

# Chapter 7. Sex Determining Mechanisms in Vertebrates

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## 7. 1. Summary

Vertebrates have various sex determining mechanisms. These have been broadly classified as either genotypic sex determination (GSD) or environmental sex determination (ESD). This terminology, however, may obscure the fact that mixtures between genotypic and environmental sex determination exist, or that genotypic and environmental sex determination may, in fact, be the extremes of a continuum. Sex ratio evolution plays an important role in the evolution of sex determining mechanisms.

## **7.2 Introduction**

This chapter starts with the proximate aspects of sex determining mechanisms (section 7.3). We introduce the traditional classification of sex determining mechanisms that exist in vertebrates (section 7.3.1) and the distribution of mechanisms among extant vertebrate taxa (section 7.3.2). At phylogenetically shallow levels, different mechanisms are present. We describe how the existence of either male or female heterogamety, or environmental sex determination is usually established for individual species or taxa (section 7.3.3). Cases of mixed sex determination, *i.e.* combinations of genotypic and environmental sex determination are also observed (section 7.3.4) and we caution this phenomenon has implications for sex identification by molecular markers (section 7.3.5). We stress that phenotypic sex generally has environmental and genetic components and discuss a model that attempts the unification of sex determination by stating that sex determination in all vertebrates is mediated by differential growth of the embryo (section 7.3.6).

In the second part of the chapter we discuss the evolution of sex-determining mechanisms. Evolution from one system to another can be quite rapid (section 7.4). We stress that sex ratio selection plays an important role in the evolution of sex determining mechanisms (section 7.4.1). This usually leads to sex determining mechanisms that produce an unbiased sex ratio, but under some conditions mechanisms that bias the sex ratio are favoured. We conclude the chapter with an illustration of how one can investigate verbal models of the evolution of sex determination by means of mathematical models. We present a simulation model with which we

analyse a hypothesis for the evolution from ESD to GSD attempting to account for male heterogamety in some taxa and female heterogamety in others (section 7.4.2).

### 7.3. Proximate aspects of sex determination

#### 7.3.1. Traditional classification of sex determining mechanisms

Sex determination is traditionally classified as either ‘genotypic’ or ‘environmental’. The term genotypic sex determination (GSD) signifies that the sex of a zygote is determined entirely by its genotype; the sex of an individual is fixed at fertilisation. The most common type of GSD involves sex chromosomes. If the male is the sex with two different sex chromosomes, this is termed male heterogamety, and the sex chromosomes are referred to as X and Y (females are XX, males are XY). Likewise, if the female is the sex with two different sex chromosomes, this is termed female heterogamety and the sex chromosomes are Z and W (females are ZW, males are ZZ). In polygenic sex determination, which is less common, sex is determined by a number of genes, each with minor effect, distributed throughout the chromosome complement.

The term environmental sex determination (ESD) signifies that the sex of an individual is determined irreversibly by the environment experienced during early development. Where the decisive environmental factor is temperature, we refer to this as ‘temperature-dependent sex determination’ (TSD). Sex determination may also be influenced by pH (in fish: Römer & Beisenherz 1996), and by social conditions or relative juvenile size (in fish: Francis & Barlow 1993, Holmgren & Mosegaard 1996). Although we restrict this chapter to the discussion of primary sex determination, we mention here that in many fish species sex change is part of their natural life history, and is often induced by environmental stimuli (Francis 1992).

#### 7.3.2. Distribution of sex determining mechanisms among vertebrate taxa

Figure 7.1 shows the phylogeny of extant vertebrate taxa along with the reported sex determining mechanisms. Sex determination by sex chromosomes is universal in birds (female heterogamety) and mammals (male heterogamety) and is present in both forms (male and female heterogamety) among reptiles, amphibians, and fish. ESD is common among reptiles, and also exists in amphibians and fish. These data should not be treated as final, since the interpretation of sex-specific markers is not entirely clear (sections 7.3.3.1 & 7.3.4.), and some of the studies

reporting ESD have been conducted at temperatures outside of the range normally experienced by the species under study (Hayes 1998). For some of these cases it remains to be shown to what extent sex is environmentally determined in the wild (sections 7.3.3.2 & 7.3.4.). Polygenic sex determination (not in figure) has been reported in some fish species, *e.g.* *Xiphophorus helleri* (Price 1984), and in *Menidia menidia* (Lagomarsino & Conover 1993).

At phylogenetically shallow levels, different mechanisms may be present. For example, male and female heterogamety occur in the amphibian sister families Hylidae and Bufonidae respectively. Moreover, ESD and both male and female heterogamety exist within the reptilian family Gekkonidae. None of these mechanisms appears to have evolved only once. Instead of the conservatism of such a basic function as sex determination, as might have been intuitively expected, sex-determining mechanisms seem to be evolutionarily flexible (Chapter 8). Even mammals, in which the X and Y sex chromosomes are generally supposed to be conserved, variation in sex determining mechanisms occurs (reviewed in Fredga 1994, Jiménez *et al.* 1996, McVean & Hurst 1996, Mittwoch 1996a).

### 7.3.3. Evidence for various sex-determining mechanisms

#### 7.3.3.1. Genotypic sex determination: male or female heterogamety

Evidence for male or female heterogamety in a species traditionally comes from investigating the karyotype. Sometimes the different sex chromosomes can be recognised by their size. If they seem similar, cytological techniques, such as C-banding (*e.g.* Schmid *et al.* 1988, 1992, 1993) are used. However, in many species sex chromosomes appear to be morphologically indistinguishable from autosomes. In these cases, breeding experiments may indirectly establish heterogamety, *e.g.* if sex-linked marker genes exist as in the guppy (Winge 1932). An alternative approach is the analysis of offspring sex ratios, of either artificially induced gynogenetic females, or artificially induced triploids, or crosses between two individuals of the same genetic sex of which one is artificially 'sex-reversed' by hormone treatment (Bacci 1968, Richards & Nace 1978, Price 1984). In the 1980s, H-Y antigen, a minor histocompatibility antigen specific for the heterogametic sex, was proposed as a tool to identify the heterogametic sex (Engel & Schmid 1981, Engel *et al.* 1981). This gave conflicting results in at least one case: in the turtle *Siebenrockiella crassicollis* the female is H-Y positive (Engel *et al.* 1981) while a cytogenetic study identifies the male as the heterogametic sex (Carr & Bickham 1981). During the last

