Chapter 15. Sex Determination and Differentiation

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Sex Determination

The goal of evolutionary behavioral analysis is to understand the source of variation in behavior within a species. This search must include a discussion of proximate mechanism. Proximate mechanisms begin with an understanding of how genes get translated into proteins, how such proteins build organs that produce hormones, how proteins and hormones get assembled into regulatory networks, and how such networks govern the physiological and developmental events that result in behaviors. It is at this point in the development of an organism that the system can no longer be described in terms of genetics *per se*, but a complete explanation of proximate mechanisms must resort to **epigenetic** explanations of the development of behavior.

epi = above + *genetics* = the genotype

No developmental process is better characterized than the epigenetic networks that lead to sex determination and differentiation of vertebrates. The genes that initiate these epigenetic cascades are quite well characterized, as are the developmental and endocrine machinery that serve to regulate the developing phenotype. Moreover, the simple switches underlying sex determination trigger many classical examples of neurodevelopment and behavior:

1. Alternative Male Phenotypes,
2. Bird Song,
3. Mammalian Parental Care.

There are many possible sex determination mechanisms in the animal kingdom and we will consider two that are found in the vertebrates:

1. Chromosomal sex determination (XY mammals, ZW birds)
2. Environmental sex determination (many reptiles).

Processes of sex determination and sex steroid hormones have an organizing effect on the development of an organism. This **organizing** effect of steroid hormones builds a phenotype that can respond to later acting **activational** effects of the sexual differentiation cascade. For example, testosterone has an organizing effect on early embryonic development that initiates the basic anatomies of females and males in a given species. However, the full expression of sex differences in most vertebrates requires that testosterone also activate many aspects of the phenotype during the process of maturation.

In addition to these early and late acting effects of steroids, the maternal environment or reproductive decisions by the mother can also have a dramatic effect on the developing phenotype that bias the kind of behaviors seen during the rest of the offspring's life. Finally, some animals are not restricted to a single sex for life and they can undergo a sex change that is triggered by the environment relatively late in life. **Sex determination** and the behavioral phenotypes that result are due to **genetic**, **epigenetic**, and **genotype-by-environment interactions**.

Chromosomal Sex Determination

The basic mammalian sex determination mechanism arises from the possession of a small but important gene located on the Y-chromosome. The Y-chromosome has very few functional genes and nearly all of the important genes for development of early embryonic form are spread among the remaining somatic chromosome or on the X chromosome. There are indeed hundreds of genes that are necessary to construct the basic reproductive system of vertebrates during early development, but the trigger to take on a female or a male form arises from **testes determining factor**, the ***sry*** gene. For years, biologists speculated that such a factor must reside on the Y chromosome and it has now been isolated in at least a few vertebrates. This factor was isolated, along with its interactions with other genes like *sox9*. Sex-determining loci induce testis development and turn on genes for maleness (*sry* gene interacts with *sox9* to effectuate gender determination) (Koopman et al. 2001).

The presence (XY) or absence (XX) of *sry* takes the embryo down two alternative pathways -- male versus female. If the gene is absent, then the organism develops into a female with ovaries and a system of endocrine glands that regulate female reproduction and behaviors. If the gene is present, the organism develops testes rather than ovaries, and the testes in turn produce testosterone, which further organizes the neuro-endocrine system. Building ovaries *vs*. testis requires different genes.

Organizational Effects

Nearly all of the sex differences in neural development and resulting behaviors are triggered by the action of steroids (Goy and McEwen 1980).

1. During early vertebrate development, the testes produce a small quantity of testosterone.
2. This testosterone is circulated by the bloodstream and organizes many aspects of neurodevelopment.

Activational Effects

With the proper neural circuitry in place, the appropriate muscle development, the correct organ systems built (e.g., sexual organs), the body is ready for the activational events that typically begin with the first breeding attempt. It is at this time that the latent sexual tendencies are awakened in the juvenile. The triggers for maturation can be genetic, or environmental. Whatever the cause or trigger, sexual maturation requires hormones to activate physiological and neurophysiological systems. In the case of male and female vertebrates, a part of the brain called the pre-optic area begins producing a neurochemical called **gonadotropin-releasing hormone** (**GnRH**).

GnRH acts on the cells in the pituitary and the pituitary begins secreting gonadotropins. **Gonadotropins** act on the gonads and stimulate them to produce steroids: testosterone in males, and estrogens in females. This second wave of steroids serves to refine the developing sexual phenotypes. Recall that the first wave of steroids during embryogenesis organized the animal and triggered development of the **primary sex characters:** male versus female organ systems developed. In this second wave of activational effects the **secondary sexual characteristics** (every other sex difference) begin to develop under the influence of these gonadal secretions (Fig. 15.1, see also Chapter 8, 9).

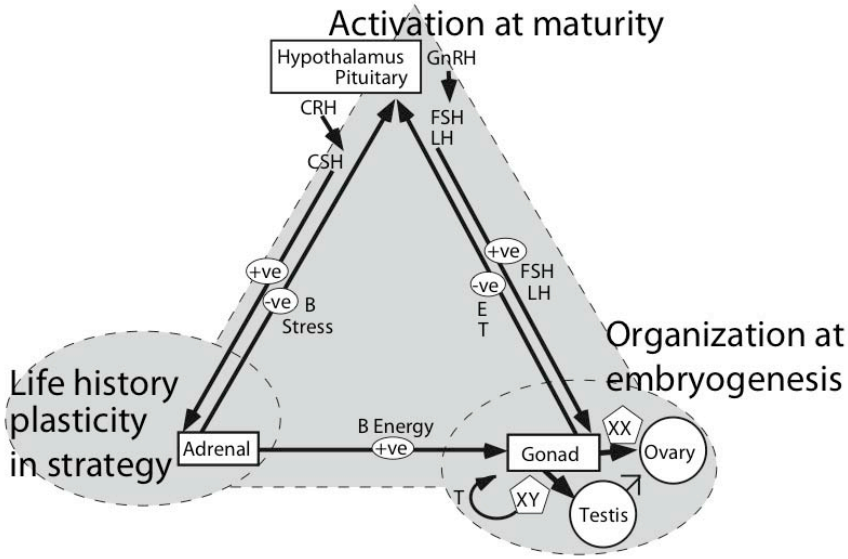


Figure 15.1. The key elements of gender determination involve early events of organization in which the gonad develop is turned on to male (*sry* gene) versus female. After these organ systems are built, the male and female genotypes are activated at maturity when the gonadotropins FSH and LH are secreted into the body for the first time. This organ system interacts with the adrenal to effectuate reproduction (see Chapter 8).

Maternal Effects

Maternal effects are broadly defined as the impact that the mother and her phenotype have on the phenotype of the offspring. This is quite distinct from the genetic effects that a mother has on her offspring's phenotype. Lets look at the definition a little more slowly.

1. "Mother and her phenotype" makes no distinction regarding what caused mom's phenotype, it could have been genetic or it might have been environmental.
2. "Impact of mother's phenotype on offspring" -- again here we are talking about offspring phenotype.

Just to be precise, **a maternal effect** includes all those attributes arising from mom that are not due to direct genetic inheritance (additive gene effect, Chap. 2). Maternal effects are an important source of the variation among individuals in fitness. In mammals, the environment of the womb, can have a dramatic effect on offspring behaviors, as can the quality of nursing which is the hallmark of the mammalian condition.

Why is this distinction important for behavior?

A female parent can impart non-genetic changes to her offspring that make them better able to cope with their environment. Rather than encode all information on how to build the offspring in the genes, placing some control into mom's decision-making machinery may produce greater fitness (e.g., better progeny). The female may be able to predict the environment of the offspring. In this case she should impart some of this information as a jumpstart to give her offspring an edge. However, this information should not necessarily be a permanent change that genetics might entail. The offspring in its own time might need to do a similar service for its offspring and a genetic effect would last too long. What if the offspring had to elicit different behaviors? The solution is to build a system that allows for **plasticity**. Below I describe a few case studies where such plasticity is important. The cues that the female has are easy to identify. In these examples, mothers integrate key environmental cues and alter the phenotypes of her offspring accordingly.

Positional Effects and the Mammalian Uterus

Rats and mice produce multiple offspring in a litter. These developing embryos are strung out along each of the two horns of the bicornate (=two-horned) uterus like a strand of pearls. All embryos are attached to the wall by a placenta, and compounds are free to circulate between the offspring and mom, and between mom and the offspring through the diffusion. In addition, compounds move into the amniotic fluid between embryos and are taken up by adjacent embryos. Steroids freely move from mom to progeny and progeny to progeny. The dose of testosterone that a male embryo produces during the early organization of the sexual reproductive system is not transmitted full strength to the sister that may be next to him, but a certain amount of the hormone does reach the female offspring. Because steroids are very potent hormones, even in fairly small doses, the amount that a female receives from her prenatal brother is enough to alter her behavioral phenotype (Fig. 15.2).

A female that is between two males in the uterus is designated:

M-**F**-M = 2M female,

and a 2M female has a different phenotype compared to a female that is between two females, which is designated:

F-F-F = 0M female.

The most obvious external manifestation of this early androgen exposure is seen in the distance between the anus and genitals, the anogenital distance, which for M-**F**-M females is larger than F-F-F females. Males normally have a long anogenital distance.

The most interesting effects are seen on behavior. Female rats exposed to male androgens exhibit more mounting behavior. Female mice are more aggressive.

The effects on aggression can even be seen in 2M males (e.g., M-**M**-M) relative to 0M males (e.g., F-**M**-F). If these animals are castrated after this early exposure and then given T supplements later in life during the activational period for testosterone (T), 2M males are more aggressive than 0M males. The castration is used to remove any differences in feedback that might occur between a 2M male's gonads and brain during adult life compared to a 0M male. By giving both types of males the same levels of exogenous hormone at maturity, vom Saal ensured that the responses seen in adult animals are due to changes arising from the **organizational period** only (when a 2M male was between two males and he received a higher dose of T than did a 0M male that was between two females). They do not want to confound the comparison with differences in T production by 2M or 0M males (Fig. 15.3).

In addition, the 0M males received higher doses of estrogen and this has an effect on its behaviors. What might those effects be?

