. For example, let T_0 be the level of androgen in serum. Then the signal at the end of the pathway can be written as

$$\begin{aligned} T_{\text{EndPoint}} &= T_0 \times (1 + \varepsilon_1\chi_1) \times (1 + \varepsilon_2\chi_2) \times \cdots \times (1 + \varepsilon_n\chi_n) \\ &= T_0\Pi(1 + \varepsilon_i\chi_i), \end{aligned}$$

where χ_i is the effect of the epi-mark controlling the ith step and ε_i scales the relative importance of the ith step in the pathway. When the values of ε_i are positive for male fetuses and negative for female fetuses, androgen signaling will be boosted in males and blunted in females.

Epi-marks can cause substantially elevated androgen signal in males compared to females at the end of the pathway (T_{EndPoint}) even when the levels of androgen in serum (T_0) overlap between the sexes to a limited degree (as appears to occur at nontrivial rate in humans and the rat model system).

As described above, additional canalization occurs when T is converted to a more potent metabolite (like DHT or E) but the androgen mimic is not recognized by the enzyme responsible for this conversion. To incorporate canalization against androgen mimics we can add a second term ($+ T_{M(e)}$) to our previous equation,

$$\begin{aligned} T_{\text{EndPoint}} &= T_0 \times (1 + \varepsilon_1\chi_1) \times (1 + \varepsilon_2\chi_2) \times \cdots \times (1 + \varepsilon_n\chi_n) + T_{M(e)} \\ &= T_0\Pi(1 + \varepsilon_i\chi_i) + T_{M(e)}, \end{aligned}$$

where $T_{M(e)}$ represents the effective concentration of the androgen mimic (its concentration multiplied by its relative ability to functionally substitute for T). If the androgen mimic is not converted to the more potent metabolite of T, and this conversion is required to achieve sufficient androgen signaling (T_{EndPoint}) in males, then XX fetuses will be protected from inappropriately expressing the male form of a trait, i.e., $(T + T_{M(e)})_{\text{Female}} = T_{\text{Male}}$ but $(T_{\text{EndPoint}})_{\text{Female}} \ll (T_{\text{EndPoint}})_{\text{Male}}$.

APPENDIX 2

Invasion of Genetic Mutations that Code for Epi-Marks that Canalize Sexual Development

Autosomal mutant. Consider a genetic mutation that, exclusively in one sex (say females), induces an epi-mark that increase fitness (w) by s. The induced epi-mark escapes erasure between generations with probability q. If the epi-mark is transmitted to offspring of the opposite sex of the parent (here, sons) it decreases fitness by σ. Due to the transgenerational effects we need to consider the number of grandchildren of a mutant, when calculating the invasion criteria. The expected number of copies of a mutant allele in the grandchildren generation is:

	From fathers		From mothers		
fraction	1/2		1/2		
w	1		$1 + s$		
	Sons	Daughters	Sons		Daughters
Epi-mark present	no	no (but carriers of gene)	Yes	no	irrelevant
fraction	1/2	1/2	$q/2$	$(1 - q)/2$	1/2
w	1	$1 + s$	$1 - \sigma$	1	$1 + s$

$$w = 1/2 \times 1 \times [1/2 \times 1 + 1/2 \times (1 + s)] + 1/2 \times (1 + s) \times [q/2 \times (1 - \sigma) + (1 - q)/2 \times 1 + 1/2 \times (1 + s)] = 1 + s - q \times \sigma/4$$

To invade:

$$1 < 1 + s - q \times \sigma/4$$

$$s > q \times \sigma/4.$$

X-linked mutant. Such mutant finds itself in a female with probability 2/3 and in a male with probability 1/3. We first consider the case with a feminizing effect:

	From fathers	From mothers		
Fraction	1/3	2/3		
W	1	$1 + s$		
	Daughters	Sons		Daughters
Epi-mark present	no (but carriers of gene)	yes	no	irrelevant
Fraction	1	$q/2$	$(1 - q)/2$	½
W	$1 + s$	$1 - \sigma$	1	$1 + s$

$$w = 1/3 \times 1 \times [1 \times (1 + s)] + 2/3 \times (1 + s) \times [q/2 \times (1 - \sigma) + (1 - q)/2 \times 1 + 1/2 \times (1 + s)] = 1 + 4/3 \times s - 1/3 \times q \times \sigma$$

To invade:

$$1 < 1 + 4/3 \times s - 1/3 \times q \times \sigma$$

$$s > q \times \sigma/4.$$

And next the case with a masculinizing effect:

	From fathers		From mothers	
fraction	1/3		2/3	
w	$1 + s$		1	
	Daughters		Sons	Daughters
Epi-mark present	yes	no	no (but carriers of gene)	no
fraction	q	$(1 - q)$	1/2	1/2
w	$1 - \sigma$	1	1 + s	1

$$w = 1/3 \times (1 + s) \times [q \times (1 - \sigma) + (1 - q) \times 1] + 2/3 \times 1 \times [1/2 \times (1 + s) + 1/2 \times 1]$$
$$= 1 + 2/3 \times s - 1/3 \times q \times \sigma$$

To invade:

$$1 < 1 + 2/3 \times s - 1/3 \times q \times \sigma$$
$$S > q \times \sigma/2.$$

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