decade researchers have been trying to establish heterogamety by searching for sex-specific DNA. This has involved screening for sex-specific presence of Bkm-related satellite DNA, characterised by repetitive GATA sequences (e.g. Demas *et al.* 1990, Nanda *et al.* 1992). Bkm (banded krait minor) was originally isolated from the W-chromosome of the snake *Bungarus fasciatus* (banded krait), and high concentrations of Bkm-related sequences appear to be linked to the W- or Y-chromosome in many species (Jones & Singh 1981, 1985, Singh & Jones 1982). Species have also been screened for sex-specificity of genes related to the human Y-linked genes *SRY* and *ZFY* (e.g. Ganesh *et al.* 1997). *ZFY* (Zinc Finger Y, Page *et al.* 1987) and *SRY* (Sex-determining Region Y, Sinclair *et al.* 1990; *Sry* in mouse, Gubbay *et al.* 1990) had both been proposed as candidates for the male determining gene *TDF* (Testis Determining Factor). *ZFY* eventually fell out of favour (Palmer *et al.* 1989), but an important role of *SRY/Sry* in mammalian sex determination has been well established (Koopman *et al.* 1991, Goodfellow & Lovell-Badge 1993). There are some exceptions, however: the gene appears to be absent in the mole voles *Ellobius lutescens* and *E. tancrei* (Fredga 1994). *SRY*- and *ZFY*-homologues have been conserved throughout the vertebrates. A male-biased distribution of *SRY*- and *ZFY*-related genes has been found in the lizard *Calotes versicolor* (Ganesh *et al.* 1997), but not in any other non-mammalian species studied so far (Bull *et al.* 1988, Griffiths 1991, Tiersch *et al.* 1991, Valleley *et al.* 1992, Coriat *et al.* 1993, 1994).

Evidence for polygenic sex determination is provided by variable sex ratios and the heritability of this trait (Scudo 1967). In a few turtles with ESD heritabilities of sex ratios at the pivotal temperature have been measured (Bull *et al.* 1982, Janzen 1992).

#### 7.3.3.2. Environmental sex determination

The existence of ESD in a species can be established experimentally when sex ratios vary according to the environment in which offspring are reared. Especially in reptiles the effect can be extreme (Figure 7.2): in some lizards and in alligators, eggs incubated at low temperature give rise to 100% females, and eggs incubated at high temperatures give rise to 100% males. In many turtles it is the other way round: 100% males at low temperatures and 100% females at high temperatures. In other turtles and in crocodiles, incubation at intermediate temperatures leads to 100% males, whereas both low and high temperatures lead to females only (review in Bull 1983). In all these cases there is only a very narrow temperature range at which both sexes are produced. The temperature at which this is the case, however, may vary within a species and is heritable

(Bull *et al.* 1982, Janzen 1992). In fishes the sex ratios usually vary less extremely with temperature, but nevertheless temperature-dependent sex determination has been established in various species (*e.g.* Conover & Heins 1987, review in Francis 1992, Römer & Beisenherz 1996, Goto *et al.* 2000).

A difficulty that arises with the interpretation of experiments that test for environmental sex determination is the possibility of differential mortality. If a biased sex ratio is found as a result of an experimental manipulation of environmental conditions at rearing, *e.g.* incubation temperature, and one wants to conclude that sex is determined environmentally, it needs to be assessed to what extent biased mortality could have been responsible for the result. One way to deal with the problem is to ‘assume the worst’, that is, that all dead individuals are of the sex least favourable for the hypothesis. These ‘data’ can then be included when testing statistically for sex ratio bias. However, this procedure is often too conservative. Researchers should take care that the conditions of the experiment (other than the experimental manipulations) are as conducive to survival of the tested individuals as possible, and identify the sex of the individuals before much mortality has taken place.

Although the possibility of ESD has been established for many species by manipulation of rearing conditions in the laboratory, very few studies have investigated to what extent ESD operates in the wild. The European pond turtle *Emys orbicularis* has been shown to exhibit ESD in the laboratory (Zaborski *et al.* 1982). However, a study of wild populations of this turtle revealed that the sex of only 17% of wild individuals was determined by the temperature (Girondot *et al.* 1994) (see also section 7.3.4.).

#### 7.3.4. Mixed sex determination

A combination of environmental and genotypic sex determination, sometimes with major genetic factors, can be present within the individual. Examples are the fish species *Menidia menidia* (Conover & Heins 1987) and *Limanda yokohamae* (Goto *et al.* 2000), and the turtle *Emys orbicularis* (Zaborski *et al.* 1988, Girondot *et al.* 1994). Some other reptiles with ESD show signs of heterogamety too (*e.g.* Engel *et al.* 1981, Nakamura *et al.* 1987, Wellins 1987, Ewert *et al.* 1990, Demas *et al.* 1990). Recently, a form of temperature-dependent sex determination has been reported in poultry (Ferguson 1994a,b), while all birds are known to have ZZ/ZW sex chromosomes.