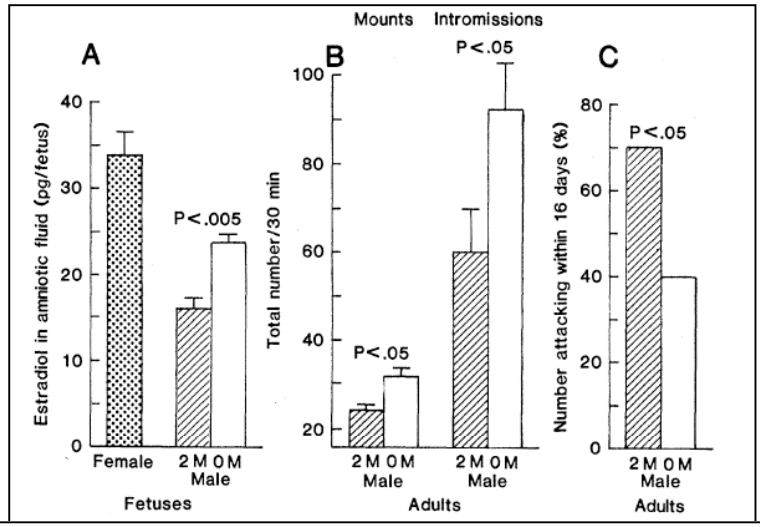


Figure 15.2. (A) Mean concentrations (± SE) of estradiol in the amniotic fluid (expressed as picograms per fetus) of female (N = 10 pools) and 0M (N = 5 pools) and 2M (N = 5 pools) male fetuses on day 17 of gestation. (B) The total number of mounts and intromission (thrusting movements) made by 90-day old 0M and 2M male mice during a 30-minute test with a sexually receptive female (± SE; 20 animals per group). (C) The percentage of neonatally castrated, 90-day-old 0M and 2M males (20 per group) that showed a 5-second sustained biting attack toward a 1M male intruder. The mice were tested for 10 min on alternate days for 16 days after a 10-mm Silastic capture containing 5 mg of T in 0.02 ml of oil was implanted in the neck region. (from vom Saal 1981).

Similar effects are likewise observed on OM and 2M females in laboratory trials implying effects organizing effect of T on female behavior. Moreover, these studies have been extended to field studies.

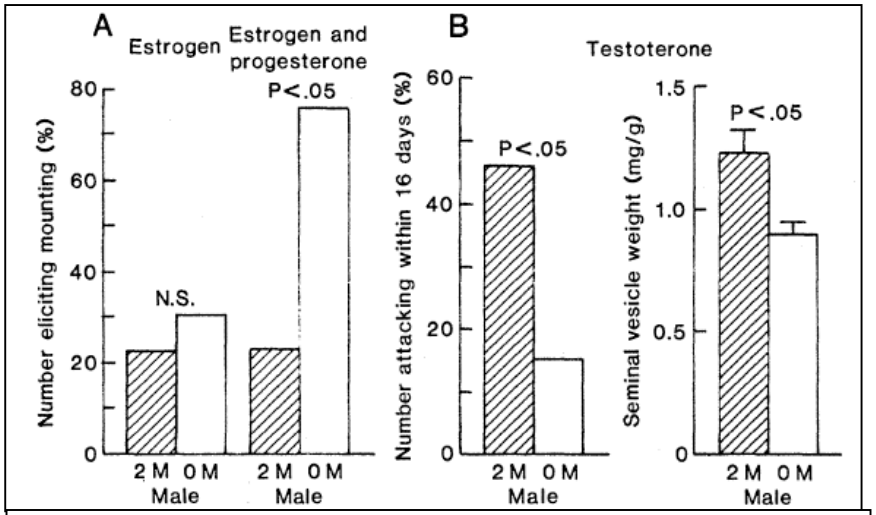


Figure 15.3. (A) The percentage of neonatally castrated, 90-day-old 0M and 2M male mice that elicited mounting by a study 1M male during a 30-minute test during the 3rd and 4th weeks of hormone treatment. All males received progesterone in oil 4 hours before being tested, while the other males received only oil (15 males per treatment condition). (B) The percentage of neonatally castrated, 200-day-old 0M and 2M males (30 per group), previously tested for female sexual behavior, that showed a 5-second sustained biting attack toward a 1M male intruder. The mice were tested for 16 days after a Silastic capsule contained testosterone was implanted in the neck region. After the 0M and 2M males had been exposed to testosterone for 35 days, all the animals were weighed and the seminal vesicles were weighted after the fluid was removed by blotting. Seminal vesicle weights are expressed as mg of tissue per gram of body weight.

Can such effects be viewed in an adaptive context?

Under conditions of crowding or stress, it might benefit a female to produce more aggressive females or males. If a female mouse (or rat) could manipulate the intrauterine position of her offspring she could impart a one generational effect on her offspring that might be advantageous for their survival or reproductive success. In a large-scale field experiment, Zielinksi et al (1992) found that 0M and 2M females differed in territorial behavior. 2M females defended significantly larger territory than 0M females and territory area of 2M females was comparable in size to spring male territories (Fig. 15.4). No other differences in reproductive success or survival were observed, thus, such territoriality might be beneficial under high-density conditions.

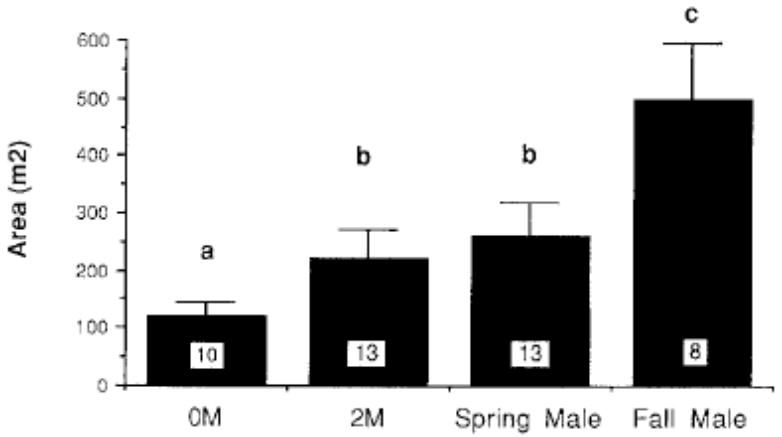


Figure 15.4. Territory area of 0M and 2M females compared to male territory areas observed in the spring and fall (Zielinksi et al 1992).

Inheritance of acquired characteristics and intrauterine position

Following up on the studies by Vom Saal et al. (1980), Clarke et al (1993) found an amazing persistent effect of positional effect on the daughters of 2M vs. 2F positional effects. The daughters from a 2M event were 1.73 times more likely to produce daughters that were 2M. Conversely daughters from 2F females were 0.6 times less likely to have a 2M daughter. Both effects reinforce the positional effects across generations resembling a form of inheritance (e.g., 2M females tend to

produce more 2M daughters, 2F females tend to produce less daughters) that is purely due to epigenetic inheritance of uterine position.

Besides these effects, Clarke and Galef (1990) have found that Mongolian gerbils also have a pronounced asymmetry in son *vs*. daughter production from each horn of their bicornate uterus (Fig. 15.5). In particular, the left horn tends to produce more daughters and the right horn more sons. Amazingly, Hippocrates in the 5th century made similar observations 2500 years ago! This maternal effect tends to concentrate males in the same horn, amplifying their maleness, and at the same time concentrate females in a different horn, amplifying their femaleness.

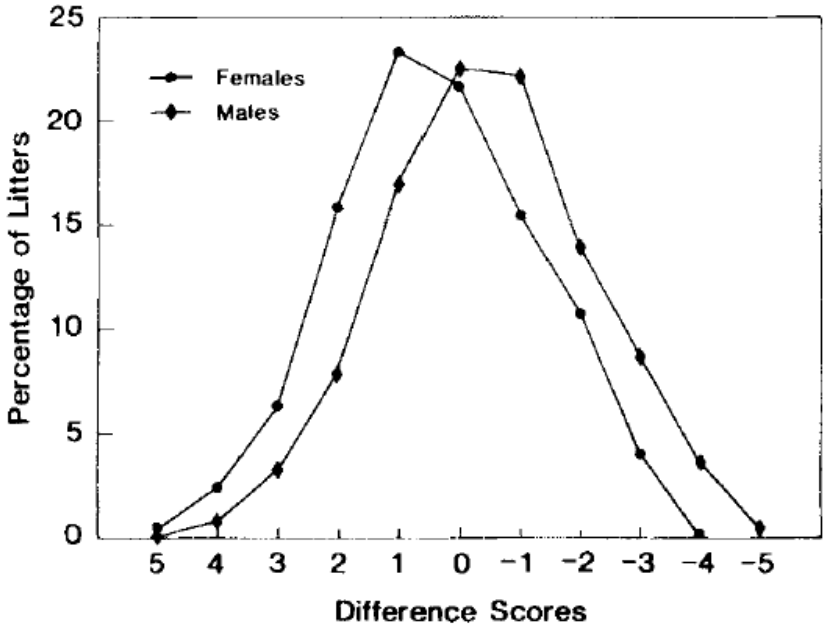
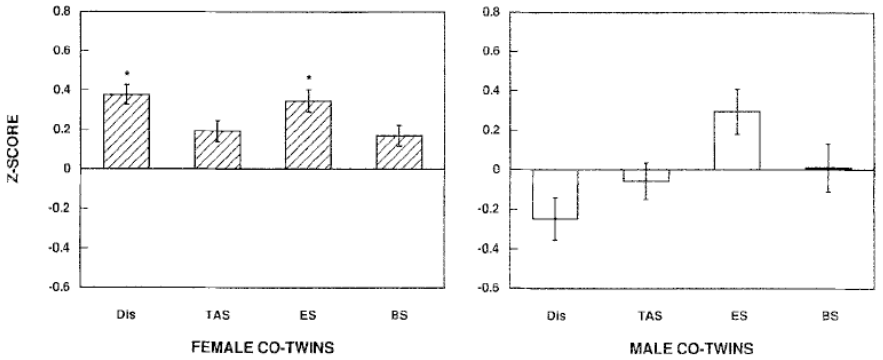


Figure 15.5. Female Mongolian gerbils produce more females on left and more males on right uterine horns. (from Clarke et al. 1991).

Are organizational effects present in human fraternal twins?

Fraternal twins in humans afford the opportunity to study the effect of prenatal hormones (E from female co-twin on brother and T from males on sister). Male and female humans generally differ on many

Figure 15.6. Effect sizes for age-adjusted sensation seeking scale (SSS) for female and male opposite-sex twins compared to same-sex twins. Effect sizes were based on age-adjusted scores and were calculated separately for females and males as mean *z* score for opposite-sex twins – mean z score for same-sex twins)/standard deviation for the same-sex twins (SE are shown). SSS subscale abbreviations: Dis, Disinhibition, TAS, Thrill and Adventure Seeking; ES, Experience seeking; BS, Boredom susceptibility.



psychosocial scales and one pronounced measurable difference is "thrill-seeking behaviors" typically thought to arise under the organizing effect of testosterone. Female co-twins differ significantly from a large group of female-female twins. Thus, positive z-scores of co-twin females with a brother reflect higher scores than the control a large group of co-twin females with sisters (e.g., the zero line). In contrast, male co-twins with a sister are not consistently different in the four metrics used to categorize thrill seeking. Thus, foetal exposure to T generates a more male-like behavioral profile with respect to psychosocial behaviors in humans (Fig. 15.6).

Ontogenetic conflict: the traits under selection in each sex

Males and females reflect the core morphs of sexual species. Recent advances in our understanding of life history trade-offs have identified different patterns of selection on the sexes as a source of additive genetic variation (Rice and Chippindale 2001). Genetic trade-offs that promote functional trade-offs in organismal design between the sexes are referred to as **intersexual ontogenetic conflict (OC)** or **intralocus**

OC (Rice and Chippindale 2001). Alleles that are favored due to sexual selection on male morphology and physiology are of limited value during natural selection on female morphology and physiology, and vice versa (OC was also discussed in Chapter 11).

Alleles should reach an optimum in each sex were it not for the fact that females and males repeatedly hybridize; they share genes in a common genome, except sex chromosomes (Y is restricted to males and X is found in females 2/3 more often than males; Gibson et al. 2002). Sex-limiting steroid hormones that govern female and male traits can ameliorate OC (Sinervo and Calsbeek 2003), or OC loci can be sequestered on sex chromosomes (Gibson et al. 2002). Gene promoters (e.g., HREs, see above) differentially control transcription and translation in the sexes (Freedman and Luisi 1993; Zajac and Chilco 1995; Sanchez et al. 2002). Sex chromosomes, which initiate sex determination via gene cascades (e.g., *sry*, *sox9*), are unlinked to autosomal genes where HREs reside.

Most life history analyses are restricted to one sex (i.e., female). The action of OC is rarely studied, despite its importance to life history. Demonstrations of OC are restricted to studies of fruit flies in the lab (Pishcedda and Chippindale 2006), or natural systems with pedigree on both sexes [red deer (Foerster et al. 2007), lizards: (Calsbeek and Sinervo 2004; Sinervo and McAdam 2007)]. OC can be revealed as a negative genetic correlation between fitness of sires *vs*. daughters (Foerster et al. 2007) (Fig. 15.8), or reciprocal lines of descent (dam-son, sire-daughter, Pischedda and Chippindale 2006) (Fig. 15.7). Despite use of the term *intralocus OC*, no study has yet tied conflict to one gene. Pedigree studies can reveal specific traits under OC [e.g., clutch size: (Sinervo and McAdam 2007), male size (Calsbeek and Sinervo 2004), or dorsal pattern (Forsman and Appelqvist 1995; Lancaster et al. 2007)]. If gene maps were constructed for pedigrees, we could resolve the loci that generate OC due to either *intrinsic* epistasis (e.g., hormone-gene promoters) or *extrinsic epistasis* (e.g. variation in optima for sender-receiver loci in each sex). Another example of a specific trait under ontogenetic conflict was given in Chapter 11 (body size of male *Uta*).

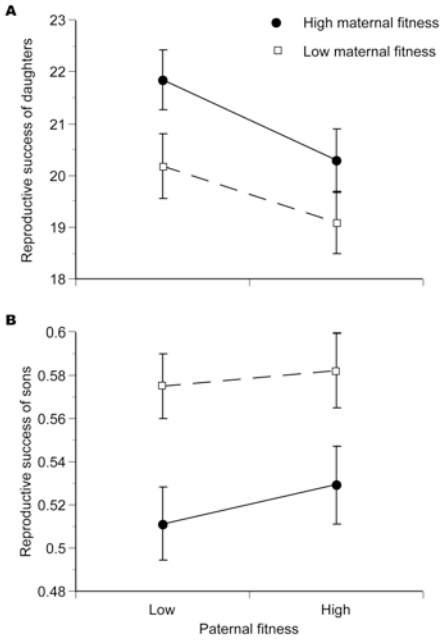
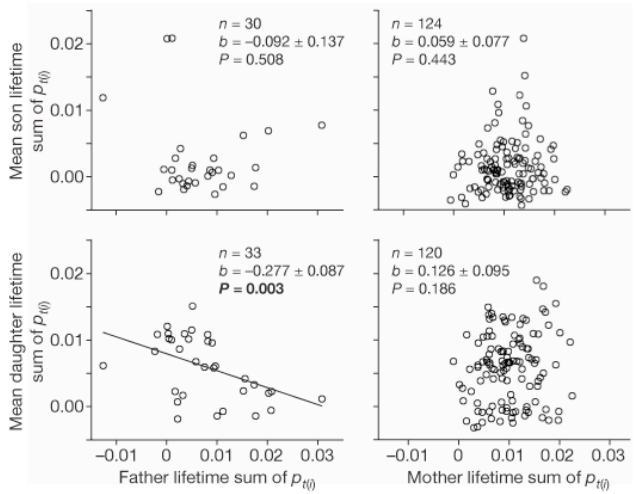


Figure 15.7 The fitness of daughters (A) versus (B) sons in lines selected for either high versus low maternal fitness or high versus low paternal fitness. Notice the conspicuous negative correlation across the sexes in both maternally and paternally selected lines of Drosophila melanogaster (Pischedda and Chippindale 2006).

Figure 15.8 The fitness of sons versus daughters expressed as a genetic correlation with mother versus father's liftetime reproductive success in the red deer, *Cervus elaphus*. Notice the conspicuous negative genetic correlation between fitness across the fathers and their daughters. (from Foerster et al. 2007).



Mate choice and maleness and females

Drickamer et al (2001) tested whether male and female house mice are choosy about the kind of female or male with which they associate. In an elaborate field experiment, they "baited" traps with the scent from a 2M male, 0M males, 2M females and 0M females (based on high measures of Anogenital distance AGD or low AGD) and then measured which type of male and female was caught in the traps (Table 15.1).

They made explicit predictions for each sex. In particular, females should prefer males with a larger AGD because they defend larger territories, with more resources, and are better parents than males with smaller AGD. In addition, males should prefer females with smaller AGD because these females produce more offspring and are better parents than females with small AGD. They also predicted that males should also avoid males with a larger AGD, and females should avoid females with a larger AGD because these mice are more aggressive than each of the respective sexes with small AGD. The results of the baiting experiment confirmed all of these predictions. Thus, maternal positional effects are potentially under strong mate choice in natural populations.

Table 15.1. Mean (± SE) ratios for captures in odorized traps/available odorized traps for female and male house mice of high and low anogenital distance (AGD) responding to traps odorized by males and females of high and low AGD. Higher ratios indicate a greater tendency for mice to be captured in traps of a particular odor type by sex and AGD category (Drickamer et al. 2001).

	Male odors		Female odors	
	High AGD	Low AGD	High AGD	Low AGD
Females as responders				
High AGD	0.148 (0.017) $t = -5.04$, $p < 0.0001$	0.059 (0.010)	0.049 (0.007) $t = 3.22$, $p = 0.0029$	0.084 (0.008)
Low AGD	0.136 (0.011) $t = -3.88$, $p = 0.0005$	0.072 (0.010)	0.052 (0.008) $t = 3.00$, $p = 0.0055$	0.088 (0.009)
Males as responders				
High AGD	0.083 (0.013) $t = 2.63$, $p = 0.0150$	0.117 (0.014)	0.076 (0.008) $t = 4.09$, $p = 0.0005$	0.125 (0.012)
Low AGD	0.044 (0.012) $t = 7.21$, $p < 0.0001$	0.163(0.022)	0.068 (0.017) $t = 7.14$, $p < 0.0001$	0.178 (0.013)

Testosterone and Yolk in Birds

Female canaries have been shown to deposit testosterone into yolk, and the amount of T that the females deposit in eggs varies with the order of laying rather than as a positional effect. The hormone testosterone is lipophilic, thus it is readily put into the lipoprotein matrix during egg production in females (either actively or passively as yolk is pumped into the eggs by nurse cells).

The amount of testosterone put into eggs was independent of the sex of the offspring. Females increased yolk T on later laid eggs (Fig. 15.9).

Such hormones have a dramatic effect on offspring dominance or social rank. Schwabl (1993) scored social rank by measuring:

1. the order in which birds started to feed after food deprivation,
2. the frequency with which individuals supplanted each other from the food dish.

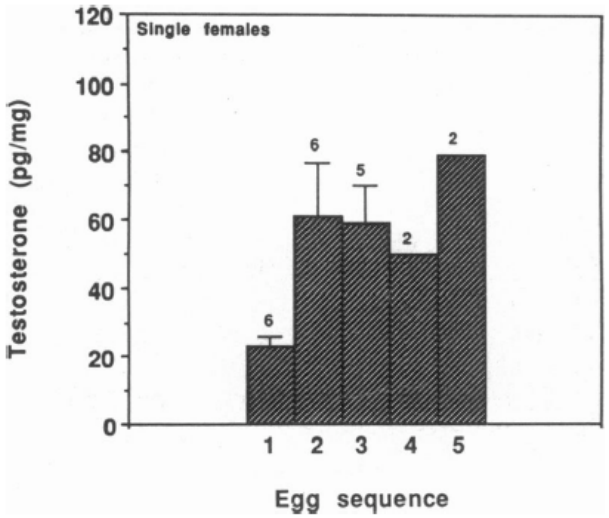


Figure 15.9. Testosterone concentrations (picograms per mg of yolk; means ± SE) of eggs laid by three female canaries kept without a male as a function of the order in which eggs were laid (Schwabl 1993).

Both male and female offspring experienced elevated social rank if they received an extra dose of testosterone from the mother (Fig. 15.10).

This yolk has a very strong adaptive consequence for the progeny. Late laid eggs will likewise hatch later than their sibs, and thus their sibs will already be larger. Thus, the yolk T makes the later hatched young more aggressive and more able to fight for resources in the nests. A number of subsequent studies have shown that females adjust levels of yolk T in response to the attractiveness of the male linking this maternal effect to sexual selection and mate choice.