In a study on the pond turtle *Emys orbicularis*, all individuals from eggs incubated at 25-26°C became males, and all individuals from eggs incubated at 30-30.5°C became females (Zaborski *et al.* 1982). The gonadal cells of all males typed H-Y negative, as did blood cells of half of them; blood cells of the other half typed H-Y positive. In all females, the gonadal cells typed H-Y positive, but blood cells typed H-Y positive in only half of them, and negative in the other half. Zaborski *et al.* (1982) consider the animals with H-Y negative blood cells as genotypic males, and the animals with H-Y positive blood cells as genotypic females. Thus, the H-Y blood cell negative phenotypic females at the high temperature are 'sex-reversed' genotypic males, and the H-Y blood cell positive phenotypic males at the low temperature are 'sex-reversed' genotypic females. Apparently this turtle has a form of GSD, probably with female heterogamety, since the female is the H-Y positive sex. The phenotypic sex, however, does not correspond with the genotypic sex in half of the individuals reared at the two extreme temperatures. This implies that the genetic status can be totally overruled by the influence of temperature, and the H-Y type of the gonadal cells can be completely reversed in accordance with the developing sex of the gonad. Girondot *et al.* (1994) found that, in a natural population of *Emys orbicularis*, both 'sex-reversed' individuals and individuals whose phenotypic and genotypic sex match, occur among males as well as females. There are indications that the fish *Scardinius erythrophthalmus* has a similar sex determining system; Koehler *et al.* (1995) found all males and half of the females to be homogametic, whereas the other half of the females were heterogametic. Moreover, a similar situation might exist in other turtle species in which heterogamety has been inferred from H-Y typing (Engel *et al.* 1981, Nakamura *et al.* 1987, Wellins 1987) while TSD has also been inferred, from laboratory experiments with eggs incubated at different temperatures (Bull *et al.* 1982, Yntema 1976, 1979).

In poultry, a comparable situation may exist. In the experiments described by Ferguson (1994a,b) poultry eggs were treated with abnormally high or low temperatures during incubation. He found that approximately 10% of the hatched birds had a sexual phenotype (confirmed by macroscopic and histological examination) that was different from their sexual genotype (confirmed by a W-specific molecular marker). Apparently, the influence of temperature can overrule the influence of the sex determining genes in at least some individuals. It is not known whether any 'sex-reversed' poultry would naturally occur or, if so, at what frequencies.

In the examples described above, sex determination seems to be governed by sex chromosomes (*i.e.* a major genetic factor) as well as an influence of temperature. A study of the silverside *Menidia menidia* (Lagomarsino & Conover 1993) suggests that in this fish, sex

determination is controlled by an interaction between major genetic factors, polygenic factors, and temperature, and that the relative importance of each component differs with latitude. This study examined family sex ratios at two different temperatures for two different populations. In the high latitude population, the sex ratios tended to fall into distinct classes, as expected from Mendelian segregation of a major sex factor(s). In this population temperature had no influence on sex ratios. In the southern population, temperature had a highly significant influence on sex ratios, and sex ratios did not conform to Mendelian ratios. High latitude populations appear to have evolved a major sex-determining factor(s) that overrides the effect of temperature, and this factor(s) is lacking in low latitude populations.

#### 7.3.5. Consequences for measuring sex ratios

The finding of mixtures between environmental and genotypic sex determination has implications for the practice of identifying the sex of individuals, of species that supposedly exhibit GSD, using molecular markers. For example, when behavioural ecologists are confronted with biased sex ratios, they want to know whether the bias is caused by differential mortality of the sexes or whether the primary sex ratio is biased. It is desirable to know the sex of individuals at as young an age as possible, long before the sex can be identified by external morphology, and without having to sacrifice the individuals. Various molecular methods have recently been developed to establish primary sex ratios in behavioural ecological studies of birds (Griffiths *et al.* 1996, Ellegren & Sheldon 1997). A molecular marker is judged to be sex-specific (or even W- or Y-linked) if it is consistently present in one sex and absent in the other in a large enough sample of known males and females. If, however, naturally 'sex-reversed' individuals occur under the influence of certain environmental conditions, this method does of course not apply. Markers should therefore be tested under a wide range of environmental conditions. In Ferguson's (1994a,b) experiments, 10% of poultry were 'sex-reversed' when exposed to pulses of lower temperature during incubation. In some bird studies on sex ratios in nature (Daan *et al.* 1996), the deviation from a 1:1 sex ratio was of the same order of magnitude. It is possible that, especially in case of adaptively biased sex ratios, temperature-induced 'sex-reversal' may be the very mechanism that parent birds use to control offspring sex ratios. Incubating parents may expose their eggs to pulses of different temperatures. If this is the case, primary sex ratios cannot be established with molecular markers. It is, nevertheless, reassuring that one bird study in fact demonstrated extremely biased primary sex ratios with molecular techniques (*e.g.* Komdeur *et al.*

1997), implying biased ratios of genetic sex, and not ‘sex-reversal’. Although no evidence exists that supports the notion that TSD is operating in birds in the field, until we know more about temperature-induced ‘sex-reversal’ in birds, caution is recommended.

Another area where researchers have tried to establish a non-fatal method of identifying sex at an early age is in endangered species of sea turtles. Sex cannot be identified by external morphology before four years of age. Wellins (1987) found that blood cells of males typed H-Y positive, consistent with male heterogamety. These turtles, however, are known to have temperature-dependent sex determination, implying that a situation similar to that of the European pond turtle (section 7.3.4.) may exist. Therefore, one cannot be sure whether the H-Y status of blood cells of an individual always corresponds to its phenotypic sex. The frequency of natural ‘sex reversals’ should first be determined. Another study (Demas *et al.* 1990) found male specific Bkm-related DNA in sea turtles. The sample was small, however, and the natural frequency of ‘sex-reversed’ individuals has not been investigated. Moreover, the relation between Bkm-related DNA and phenotypic sex is not clear. Demas *et al.* (1990) mention the possibility that the DNA is altered in accordance with the developing sex, as induced by the temperature. More information is needed before it can be decided whether sex can be reliably identified by molecular markers in these species.

In fish aquaculture, it is also desirable to identify the sex of individuals at an early age. While molecular techniques have become much more commonplace (*e.g.* Coughlan *et al.* 1999), we again stress that hopes of relying on molecular markers may be too high. Fish are notorious for having labile sex determination (Francis 1992): environmental sex determination, socially induced sex determination, and sex change induced by various stimuli have been documented. Reports of sex-specific molecular markers in, *e.g.*, salmon (Devlin *et al.* 1991) are alternated with reports of ESD in salmon of the same genus (Craig *et al.* 1996). Thorough study is needed of the relation between phenotypic sex and genetic constitution. Laboratory studies, with controlled environmental conditions, are powerful. Knowledge of the situation in the field, however, is indispensable.