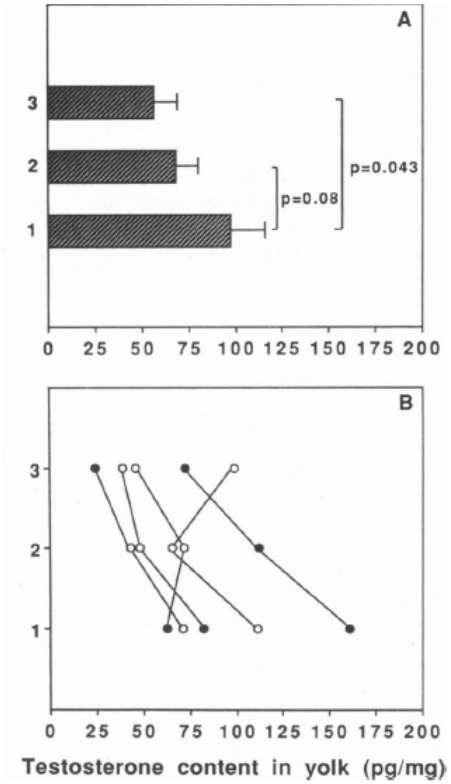


Figure 15.10. Concentration of maternal T (means ± SE) measured in eggs from which sibling juvenile canaries of different social rank hatched. High (1), intermediate (2) and low (3) social rank was assigned from observations of access to food. Levels of significance of T egg concentration between birds of different ranks are indicated next to brackets. T concentration of eggs and the social rank of the individual birds of each of these cohorts. Note the variable composition of the groups of males (●) and females (○).

Environmental Sex-Determination in Reptiles

Many turtles, lizards, crocodilians, and a few snakes have a form of sex determination that depends on the incubation temperature of eggs. Given that the female is responsible for the location in which she buries her eggs, she can control the sex ratio of her clutch. For example, some turtles have eggs that turn into males when they are incubated at low temperatures, and females when they are incubated at high temperatures. There is also a temperature at which the eggs develop into males and females with a 50:50 ratio. This temperature is referred to as the threshold temperature. Other species of turtles produce males at low temperatures, females at intermediate temperatures, and males again at the highest temperature.

Fred Janzen (19950 produced snapping turtle eggs and hatchlings that were incubated at:

1. the male temperature (e.g., only males were produced in these clutches),
2. the female temperature (e.g., only males were produced in these clutches) and
3. at the critical temperature (e.g., 50:50 ratio).

Janzen then released these turtles into the wild and assayed their survival. Those, hatchlings that were produced at the all male temperature or the all female temperature had higher survival than the males and females produced at the critical temperature (Fig. 15.11).

If females lay eggs in a nest that will be largely exposed to the critical temperature, those offspring will be at a severe disadvantage compared to offspring at either the all male or all female temperature. He speculates that the

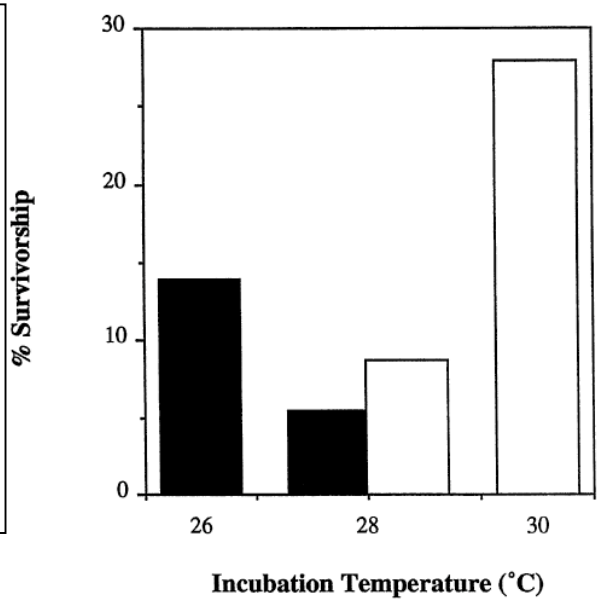


Figure 15.11. Relative survivorship of hatchling snapping turtles as a function of gender and incubation temperature. The solid bars and female hatchlings indicate male hatchlings by the open bars. Note that female turtles from high temperatures and male turtles from low temperatures both have higher survivorship than either of their consexuals from the intermediate incubation temperature. (from Janzen 1995).

offspring that are right at the critical temperature may be incurring developmental problems because they are on the knife-edge of becoming male or female. In contrast those that are at definitive male and female temperatures have a more "stable development." Thus, females should produce either an all male clutch or an all female clutch.

In another species of turtle (actually a box turtle), Wilhem Roosenburg (1996) has found that females that lay small eggs should lay in places were those eggs will develop into males. Small eggs do not reach a very large size at maturity, and male turtles do not have to be all that big -- even a small male can fertilize a female. There isn't a premium on male size that might arise for male-male contests. In contrast, fitness of female offspring at maturity is directly dependent on how large the turtle is at maturity -- bigger females produce more offspring. If a mother is going to produce a clutch with very large eggs, then she should lay those eggs in a warm place where they will develop into female offspring. Roosenburg speculates that female nest site selection should be plastic depending on the size of a female's eggs. If she has big eggs, perhaps because it was a good year for her, then she should produce a female biased clutch. If she has small eggs, perhaps because it was bad year, then she should produce a male biased clutch.

Sex-ratio adjustment can also occur via environmentally induced maternal affects with adaptive consequences. Progeny gender is under environmental influence in numerous species of reptiles (Harlow 2000, Harlow & Taylor 2000, Elf *et al*. 2002, Milnes *et al*. 2002, Shine *et al*. 2002) and is thought to have an adaptive explanation (Shine 1999). For example, turtles are able to manipulate progeny sex by varying the depth at which eggs are buried in nests (Packard *et al*. 1987, Morjan & Janzen 2003). The adaptive significance of environmental sex determination has been a subject of much debate (reviewed in Shine 1999), but it is generally thought that females can maximize their fitness by giving progeny an opportunity to develop into the gender that will perform best given the environmental conditions. One problem with this argument is that temperature-dependent sex determination will necessarily lead to a confounding effect between environmental and progeny gender effects. Shine (1999) points out, and we (Calsbeek and Sinervo 2008) agree, that hormonal manipulations that override temperature effects will be an important next step towards understanding the fitness effects of sex-ratio adjustment in these taxa. Females should manipulate the sex ratio of her clutch to ameliorate the potential ontogenetic conflict arising from both her genotype and the genotype of her sire (Calsbeek and Sinervo 2008). For example, in turtles, females are often larger and thus a small-bodied female might oviposit in soil with a temperature that will generate all-male broods. Conversely, a female that mated with a large male might oviposit in soil with a temperature regime that will generate all-female broods.

Homosexuality in Humans

The biological determinants of homosexuality in humans or other forms of gender preferences/sexual orientation like transexuality, bisexuality or lesbianism are the subject of heated debate in our modern culture. While little data is available on the latter human predispositions, recent data on homosexuality provides support for at least two biological causes that might play a role in homosexuality. The reason data is available on homosexuality may be because the prevalence of the behavior is purported to be relatively high (up to 8-10%), which is interesting.

i. Genetic determination. Family studies of brothers and twins report that homosexuality is more common in brothers of homosexual subjects (Bailey and Zucker 1995). A study by Hamer et al. (1992) reported an elevated levels of homosexuality in maternal lines. Gene mapping studies (Hamer and Copeland 1995) suggested the role of a genetic factor isolated to the distal short arm of the X chromosome (region Xq28). In an attempt to repeat this work, Bailey et al. (1999) were unsuccessful casting doubt on a simple genetic basis for homosexuality.

ii. Immunoreacton hypothesis. A classic finding that has been repeated in several studies is a birth-order effect. Later born sons have a higher incidence of homosexuality than early-born sons. Blanchard (1997) posited quite that such an effect might arise from an immunological reaction of a mother to foreign male-function proteins to which she had not been previously exposed during her own development.

Side Box 15.1. Alert – Endocrine disruptors in your drinking water

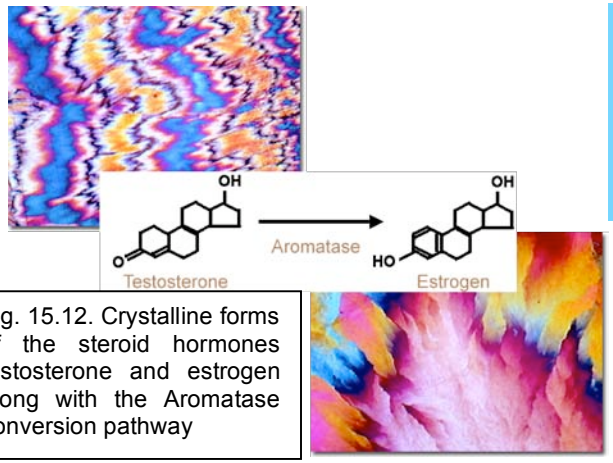


Fig. 15.12. Crystalline forms of the steroid hormones testosterone and estrogen along with the Aromatase conversion pathway

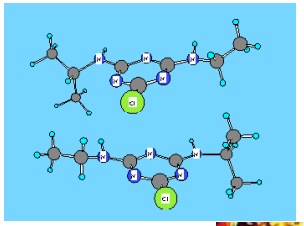


Fig. 15.13. Molecular structure of the weed killer Atrazine, an endocrine disruptor, and its crystalline form.

In a landmark paper, Tyrone Hayes of UC Berkeley raised leopard frog tadpoles under conditions of Atrazine measured in the parts per billion and compared gender development to controls (in normal water). The EPA considers these levels of Atrazine safe, and levels around agricultural areas where the weed killer Atrazine is used routinely exceed several parts per billion in the ground water. Male tadpoles treated with Atrizine developed a **gynandromorphic** testes with both male spermiogenesis and female ovagenesis occurring side-by-side. Levels of T produced by mature males that were treated with Atrazine were comparable to levels produced by females and far lower than levels of T produced by control males.

Why should you be concerned? In humans, across all industrialized nations, sperm production in males has declined by 50 percent since the 1950's due to an unknown cause. It does not take a chemical engineer to make the link that something might be happening to our drinking water.

The way endocrine disruptors like Atrazine work is quite insidious. Disruptors compete for active sites on aromatase, or on binding sites on the Estrogen Response Elements (ERE) or other hormone response elements (HRE), which are protein complexes that carry E or steroid hormones like T to DNA to trigger gene transcription. Needless to say the chemical industry was not amused and they attacked Tyrone Hayes' work. Nevertheless, the effect of chemicals in the environment could have potent effects on gender development in vertebrates. Environmental estrogens have been purported to affect gender development in alligators and other vertebrates like frogs.

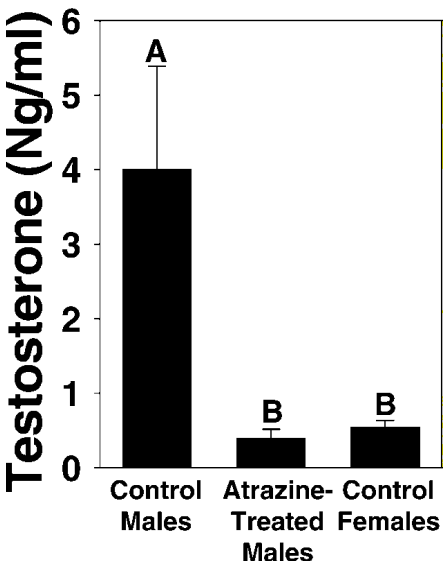
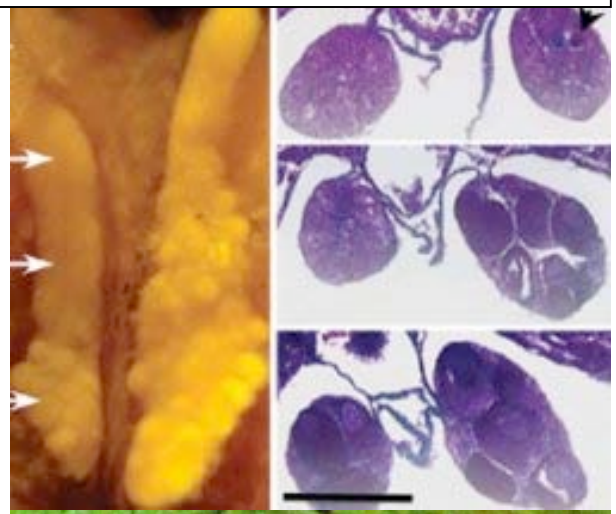


Fig. 15.14. Testosterone production by Control males, Atrazine treated males, and control females (ng/ml). (from Hayes et al. 2002).

Fig. 15.16. Photograph of a leopard frog that was exposed to the weed killer Atrazine in parts per billion concentrations illustrating the development of ova on the male testis. A section through the testis at the levels illustrated by the arrows clearly shows the presence of yolking ova on the testis.



Are you sure your drinking water is safe?

Naturally any gender modifying substance might have impacts on human sexual orientation, but these links have not yet been investigated.

Table 1 5 . 2. Reported fecundities of subjects' relatives from the maternal and paternal lines (p-Value calculated using the Mann–Whitney test; n.s., not significant.)

		homosexuals			heterosexuals			
relative class	likelihood of sharing X chromosome	N	mean fecundity	s.d.	N	mean fecundity	s.d.	p
mothers	1	98	2.69	1.30	100	2.32	1.05	0.02
first-borns' mothers	1	32	1.94	0.88	52	1.77	0.61	n.s.
maternal aunts	0.75	95	1.98	0.98	121	1.51	0.97	0.001
maternal uncles	0.25	114	1.75	0.91	117	1.73	0.94	n.s.
maternal grandparents	0.5	91	3.55	2.57	100	3.39	1.85	n.s.
sons and daughters of maternal grandparents [a]	0.25–1	307	2.17	0.85	338	1.83	0.72	0.001
paternal aunts	0	111	1.75	1.07	129	1.94	1.13	n.s.
paternal uncles	0	134	1.75	0.95	135	1.67	0.94	n.s.
paternal grandparents	0	88	4.03	2.72	99	3.81	2.43	n.s.
sons and daughters of paternal grandparents (excluding father) [b]	0	239	1.77	0.85	264	1.80	0.89	n.s.

[a] Cumulative fecundity of mothers, maternal aunts and maternal uncles.
[b] Cumulative fecundity of paternal aunts and paternal uncles.

In the immunological hypothesis, specific male-function proteins that are triggered by *sry*, *sox9*, or some other gender specifying gene, which are produced by the growing male foetus. Thus, foreign proteins from the 1st sons of a female leak across the placenta. In the female's bloodstream these foreign proteins elicit an immunological response. Levels of antibodies buildup in females such that in later born sons, antibodies travel across the placenta from the mother and bind to these male proteins altering their efficacy in effectuating organizational events and thereby altering sexual orientation. One of the primary hypotheses for a male-function protein expression would of course reside in the brain. Early studies seem to suggest that there are indeed differences in brain morphology of homosexual and heterosexual males, however, these studies were conducted on AIDS patients in the homosexual group, and thus it is unclear whether the neurological differences are due to AIDS or homosexuality *per se*.

Homosexuality presents a Darwinian paradox of sorts if the genetic hypothesis is true. That is a genetic factor for homosexuality ought to decrease in the population, if homosexuals produce fewer progeny. Given old stigmas regarding the expression of the behavior in many human cultures (at least modern cultures), the age of outing however, for many homosexuals is often well after the period during which progeny might be produced. Thus, this paradox is only weekly supported by modern behavioral observations, at least those 'outings' that are anecdotally reported in the press.

Camperio-Ciana et al. (2004) sought to replicate the previous work with an ingenious survey distributed outside nightclubs that were frequented by homosexuals and other nightclubs that were frequented by heterosexuals. In their study, the asked information on patrilineal and matrilineal fitness measures (e.g., number of brothers, sisters, male and female cousins from either the mothers side or the fathers side). Moreover, they also asked the basic question of sexual orientation. They found that homosexuality was not only consistent with a combination of the *genetic hypothesis* and *immunological reaction hypothesis*, but also that *ontogenetic conflict* of the sexes also explained the evolutionary persistence of homosexuality in humans (Rice 1999).

Female lineages in which homosexuality was prevalent had higher fertility compared to lineages in which homosexuality was absent (Table 15.2). This finding suggests that a gene may control homosexuality and that the fitness benefit of high fertility is balanced by the reduced fitness of homosexual sons due to the predicted effect of the behavior on the likelihood of paternity of a homosexual that expressed this behavior early in life. The maternal effect (putative genetic) contributed to 14.7% of the variation and birth-order contributed to 6.7% of the variation in homosexual *versus* heterosexual orientation. Therefore, they also suggest that there is considerable unexplained variation, which might be attributable to other as yet unidentified routes of transmission such as a culturally inherited route (see chapter 5 on Darwin finch bird song for an example of cultural transmission). Camperio-Ciana et al. (2004) also point out that the immunological hypothesis is consistent with it arising as a consequence of the genetic effect on fertility. Higher fertility in

females would be predispose them to produce more sons and thus would incur a greater incidence of immunological reaction to sons on later births.

Activational effects and contro of secondary sexual characters

Sexual differentiation and the acquisition of secondary sexual characters is a two step process in which the embryo is organized by the effects of steroids, and then at maturity, the organism's development of secondary sexual characters are activated. I will describe three case studies that illustrate the development of alternative males, development of male bird song, and development of mammalian parental care. The example of the stoplight parrotfish sex change or the alternative male phenotypes in the midshipman is useful because it illustrates that distinctions between maleness and femaleness are often quite fuzzy. Development of bird song in males is demonstrative of the activational effects on neural circuitry. Finally, the example of mammalian maternal care illustrates that a single gene that affects the nervous system, although the gene seems to control a suite of maternal behaviors, can influence some complicated female behaviors.

The Stoplight Parrotfish Sex change

The stoplight parrotfish (*Sparisoma viride*) undergoes a sex change late in life. All individuals start out as females and then later transform in to males. This species is referred to as a **protogynous hermaphrodite**. The little twist in this story is that some females transform into terminal phase territorial males. Other females transform into sneakers first, then they transform into terminal phase males.

When a female changes into a male (any kind), estradiol levels decrease and levels of 11-ketotestosterone increase. Females that have transformed into terminal phase males maintain very high levels of 11-ketotestosterone, whereas females that have transformed in sneakers maintain moderate levels of testosterone, and estradiol. Indeed, injections of 11-ketotestosterone cause females to transform into males by changing gonads and color. Additional details on this example are found in Chapter 9. This example is also a model of how organizational events are not just restricted to embryos, but can also occur during sex change later in life.

Alternative Males in Plainfin Midshipman Fish

Male Plainfin Midshipman Fish come in two alternative male phenotypes (Bass, 1996):

1. A larger Type I male that actively defends a nest and attracts females with a humming vocalization, and
2. a smaller, female-sized Type II male, that has extra large testes and does not defend a nest, but attempts to sneak into a Type I male's nest and fertilize a spawning female's eggs. If he cannot get into the nest, he sits at the entrance, ejaculates sperm, and fans it into the nest.

Levels of testosterone control the difference in these secondary sexual characters:

1. Type I males have the 5 times more testosterone than type II males, and they type I males also have a distinctive kind of testosterone, 11-ketotestosterone.
2. Type II males have modest levels of testosterone but no 11-ketotestosterone.
3. and female have hormones like type II males with the addition of estradiol.

Bass and his colleagues have found that a suite of morphological and neural changes are associated with the alternative males and seem to be regulated by levels of testosterone:

Type I males develop:

1. a large sonic muscle which is responsible for the singing,
2. an elaborate vocal-acoustic neural circuit in the brain.

While the sonic muscle is only found in Type I males, the neural circuits are found in both Type I males, Type II males, and females. What differs among the three is the pacemaker-motor neuron circuit that fires at a frequency that is 15 to 20 percent higher in Type I males compared to Type II males or females. The nerve cells and cell bodies of this circuit are also up to three times larger in Type I males. This song is necessary to attract females.

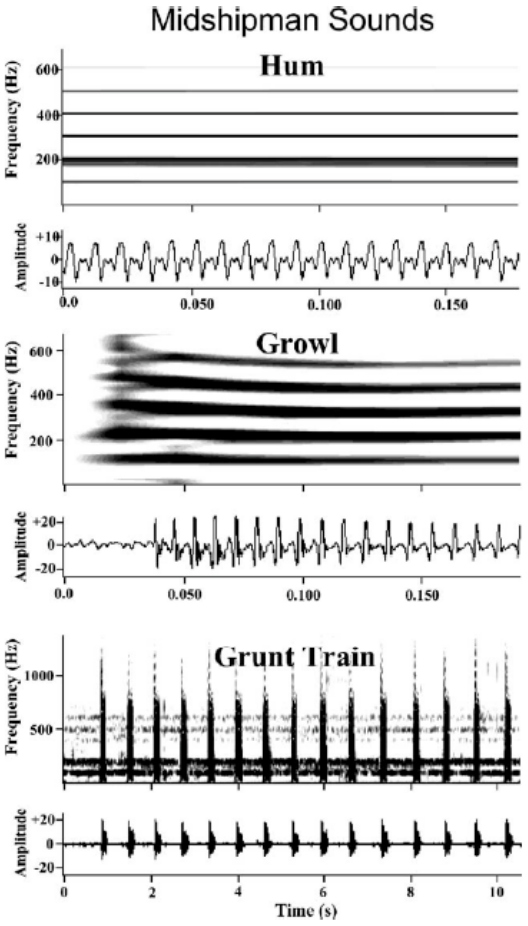


Figure 15.17. Acoustic signals of the plainfin midshipman fish, *P. noctotus*. Shown here are spectograms (top) and oscillograms (bottom) of a representative hum, growl, and grunt train from the nest of a type I male.