#### 7.3.6. A universal mechanism: a model

Here we describe a model, put forward by Kraak and de Looze (1993), in which we view ESD and GSD as the two extremes of a continuum. Both environment and genes determine phenotypic sex, but the extent of their contribution varies. When genes dominate, sex is said to



be genetically determined, and when environmental influences dominate, sex determination is called environmental. Several authors have suggested that growth rate may be a universal organising principle of sex determination (Mittwoch 1971, 1996a, Kraak & De Looze 1993) by acting as the main trigger of sexual differentiation in a critical period during early development. This idea may contribute to viewing ESD and GSD as part of the same mechanism. Mittwoch was the first to propose that the sex chromosomes give rise to quantitative phenotypic differences in growth rate that result in two qualitatively different classes of individuals, *i.e.* females and males (*e.g.* Mittwoch *et al.* 1969, Mittwoch 1989, 1996a). More specifically, she suggested that the mammalian Y-chromosome carries growth enhancing allele(s), and that for testis development to occur, the embryonal gonad will need to reach a threshold size by a critical time in development, failing which the gonad will become an ovary (Figure 7.3a) (Mittwoch 1969, 1996a). Figure 7.3b illustrates the opposite threshold mechanism, which might operate in birds: fast growing gonads become ovaries, and the W-chromosome may carry the growth promoters (Mittwoch 1971, 1986). Kraak and De Looze (1993) suggested the unification of sex determining mechanisms for all vertebrates, by proposing that also in other vertebrates one or the other of these threshold mechanisms is operating. The proximate cue for differentiation of the gonads into either testes or ovaries, in all vertebrates, is thought to be the size or stage reached at a critical time in development. Growth rate, in turn, is a quantitative phenotypic trait caused by environmental influences (*e.g.* temperature) and genetic factors, with ESD at one end of the continuum and GSD at the other. In species with ESD the relation between sex and growth is thought to be adaptive: the sex that benefits most from fast growth should arise under fast growth conditions (Charnov & Bull 1977, Head *et al.* 1987, Ewert *et al.* 1994, Shine 1999, but see Janzen & Paukstis 1991b). Sex determination can then be viewed as a condition- or state-dependent strategy (*sensu* McNamara & Houston 1996).

Any environmental influence on growth at the proper time is, in this view, sex determining. Any gene that has an effect on growth in this period, may it be minor or major, is a (minor or major) sex determining gene. It could be the case that *Zfy* or *Sry* act as growth factors (but see Burgoyne *et al.* 1995). Sex determination is in principle polygenic. Heterogamety may be caused by linkage of several growth promoting alleles on one of the chromosomes in a pair (Kraak & De Looze 1993). Or, one or a few genes may have strongly sequestered the process of gonadal differentiation, as in mammals, by influencing growth rate at the right time and the right place. Even in the latter case, effects of minor growth genes, and/or environmental effects may contribute to a resulting growth rate that induces 'sex reversal'. The term 'sex reversal' is not

strictly appropriate. The term is used to indicate that the phenotypic sex of an individual is not in accordance with its genotype. But here, 'genotype' refers only to sex chromosome constitution; if it referred to all sex-chromosomal and autosomal genes, the individual's phenotypic sex could, in fact, be in accordance with the genotype. This idea is supported by the finding that autosomal deletions resulting in slow growth can give rise to XY females in mice (Cattanach *et al.* 1995). Furthermore, in true hermaphroditism in humans (*i.e.* the presence of ovarian and testicular tissue in the same individual), the ovarian tissue occurs more often on the left side while testicular tissue is more often present on the right side, and in normal mammalian embryos right gonads grow faster than left gonads (Mittwoch 1996b,c). Whatever the reason ('environmental?') for this asymmetry in growth rate, this may mean that in rare individuals the size of the left gonad has remained just below the critical threshold, while the right has just exceeded the threshold, at the critical time.

Evidence supporting this model of sex determination, *e.g.* that early mammalian embryonal growth is related to the presence of Y-linked genes, has been extensively reviewed by Hurst (1994), Mittwoch (1996a) and Erickson (1997); see also Roldan and Gomiendo (1999). Others present evidence and propose models for reptiles with TSD, in which the effect of temperature on asynchronous (heterochronic) development plays a role in sex determination, and speculate on the universal validity of such models for vertebrate sex determination (Haig 1991, Smith & Joss 1994, and see Johnston *et al.* 1995). Several studies on ESD in fish implicate a relation between growth and phenotypic sex (Blázquez *et al.* 1999, Goto *et al.* 2000). Models that do not focus on the influence of temperature on growth have been proposed by Deeming and Ferguson (1988, 1991). In their view, the dose of a particular molecule determines sex. In GSD the dose is genetically specified. In ESD, the efficiency of gene transcription, or translation, or the stability of the mRNA or gene product, or the activity of the gene product, is determined by environmental conditions. Some evidence *contra* the importance of growth in sex determination is the fact that numerous studies on reptile eggs show that water availability during development significantly influences embryonic growth rate (reviewed by Packard 1991), yet no effect of water availability on sex determination has been demonstrated in these species (Packard *et al.* 1989).

The model of sex determination outlined above can easily account for all combinations of ESD and GSD. According to this view, growth genes anywhere in the genome influence sex determination, and thus tend to be sex specific; but not consistently so, due to additive genetic and environmental effects on growth. It can explain that even in birds, where sex determination

is strongly canalised by factor(s) on the sex chromosomes, temperature can sometimes override the genetic status. It can potentially explain the as yet unexplained ‘sex reversed’ humans, *e.g.*, females with an intact *SRY* gene but a deletion at the short arm of chromosome 9 (Bennett *et al.* 1993, Raymond *et al.* 1999), or other XY females and XX males that cannot be accounted for by *SRY*-mutations (Kusz *et al.* 1999). This is because any factor that sufficiently disturbs normal growth of the embryonal tissues may cause a change in whether the gonad reaches the threshold or not, and hence gonadal differentiation.

#### 7.4. Evolution of sex-determining mechanisms

Sex determining mechanisms can evolve quite rapidly, even though some of the genes involved in the process are quite conserved (reviewed by Marín & Baker 1998, Chapter 8). Several models have been proposed to account for the evolution from one system of sex determination to another (reviewed by Bull 1983, Werren & Beukeboom 1998). In section 7.4.1. we stress the importance of sex ratio selection, which plays a decisive role in all such models. In section 7.4.2 we address the question of how mathematical modelling techniques can be used to examine ideas about sex determination. We give a worked example of a simulation model to show the kind of approach that might be taken. The simulation model analyses the verbal hypothesis of Kraak and de Looze (1993) for the evolution of sex specific heterogamety in vertebrates. We will not deal with evolutionary processes that take place after the establishment of heterogamety, such as the degeneration of Y-chromosomes and the evolution of dosage compensation, because these have been treated elsewhere (Charlesworth 1996, Charlesworth & Charlesworth 2000).

##### 7.4.1. The importance of sex ratio selection

Because sex determining mechanisms control the inheritance of sex, they also determine the primary sex ratio among offspring. A 1:1 sex ratio is usually advantageous (Chapters 1 & 2), hence systems of sex determination tend to be most stable when they lead to an even sex ratio (Bull 1983, Karlin & Lessard 1986). Nur (1974) provided a simple one-locus-two-allele model to illustrate this. Consider a locus, with alleles *A* and *a*, that affects sex determination, but not fertility or survival. Allele *A* has frequency *x* in females and *y* in males, and a proportion *M* of the offspring become male. Thus, the frequency of *A* is given by  $p = (1-M)x + My$ . Because

females and males contribute equally to the next generation (every offspring has one mother and one father), the frequency of  $A$  in the next generation will be  $p = (x+y)/2$ , hence the change in frequency from one generation to the next is given by

$$p = p + p = (y - x)(1/2 - M). \quad (7.1)$$

The frequencies of genes that are involved in sex determining systems often differ between males and females ( $x \neq y$ ), hence (7.1) tells us that in equilibrium ( $\Delta p = 0$ ), the sex ratio is even ( $M = 1/2$ ). The beauty of this argument is that it holds regardless of how  $x$  and  $y$  affect the sex ratio  $M$ .

Sometimes sex ratios other than 1:1 are selected for (Chapters 1 & 2), and then sex determination mutations that bias the sex ratio may have an advantage. For example, in several species of lemmings a mutant X-chromosome, designated  $X^*$ , causes  $X^*Y$  individuals to develop as females instead of males (Fredga *et al.* 1976), thus causing a female-biased sex ratio. It has been argued that the  $X^*$  chromosome has a selective advantage because the high rate of inbreeding in lemmings favours a female-biased sex ratio (Maynard Smith & Stenseth 1978).

There may also be conflicts of interest over the sex ratio (Chapter 2 & 8) between parent and offspring, between parents or between nuclear and cytoplasmic genes, and this may be an important driving force of evolutionary changes in sex determination (Werren & Beukeboom 1998). For example, cytoplasmic elements are nearly always transmitted via eggs (not via sperm) and therefore favour strongly female-biased sex ratios, unlike autosomal nuclear genes that usually favour a balanced sex ratio (Chapter 9). However, we do not know of any vertebrate examples.

Sex ratio selection is also thought to explain the evolution of ESD (Bull 1983). All else being equal, selection favours a low sex ratio variance among offspring rather than a sex ratio that fluctuates with environmental conditions (Charnov 1982), as would be the case with ESD. However, if fitness varies with environmental conditions in a sex-specific way, then selection favours overproduction of the sex that benefits most given the prevailing condition (Trivers & Willard 1973, Charnov & Bull 1977). ESD is a mechanism that achieves such condition-dependent sex ratios (see Chapter 8 for invertebrate examples). ESD, in turn, influences the population sex ratio. Models have shown that, when sex depends on environment rather than genotype, the sex expressed under relatively unfavourable conditions will be more abundant (Charnov 1982, Bull 1983, Frank & Swingland 1988).

#### 7.4.2. Simulations of a scenario for the evolution of ESD to GSD

Kraak and De Looze (1993) have suggested that which of the two threshold mechanisms of Figure 7.3 is present in taxa with sex chromosomes is historically determined. In a verbal model they proposed a transition from adaptive ESD to GSD with sex chromosomes, in which genes take over the role of the environment in bringing about differential growth. They assumed vertebrate sex determination to be growth dependent, as argued above (section 7.3.6.), and ESD to be ancestral (as supported by Bull 1980, Janzen & Paukstis 1991a, Cree *et al.* 1995). According to their evolutionary scenario the sex that grows fastest and has a size advantage under ESD will be the sex with heterogametic sex chromosomes. Verbal arguments, however, are not very transparent with respect to their dependence on implicit assumptions. Often a more formal, mathematical, treatment is required in order to see on what assumptions the predicted outcome depends. We previously analysed part of the verbal argument using a two-locus simulation study (Kraak *et al.* 2000). Here we present a multi-locus simulation study that investigates the argument (Kraak & de Looze 1993) that selection would favour strong linkage of growth-accelerating alleles on one chromosome that would subsequently become the Y- or W-chromosome. This situation, in which growth genes are sex determining and at the same time have a differential effect on female and male fitness, is a special case of the situation in which selection favours sexually antagonistic genes becoming linked to a sex determining locus (Rice 1987).

We consider a diploid, randomly mating population of constant size ( $N_f + N_m = 500$ ) but varying sex ratio. The simulations start with a sex ratio of 1:1. Generations are discrete and non-overlapping. The sex of each individual is determined by the value of a phenotypic trait  $P$  relative to a threshold value  $T$ . We arbitrarily label the sex developed for  $P > T$  “male” and that for  $P \leq T$  “female” (as in Figure 7.3a). An individual’s trait value results from the additive interaction of genetic and environmental factors:  $P = G + E$ .  $E$  corresponds to random individual variations in environmental conditions and is drawn at random from a normal distribution with mean zero and variance  $VE$ . We considered  $VE = 0.25$ ,  $VE = 0.05$ , and  $VE = 0.01$ .  $G$  reflects the additive genetic effects and is the average of the 16 allelic values at 8 loci that are located on one pair of homologous chromosomes. An individual’s threshold value  $T$  is the average of the 2 allelic values at an unlinked threshold locus, and an individual’s recombination rate  $R$  between the growth loci similarly results from the allelic values at an unlinked recombination locus.