Development of Song in Birds

Sex differences in passerine birds are striking in that males typically sing and females usually do not (though see exceptions, Chapter 8, 9). Testosterone again is implicated in this fundamental difference in behavior, which raises the following questions. How are the neural circuits shaped during the development of secondary sex characters?

In considering the effects of testosterone it is important to note that testosterone *per se* is not the potent androgen, rather testosterone is metabolized into estrogen by the enzyme aromatase.

1. During early vertebrate development, the testes produce a small quantity of testosterone.
2. This testosterone is circulated by the bloodstream and organizes many aspects of neurodevelopment.
3. When the testosterone reaches the brain, it can be converted to estradiol by the enzyme aromatase, or to 5-alpha-dihydrotestosterone by 5 alpha reductase.
4. Testosterone or estradiol binds to a steroid protein carrier, which then forms a complex that can further bind directly to DNA and affect DNA synthesis.
5. Differences in DNA synthesis among cells in the brain lead to growth or death of specific neural circuits.

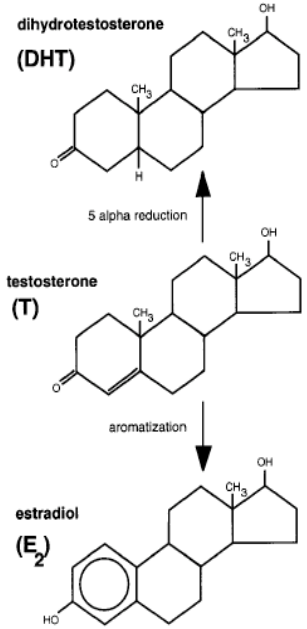


Figure 15.18. Testosterone is a major gonadal steroid hormone, from which the androgenic metabolite dihydrotestosterone (DHT) and the estrogenic metabolite estradiol (E2) are derived. Testosterone can act either directly on cells, or via either of these two metabolites.

For example, the effects of estrogen derived from aromatase's action on testosterone, stimulates the growth of brain regions directly linked to song production in male birds (See Side Box 15.2). The general action of these hormones is to masculinize or de-feminize regions of the brain necessary for male functions.

Other "male functions" ascribed to the organizational effects of testosterone includes elaboration of the **hippocampus**, which is related to spatial learning. Presumably the spatial learning is necessary for developing and holding a large territory. Because most male birds are territorial and females are not as territorial, males should have more processing capability in this regard.

It is not invariably the case that only males sing and only males need elaborate spatial maps. Many songbirds have females with fairly elaborate song repertoires. In such species one does not find the same kind of sex differences in neuroanatomy as in species with male-only songs. Presumably, the similarities between males and females in these species result from alterations in the basic vertebrate program of organizational effects.

In addition, in some species, like the brown-headed cowbird, females develop a larger hippocampus than males and this seems to be related to the spatial demands placed upon the females. Cowbirds are parasitic on the parental efforts of other bird species. They lay their eggs in other bird's nests and let the host raise their offspring. Males have no need for the spatial maps that females develop during the searches for host nests.

Thus, females have evolved a more elaborate hippocampus. Again, species-specific sex differences in neural development presumably result from some alteration in the basic program of organizational effects, which has yet to be described for the cowbirds.

In summary, the early action of testosterone or its metabolites serves to organize the brain so that it has the proper neural circuitry for later acting activational events.

Estrogen treatment of females in their youth can masculinize them and cause females to sing songs (e.g. it looks like the critical date for zebra finches is 3 to 10 days after hatching). Males get their high dose from testosterone that is aromatized to estrogen. The concentrations of estrogen in the brain can be very high.

Specific regions of the brain have very high levels of aromatase and corresponding high levels of estrogen. In particular, a region adjacent to the higher vocal center (HVC) has high concentrations of estrogen. It appears that high levels of T and then E, trigger growth and proliferation of neurons in several specific areas that have been identified in the song circuit of birds.

Testosterone and trade-offs between Singing, Polygyny, and Care

Testosterone is clearly implicated in the development of male song in juvenile birds. In addition, levels of testosterone in adult birds are also correlated with levels of parental care and the tendency to monogamy or polygamy. Species of birds that can maintain high levels of testosterone during the entire reproductive season also tend to be quite polygamous. By maintaining high levels of T, these birds keep singing and courting additional females. These males are not the best parents however, as they usually leave the female to rear the young (Wingfield et al. 1990).

To investigate these correlational pattern, Ketterson and her colleagues (reviewed in Ketterson and Nolan 1994) picked a largely monogamous species, the dark-eyed Junco, in which males and females provide parental care. They implanted half of the males with T, and the other half with sham implants. True to form, the T-implanted males sang more, ranged farther, and tried to court more females, all at the expense of parental care. The females of T-implanted males were left to carry the burden of care that normally is split between the male and the female. In the long run, such decreased care on the part of the male might be expected to cause the female to work harder and perhaps experience greater costs of reproduction. The trade-off between monogamy and polygyny in birds arises from a simple hormonal change -- T secretion.

Side Box 15.2.
Development
of song circuits

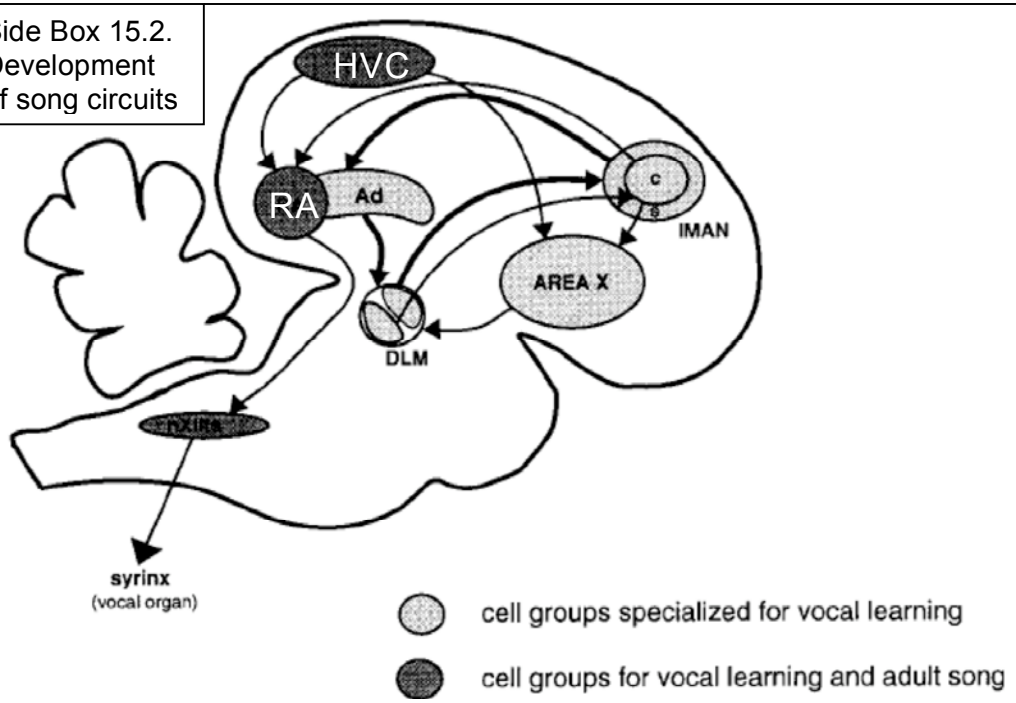


Fig. 15.19. Sagittal diagram showing some of the major circuits for song control. The circuitry that controls song learning and behavior can be conceptualized as consisting of two broad functional categories. For example, the projection from the HVC to RA is involved in the production of stable song in adult birds and is believed to play a role in later stages of vocal learning. The projection from HVC to Area X leads into a set of circuits that are apparently specialized for one or more aspects of vocal learning, but do not play a role in adult song production. Within this latter category, two major forebrain loops are shown: the $IMAN_{shell} \rightarrow Ad \rightarrow DLM$ pathway and the $IMAN_{core} \rightarrow X \rightarrow DLM$ pathway. Many other circuits are omitted for the sake of clarity.

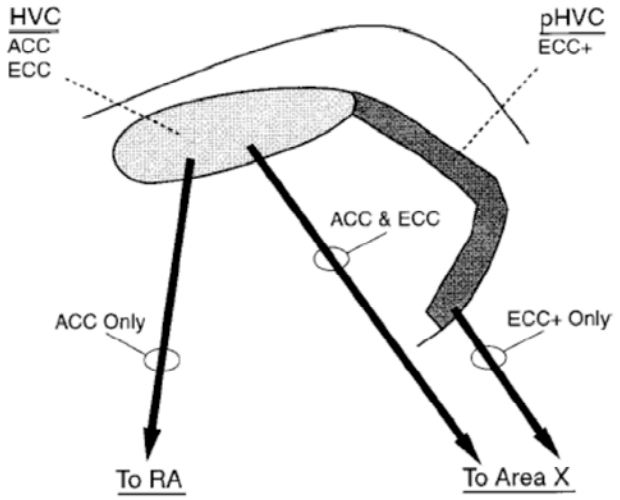


Fig. 15.20. Schematic coronal section of the HVC-paraHVC complex showing the hormonal sensitivity of different projection pathways in adult male canaries. DHT and E_2 are selectively concentrated by projection neurons from HVC and pHVC to separate efferent targets. RA-projecting neurons in the HVC concentrate androgenic hormones (DHT) exclusively, whereas X-projecting neurons in the HVC concentrate androgenic and estrogenic (E_2) hormones. ParaHVC contains only X-projecting neurons (no RA-projecting neurons), and these are exclusively estrogen-concentrating. ECC = estrogen-concentrating cells in the HVC (which are lightly labeled); ACC = androgen-concentrating cells in the HVC; ECC+ = estrogen-concentrating cells in the paraHVC (which are heavily labeled). (after Johnson and Bottjer 1995).