At the threshold and recombination loci a broad spectrum of 250 alleles is feasible, the allelic values ranging from  $-1$  to  $+1$  and  $0$  to  $0.5$  respectively. At the growth loci only two alleles are feasible, the allelic values being either  $0$  and  $0.5$  (case A), or  $-0.25$  and  $+0.25$  (case B). At the start of each simulation, all individuals are homozygous for  $T$ -alleles with value zero and homozygous for  $R$ -alleles with value  $0.5$ . In case A all individuals at the start are homozygous at each G-locus for alleles with value  $0$ . This situation corresponds to ESD since an individual's sex is purely determined by its environment. Here only growth accelerating mutations are possible (and back mutations to the growth-neutral allele). In case B the individuals at the start have a random sequence of allelic values at their G-loci. This situation corresponds to polygenic sex determination; genetic effects can be growth enhancing or growth inhibiting. Genetic variation is generated by mutation. At the  $T$ -locus, a given allele  $T_i$  changes with probability  $\mu_T$  into a new allele  $T'_i$ , where the new value is chosen at random from the interval  $[T_i - T, T_i + T]$ . The same holds true at the  $R$ -locus with mutation rate  $\mu_R$ . At the G-loci a given allele changes with probability  $\mu_G$

$\mu_T = \mu_R = \mu_G = 0.01$ . The recombination rate  $R$  of an individual determines the probability that crossing-over takes place between the two homologous chromosomes that carry the G-loci, at one location randomly chosen from the 7 locations between the 8 loci in the sequence. By such a crossing-over event parts of the two allele sequences are swapped (Figure 7.4).

In addition to its role in sex determination, the phenotypic value  $P$  also has a direct effect on viability: the probability  $W$  of survival to reproduction of an individual is linearly related to  $P$ ,  $W(P) = 0.5 + \alpha$ . It is a crucial assumption of our model that  $\alpha$  is sex specific: males are more positively affected by a high value of  $P$  than females. In our simulations  $\alpha_m = 1$ , but various values of  $\alpha_f$  were considered:  $\alpha_f = -1$ ,  $\alpha_f = -0.5$ ,  $\alpha_f = 0$ , and  $\alpha_f = +0.5$  (Figure 7.5). For each parameter combination 10 simulations were carried out, running through 50,000 generations.

The prediction of Kraak and de Looze (1993) is confirmed only when size benefits differ maximally between males and females ( $\alpha_f = -1$ , Figure 7.5a), and only if we start with pure ESD, *i.e.* all individuals being homozygous at each G-locus for neutral alleles, and the alternative alleles are growth accelerating (case A). Figure 7.6 depicts the results of a typical case of this kind ( $VE = 0.01$ ). The sex ratio remains close to 1:1 throughout the 50,000 generations (Figure 7.6a). The mean recombination rate  $R$  (of both males and females) drops at about generation 10,000 and then fluctuates around a low value (Figure 7.6b). At the same time when the recombination rate drops, mean male size  $P$  goes up (Figure 7.6c) as well as mean male heterozygosity (= fraction of G-loci at which an individual is heterozygous, Figure 7.6e). Mean

threshold  $T$  remains rather constant in males and females. At generation 25,000 most females have 0 or 1 growth accelerating allele on both chromosomes (Figure 7.6h), whereas most males have 7 or 8 growth accelerating alleles tightly linked on one homologue and 0 or 1 on the other (Figure 7.6g). We interpret this as females having two X-chromosomes, and males having one Y- and one X-chromosome, which recombine at low rates. The Y-chromosome carries growth-enhancing alleles; hence, the fast growing sex that benefits most from large size became the heterogametic sex. This result was replicated 10 times for  $VE = 0.05$ , and 10 times for  $VE = 0.01$  (at which parameter value the low recombination rate remained more stable). With  $VE = 0.25$  there is almost no selection for growth accelerating alleles, and sex determination remains almost purely environmental. When starting conditions are polygenic (case B), the outcomes are slightly different. Both males and females stay heterozygous at  $G$ -loci, but  $R$  goes down while males accumulate many growth accelerating alleles linked together on one homologue and females accumulate many growth inhibiting alleles linked together on one homologue, the other homologue being variable in both males and females.

With a slightly smaller difference in size benefits ( $\alpha_f = -0.5$ , Figure 7.5b) both sexes become/stay heterozygous at the  $G$ -loci, and with even smaller fitness differentials ( $\alpha_f = 0$  and  $\alpha_f = +0.5$ , Figure 7.5c and 7.5d) growth accelerating alleles tend to approach fixation in both sexes, while  $R$  fluctuates randomly. In case  $\alpha_f = 0$  or  $\alpha_f = +0.5$  males become heterozygous at the  $T$ -locus and get a lower mean  $T$  than do females. These patterns are similar for case A and case B.

In conclusion, if the difference in fitness effects of size between the sexes is large enough in a species with ESD, selection may favour linkage of growth accelerating genes on one homologue of a pair of autosomes in the fast growing sex. This pair of autosomes will then effectively become a pair of sex chromosomes, with the fast growing sex being the heterogametic sex. However, this occurs only under certain restrictive conditions, and it is not clear how often these conditions are met in nature. For example, a starting situation without genetic variation for growth seems unlikely. Therefore, we cannot yet conclude that the proposed scenario provides a sufficient explanation for the presence of male versus female heterogamety.

## 7.5. Conclusions

We started this chapter with the traditional classification of sex determining mechanisms as either GSD, with female or male heterogamety (or polygenic sex determination), or ESD. However, both the establishment of GSD and of ESD appear sometimes to be ambiguous. ESD

has often been established in the laboratory, but sometimes with experimental conditions that are outside of the range naturally experienced by the species. Few studies exist that show that ESD operates in the wild. One study on a turtle that exhibits ESD in the laboratory suggests that it does not occur at high frequencies naturally. More studies should investigate ESD in the wild. GSD, and in particular the heterogametic sex, is often established with molecular techniques. However, it is not clear what is the relation between the phenotypic sex of an individual on the one hand, and the genetic constitution or presence of a molecular marker in an individual on the other hand. We recommend that when molecular markers are to be used for sex ratio studies, they should be tested with sufficiently large samples and under a wide range of environmental conditions. The discrepancies between phenotypic sex and genotypic sex should be the subject of study as they might shed light on the nature of sex determination. The apparent existence of mixtures of ESD and heterogamety challenges the traditional classification. We discuss a model of sex determination that attempts to account for these cases. Other models may be plausible too. Ultimately, an evolutionary model should explain not only the existing modes of sex determination, but also their phylogenetic distribution.

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## Figure legends

### **Figure 7.1. Phylogenetic distribution of reported sex determining mechanisms within extant vertebrate taxa.**

A. Fishes, at the level of orders. B. Tetrapods, at the level of families. Taxa homogenous for sex determining mechanisms (*e.g.* birds and mammals) are collapsed. Mechanisms were classified into environmental sex determination (ESD) or genotypic sex determination (GSD) with male or female heterogamety. The evidence of heterogamety comes from breeding experiments, karyotypes, cytogenetics, H-Y antigen, or sex-specific DNA (*e.g.* Y-associated *Sry* or *Zfy*, W-associated *Bkm*). Evidence of ESD comes from laboratory studies that manipulated rearing conditions. These data should not be treated as final, since the interpretation of sex-specific markers is not entirely clear, and some of the studies reporting ESD have been conducted at temperatures outside of the range normally experienced by the species under study (Hayes 1998). Only a minority of existing species has been investigated for their sex determining mechanisms (Janzen & Paukstis 1991a); in particular relatively few fish species have been investigated. The relative rarity of ESD among, for example, amphibian families may therefore reflect the lack of studies on ESD in those taxa. Fish phylogeny is based on Nelson (1994), Lundberg (1996), Janvier (1996a,b). Tetrapod phylogeny is based on Ford & Cannatella (1993), Gaffney & Meylan (1988), Gauthier *et al.* (1988), Hillis & Green (1990), Janzen & Paukstis (1991b), Larson & Dimmick (1993), Laurin (1996), Laurin & Reisz (1995), Laurin *et al.* (1996), and Rieppel (1988). The classification of sex determining mechanisms is based on (numbers in last column): (1) Beamish (1993), (2) Docker & Beamish (1994), (3) VanEennaam *et al.* (1999), (4) Chourrout (1986), (5) Sola *et al.* (1981), (6) Moreira-Filho *et al.* (1993), (7) Bertollo & Cavallero (1992), (8) Molina *et al.* (1998), (9) Maistro *et al.* (1998), (10) Patino *et al.* (1996), (11) Craig *et al.* (1996), (12) Conover & Heins (1987), (13) Strüssman *et al.* (1996a), (14) Strüssman *et al.* (1996b), (15) Francis (1992), (16) Römer & Beisenhertz (1996), (17) Goto *et al.* (2000), (18) Schmid *et al.* (1993), (19) Hillis & Green (1990), (20) Schmid & Haaf (1989), (21) Witschi (1929), Pieau (1975), Richards & Nace (1978), (22) Schmid *et al.* (1988), (23) Schmid *et al.* (1992), (24) Mahony (1991), (25) Duellman & Trueb (1986), (26) Dorazi *et al.* (1995), (27) Janzen & Paukstis (1991a), (28) Engel *et al.* (1981), (29) Janzen & Paukstis (1991b), (30) Nakamura *et al.* (1987), (31) Demas *et al.* (1990), (32) Wellins (1978), (33) Olmo (1986), (34) Viets *et al.* (1994), (35) Caputo *et al.* (1994), (36) Gorman (1973), (37) Olmo *et al.* (1990), (38)

Volobouev *et al.* (1990), (39) Moritz (1990), (40) King & Rofe (1976), (41) Ganesh *et al.* (1997), (42) Cole (1971), (43) Cree *et al.* (1995).

**Figure 7.2. Response of sex ratio to incubation temperature in reptiles.**

These graphs represent only the approximate form of the response and are not drawn according to any single species. There are four patterns recognised at present. (A) Females develop at low temperature, males at high temperature. (B) The reverse of A, males develop at low temperatures and females at high ones. (C) Females at low and high temperatures, males at intermediate ones. (D) The hatchling sex ratio of some species is not significantly influenced by incubation temperature. From Kraak and de Looze (1993), with permission.

**Figure 7.3. Threshold model for growth dependent sex determination.**

If a threshold gonadal size or growth rate is reached by a critical time in development, those genes are activated and expressed that are responsible for sex differentiation into males in (A) and females in (B). If the threshold is not reached in time, the other sex develops. From Kraak and de Looze (1993), with permission.

**Figure 7.4. Crossing over between the two homologous chromosomes that carry the 8 growth loci with alleles ‘o’ and ‘\*’.**

The chromosomes break at location randomly chosen in the sequence of loci and the chromosome parts are swapped.

**Figure 7.5. Growth dependent survival until reproduction.**

(A)  $\alpha_m = 1, \alpha_f = -1$  (B);  $\alpha_m = 1, \alpha_f = -0.5$  (C);  $\alpha_m = 1, \alpha_f = 0$  (D)  $\alpha_m = 1, \alpha_f = +0.5$ . Male values are shown by solid lines and female values by broken lines.