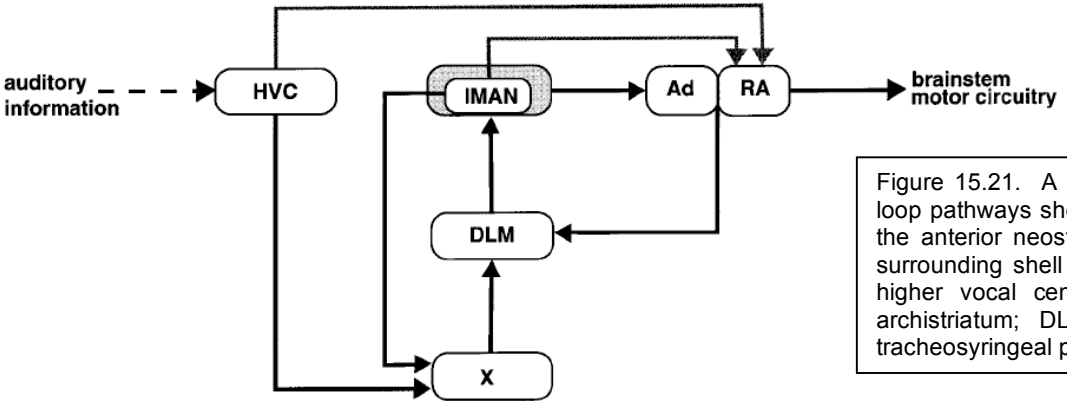


Figure 15.21. A schematic diagram highlighting the connections of the two closed-loop pathways shown above in (Figure 15.19). IMAN-lateral magnocellular nucleus of the anterior neostriatum, which is divided into a core (c) magnocellular region and surrounding shell (s) parvocellular region; X = Area X of the avian striatum; HVC = higher vocal center; RA = robust nucleus of the archistriatum; Ad = dorsal archistriatum; DLM = medial dorsolateral nucleus of the thalamus; nXIIts = tracheosyringeal portion of the hypoglossal nucleus.

Development of Maternal Behavior in Mammals

The hormones involved in the development of maternal behaviors in mammals are linked to the hormone changes in both progesterone and estrogen. Progesterone levels are typically quite high during pregnancy, but as the date of birth approaches, progesterone levels begin to fall and estrogen levels begin to rise. It is the synergistic action of both progesterone and estrogen during birth that seems to trigger maternal behaviors in female mice. Estrogen also acts synergistically with oxytocin (see Chapter 11) to stimulate milk production. High levels of estrogen and low levels of progesterone triggers nest building increases 4-6 days prior to birth. Simply presenting pups to virgin females or to males can induce the above maternal behaviors. The presence of pups triggers the following maternal behaviors:

1. retrieval response of pups when the pups are not in the nest,
2. the nursing crouch of the female.

What controls these nurturing maternal behaviors?

New evidence suggests a very simple neural pathway may be involved. Brown and her colleagues have succeeded in creating a mutation in a single gene, *fosB* that appears to extinguish nurturing behaviors in female mice. The hypothalamus in female mice has been shown to be critical for nurturing behavior in lesion experiments. It appears that the gene products of *fosB* are expressed in the preoptic area, which is located in the hypothalamus. *FosB* deficient moms do not show the following nurturing behaviors (see Chapter 2):

1. they do not retrieve young, despite normal maze running ability.
2. they do not nurse young, despite normal mammary gland.
3. *FosB* moms have normal expression of Estrogen/Progesterone, and normal olfactory abilities. They just do not nurture young.

The trigger for *FosB* expression in the preoptic area of the brain in normal mice is exposure to pups (see Chapter 2). The olfactory triggers of this neural pathway are particularly important. This is true for both males and females. The mere presence of pups can trigger such filiative behavior. An incredibly interesting, but as yet completely unstudied route of filiative behavior, has to do with the levels of filiative behavior being transmitted across generations. Progeny that experience filiative behavior in their youth may be more likely to be filiative in their adulthood towards their own pups and vice versa. This is not due to transmission of genes, but from the expression of filiative behavior per se. A cross-fostering design in a species that was polymorphic for paternal an/or maternal filiative behavior would be quite interesting (see also Chapter 11 for the role of Vassopressin and Oxytocin).

Epistasis and the origin of epigenetic effects

THE FOUR LEVELS OF EPISTASIS IN SOCIAL SYSTEMS

Ideas on epistasis are unified. Gene products build physiological systems, physiological systems interact to create signaler-receiver molecules of endocrine systems, endocrine systems control expression of multiple genes, signalers and receivers interact in social systems, and multiple genes (with variants at each locus) interact to create fitness surfaces and perhaps fitness epistasis (assessed by CS). Physiological and behavioral epistases are unified; each involves senders and receivers. Physiological epistasis is *intrinsic*, while behavioral epistasis is *extrinsic* to organisms. Steroid hormones and DNA receptors such as hormone response elements [HRE] are a core class of intrinsic signals (Freedman and Luisi 1993; Sanchez et al. 2002; Zajac and Chilco 1995). The ontogeny of gene expression also has signaler and receiver molecules that are core components of developmental systems.

Resolving any form of epistasis (Fig. 3B) involves a cross-product term (see Chapter 3) [gene epistasis: (Cordell 2002), fitness epistasis: (Whitlock et al. 1995), epistasis under FDS: see supplement to (Sinervo and Calsbeek 2006), or endocrine epistasis (Lancaster et al. 2007)].

1. Genetic epistasis: non linear interactions among alleles at many loci

Genetic epistasis is related to physiological epistasis (Sinervo and Calsbeek 2003; Sinervo and Svensson 2002). In the case of gene epistasis, one gene interacts with another, perhaps shutting off that gene or alternatively, amplifying products in non-linear way (Cordell 2002).

Epistasis of interacting endocrine systems generates non-linear gene and physiological effects. Products of one allele may shut off other gene function or initiate a cascade of genes. For example, sex-determining loci induce testis development and turn on genes for maleness (i.e., *sry* gene interacts with *sox9*) (Koopman et al. 2001).

2. Physiological and behavioral epistasis: sender-receiver interactions

Sewall Wright (1968) considered physiological epistasis (physiological or endocrine cascades) to be a universal property of genetic systems (Wade 2002). He theorized that genetic variation in epistatic networks destabilized organismal function, and that epistatic variation is fixed in most species owing to negative fitness effects. As Wright suggested, epistasis may be fixed in species lacking morphs. However, sexual species have male and female morphs, and Wright's conjecture for epistasis is likely invalidated under strong sexual selection (e.g., the ontogenetic conflict of the sexes is discussed below). Moreover, mutations in and evolution of signaler-receiver genes that control development must generate transient epistasis that ultimately becomes fixed to stabilize a phenotype (e.g., canalization).

Any species with alternative morphs within a sex will also exhibit epistasis. Morph loci alter expression of endocrine pathways (Brantley et al. 1993). Morph loci consist of key regulatory genes of endocrine systems that organize suites of behavioral, morphological, physiological and life history traits into co-adapted syndromes. For example, loss-of-function genes that create paedomorphic Axolotls (see Chapter 12) act via the hypothalamic-pituitary-thyroid (HPT) axis (Voss and Shaffer 1997). In other salamanders (e.g., *A. talpoideum*), paedomorphs and metamorphs co-occur (Semlistch 1998). The HPT axis of polymorphic salamanders may sustain epistasis at loci with which it interacts to generate morphs. Given the possibility of segregating epistatic variation, morphs are thus of great interest to life history theory. Sex chromosomes are the fundamental morph loci of all sexual species that generate OC.

3. Fitness epistasis: non linear interactions of alleles and fitness

Genetic and physiological epistases are related to **fitness epistasis** (Kelly 2000; Whitlock et al. 1995) in which non-linearity of traits and fitness can be so extreme that alternative optima arise on fitness landscapes (Fig. 1). In contrast, purely additive effects of traits (and alleles) generate much simpler (i.e. one optimum) fitness landscapes (Sinervo and Svensson 2002).

Within species, a specific morph allele that codes for morph determination may require interactions with other *strategic loci* to create ideal combinations of alleles and high fitness (Sinervo and Clobert 2003). Admixtures of multilocus morph genotypes form during sexual reproduction, revealing fitness epistasis (See Chapter 4, Levels of selection, Side Box 4.3).

A case study of the levels of epistasis

Lancaster *et al.* (2007) resolved a complex web of epistasis involving sire genotype and maternal genotype at the throat color locus of *Uta stansburiana*, the same locus in social neighbors, and unlinked dorsal pattern loci. This example (Side Box 14.3) spans all forms of epistasis listed in section II. *Uta* females add estrogen to egg yolk as a function of the sire's throat color. Dams add extra yolk estrogen if sires carry a yellow (*y*) color allele, which codes for sneaky behaviors in sons (Side Box 14.3). Yolk estrogen induces a barred dorsal pattern when *y* alleles are present in progeny. Yolk estrogen is also modulated by the presence of orange color alleles in social neighbors, but only if progeny lack yellow alleles (e.g., a yellow-sire allele pathway). Females add more yolk estrogen as orange (*o*) alleles increase in frequency in neighbors. This second pathway for yolk estrogen induces a different dorsal pattern as a function of progeny color alleles and progeny sex. In this second pathway, stripes are induced by yolk estrogen in sons *carrying o* alleles, but stripes are induced by yolk estrogen in daughters *lacking o* alleles.

Throat color and dorsal pattern loci are thus involved in gene epistasis (sire and dam gametic union), social epistasis (signaling via orange alleles in neighbors), and endocrine epistasis of dams (Side Box 15.3). Color has been mapped to a gene called OBY, after the colors orange, blue and yellow. The loci for back pattern are not yet mapped, but they are not genetically correlated with the OBY locus based on crosses.

Lancaster *et al.* (2007) also measured CS on progeny throat color and dorsal pattern by releasing them into nature. Epistatic induction of progeny dorsal patterns by dams was adaptive. Patterns of CS matched patterns of maternal induction of yolk estrogen by social neighbors and yellow sire alleles (Side Box 15.3). Without maternal induction, CS would generate much stronger LD between color and dorsal pattern loci. Thus, *epistatic gene interactions* are under *epistatic selection* to reduce genetic load arising from genesis of trait complexes due to unlinked but interacting genes. Maternal induction of progeny traits, due to cues in social neighborhoods, ameliorates this fitness epistasis. However, this generates complex forms of inheritance involving maternal effects and additive genetic effects. This fitness epistasis arises because, dorsal pattern loci and signals for social strategy, the color locus, synergize to generate a rich set of anti-predator behaviors. This system of epistasis arises in response to perceptual trade-offs in predators (Pough 1990), which generate FD and CS on their *Uta* prey (see below and Chap. 14).

The profound role of such epigenetic effects

Without maternal induction by female *Uta*, CS would generate much stronger LD between colour and dorsal pattern loci. This is because dorsal pattern loci and the throat color locus are unlinked, but synergize to enhance anti-predator behaviors (e.g., social behavior must be matched to escape behavior). The CS on *Uta* is very similar to selection on garter snake behavior and back patterns, first introduced in Chapter 3. Thus, *epistatic gene interactions* are under *epistatic selection* (i.e. CS, which is *fitness epistasis*) to reduce the genetic load arising from genesis of trait complexes due to unlinked but interacting genes. Maternal induction of progeny traits, cued by genes in neighbours, ameliorates fitness epistasis, but this generates complex inheritance involving maternal and genetic effects.

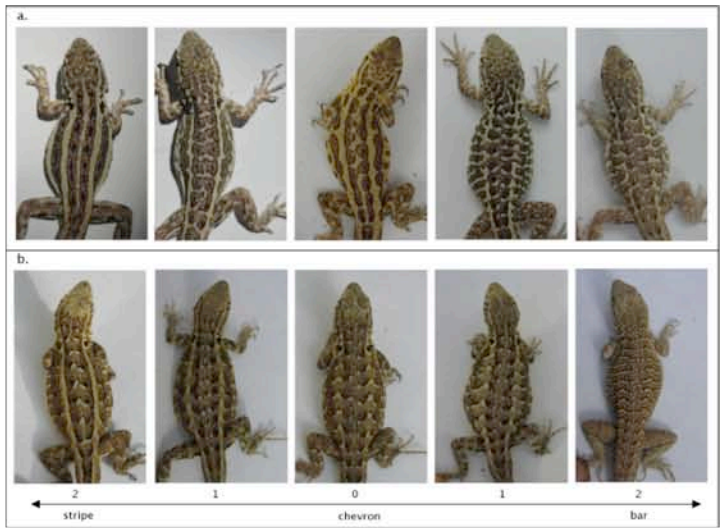
The adaptive value of particular throat and back correlations likely derives from underlying their social syndromes. O males are socially dominant and spend much of their time out in the open, displaying and actively patrolling territories from the highest vantage points available. Y males are sneakers that travel large home ranges and are often found in the grass and smaller rocks where they go undetected by O males. Yellow females tend to be more tolerant of nearby neighbors than orange females, and thus live at higher densities. These social phenotypes associated with throat color correspond to different levels and kinds of predation risk (e.g. snake vs. bird attack) and different available escape routes. Moreover, social phenotypes and antipredator behaviors are often two aspects of the same behavioral syndrome or personality type (see Chapter 17). In the absence of *y* alleles, which works with crypsis, induction of a barred pattern is of limited adaptive value. Likewise, in the absence of a high frequency of O neighbors the induction of the striped O phenotype has no adaptive value in sons, and patterns revert. In both cases, the back patterns revert to the default ground state of chevrons, which is adaptive under these conditions.

Epigenetic inheritance mechanisms in general, and prenatal steroid maternal effects in particular are more versatile and complex than previously thought. In *Uta*, prenatal exposure to estradiol has different results on offspring phenotype depending on the inducing cue and the genotype and sex of the progeny. There are in fact three distinct high fitness outcomes of prenatal estradiol exposure, induced by two distinct social cues. This level of complexity in endocrine-medicated maternal effect mechanisms has not previously been demonstrated.

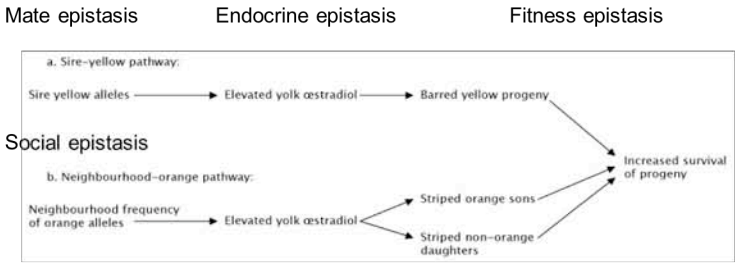
Maternal estrogen plasticity of *Uta*, which interacts with dorsal pattern and morph loci (Lancaster et al. 2007), reflects novel machinery that is built from social dynamics. Epistatic networks might often fix on maternal plasticity, resolving genetic load generated by epistatic selection. Intrauterine positional effects in mammals, in which hormones from one embryo leak into other sex progeny, trigger HREs, and inducing new behavioral types (Clark et al. 1991; vom Saal et al. 1983). Such endocrine epistasis may be adaptive in density-regulated populations because it can, for example, induce more or less aggression in those daughters that develop beside brothers *vs*. sisters in the uterus. This creates altered male and female phenotypes that can respond to density competition by purely epigenetic means. Epigenetic inheritance mechanisms such as maternal effects and indirect genetic effects contribute to rate and/or direction of evolutionary changes. Epigenetic inheritance mechanisms alter the response to selection, and thus rate of evolution, from its expected value based on direct genetic inheritance.

Box 15.3: Endocrine, gene, and fitness epistasis

A. Experimentally induced variation due to ectopic yolk estrogen.
B. Natural variation in dorsal patterns (stripe, chevron, bar).



G. Summary of epistatic networks for dorsal pattern loci, throat color loci, and yolk estrogen and cascading effects on progeny survival.



Gene/endocrine epistasis: sex Chromosomes and yolk estrogen

D. Sire yellow pathway: Endocrine epistasis on dorsal pattern of sons versus daughters, the effect of yolk estrogen.

C. Estrogen in yolk induced by social mate

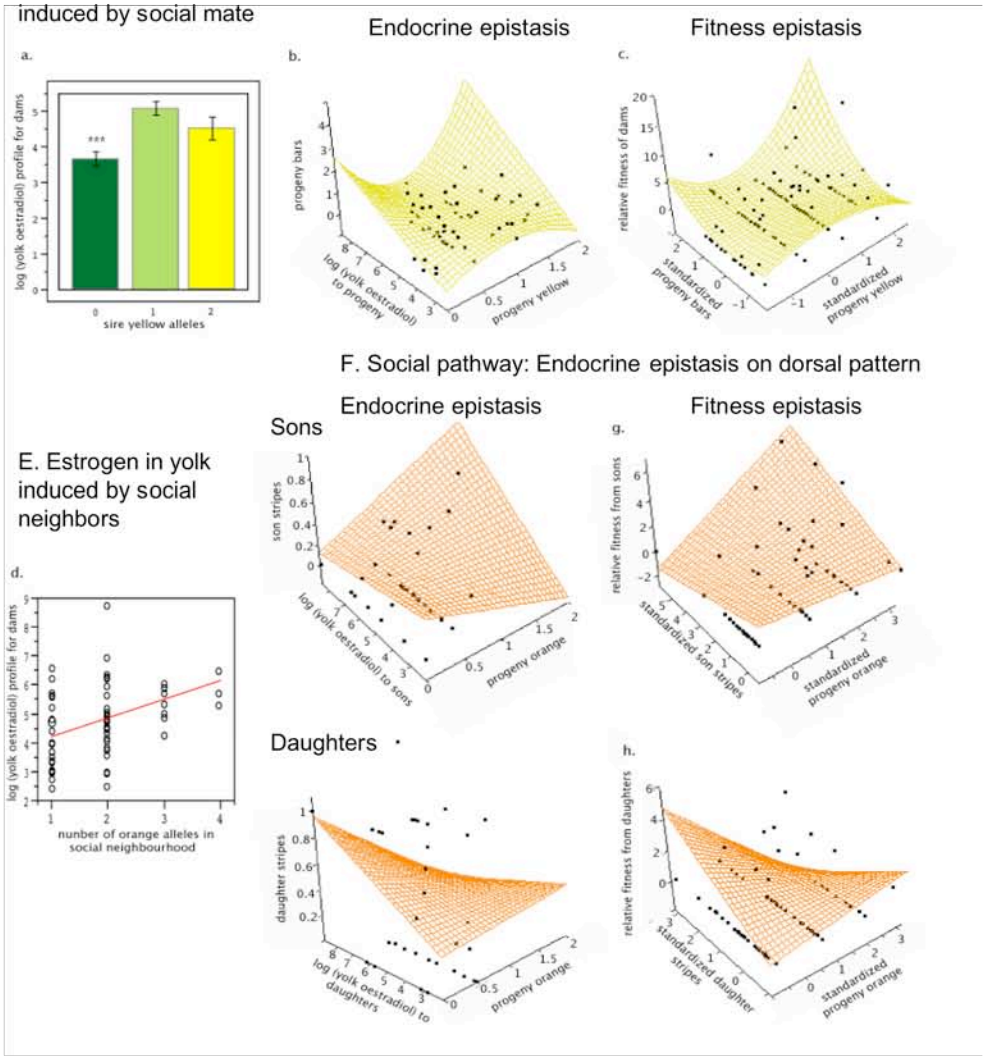


Figure 15.22. C-D) The role of the yellow (*y*) allele in transmission to sons is salient with regards to production of a well-adapted sneaker male morphology. C) Progeny receiving *y* alleles are more likely to receive yolk estrogen from their mom, which in combination with *y* alleles and dorsal pattern loci, predisposes sons to have a barred morphology, which is very cryptic in the grass habitat were sneaker males reside. This endocrine epistasis enhances survival in both sons and daughters (fitness epistasis). The other pathway, number of *o* alleles in a neighborhood, induces completely opposite patterns in sons and daughters and reflects the high survival of sons with stripes (and also if they inherit *o* alleles). E-F) Stripped O males are likewise adapted because they have high speed. Stripes aids O in escape from predators. In contrast, non-O daughters, from the same females, rely on crypsis via bar and chevron dorsal patterns. Endocrine epistasis allows dams to produce well-adapted social and anti-predator phenotypes in their progeny.

Study Questions for Sex Determination and Differentiation

1. What is an organizational effect? Describe how the relevant hormones organize the song bird brain. Be specific about which hormones are involved in the cascade of events. After these organizational events, how does a male bird learn his song?

2. What is an activational effect? Describe how maternal behaviors in mice are activated by hormone interactions. What is the environmental trigger for nurturing behaviors? Is nurturing under genetic control?

3. What is a maternal effect? How is this different from genetic alteration of phenotype? What are the advantages of a system in which the female can alter offspring via a maternal effect, compared to hard-wired genetic differences?

4. How does the phenomenon of sex change in the stoplight parrotfish span the spectrum of male and female reproductive phenotypes? Describe the mechanisms of sex change (with diag. of hormone profile) in terms of the principle male and female hormones found in fish.

5. Discuss the costs and benefits of polygyny and monogamy in male song birds and the physiological mechanisms underlying song, courtship, and the propensity for parental care.

6. Outline the major circuits involved in the sexual differentiation of a male's song center. Need a diagram for this answer.

7. How is the inheritance of 0M vs. 2M progeny in the Mongolian gerbil heritable but not genetically heritable?

8. Illustrate physiological epsistasis (i.e., endocrine epistasis) with an example. In your example discuss the adaptive value of physiological epistasis.