**Figure 7.6. Outcome of a simulation run.**

The simulation was carried out for 50,000 generations with  $N$  ,  $\mu_T = \mu_R = \mu_G = 0.01$ ,  $VE = 0.01$ ,  $\alpha_m = 1, \alpha_f = -1$ , starting with each G-locus being homozygous for allelic value 0 and the alternative allele having value 0.5. (A) The sex ratio (proportion males) through time. (B) Mean recombination rate  $R$  through time. (C) Mean male phenotype  $P$  through time. (D) Mean female phenotype  $P$  through time. (E) Mean male heterozygosity (fraction of heterozygous G-loci) through time. (F) Mean female heterozygosity through time. (G) Frequency distribution of

growth accelerating alleles on the two homologues in males at generation 25,000. (H) Frequency distribution of growth accelerating alleles on the two homologues in females at generation

Figure 7.1 A

	ESD	heterogamety:		ref.
		male	female	
Petromyzontiformes	●			1,2
Acipenseriformes			●	3
Anguilliformes			●	4,5
Cypriniformes		●	●	4,6
Characiformes			●	
Siluriformes	●	●	●	7,8,9
Salmoniformes	●	●		4,5,6,10
<i>Myctophyiformes</i>		●	●	4,5,11
Atheriniformes	●			4,5
Cyprinodontiformes	●	●	●	12,13,14
Beryciformes		●		4,15,16
Gasterosteiformes		●	●	4
Perciformes	●	●	●	
Pleuronectiformes	●	●	●	4,5
Tetraodontiformes		●		4-
Tetrapoda	●	●	●	6,15,16 4,5,17

Figure 7.1 B

		ESD	heterogamety:		ref.	
			male	female		
Amphibia	Anura	<i>Rhacophoridae</i>		●	18	
		<i>Ranidae</i>	●	●	19,20,21	
		<i>Hylidae</i>		●	19,20,22	
		<i>Bufo</i>	●		19,20,21	
		<i>Leptodactylidae</i>		●	19,20,23	
		<i>Myobatrachinae</i>			●	24
		<i>Pelodytidae</i>		●	19	
		<i>Pipidae</i>			●	19,20
		<i>Discoglossidae</i>		●	●	19,20
		<i>Leiopelmatidae</i>			●	19
	Caudata	<i>Sirenidae</i>			●	19,25
		<i>Plethodontidae</i>		●	●	19,20
		<i>Proteidae</i>		●		19,20
		<i>Salamandridae</i>	●	●	●	19,20,26
		<i>Ambystomatidae</i>			●	19,20
		<i>Pelomedusidae</i>	●		●	27,28
		<i>Chelidae</i>		●		27,29
	Chelononia	<i>Chelydridae</i>	●	●	●	27,28,29,30
		<i>Dermochelyidae</i>	●			27
		<i>Cheloniidae</i>	●	●		27,31,32
		<i>Carettochelyidae</i>	●			27
		<i>Dermatemydidae</i>	●			27
		<i>Kinosternidae</i>	●	●	●	27,28
		<i>Emydidae</i>	●		●	27,28,29
		<i>Bataguridae + Testudinae</i>	●	●	●	27,28
		<i>Varanidae</i>	●		●	27,29,33,34
		<i>Anguidae</i>	●			27
	Squamata	<i>Scincidae</i>		●		29,33,35
		<i>Teiidae</i>		●		29
		<i>Lacertidae</i>	●		●	27,29,33,36,38
		---?--- <i>Amphisbaenia</i>			●	27
		---?--- <i>Serpentes</i>			●	27
		<i>Pygopodidae</i>		●		27
		<i>Gekkonidae</i>	●	●	●	29,33,39,40
<i>Agamidae</i>		●	●		34,41	
<i>Iguanidae</i>		●	●		29,33,34,36,42	
<i>Sphenodontia</i>		●			43	
<i>Crocodylia</i>	●			27		
<i>Aves</i>			●			
<i>Mammalia</i>		●				



Figure 7.2

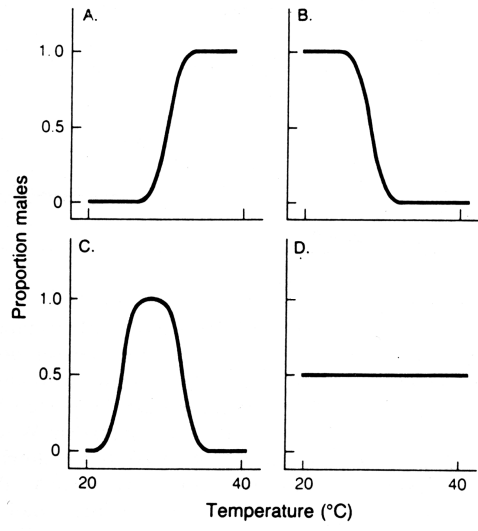


Figure 7.3

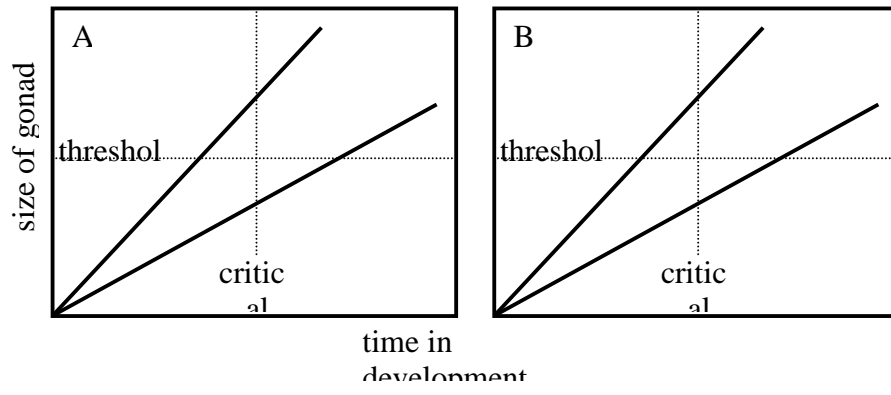


Figure 7.4

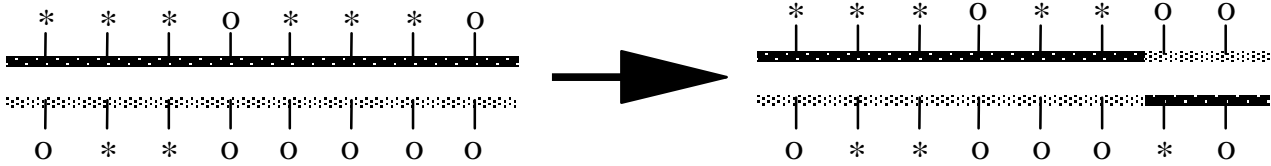


Figure 7.5

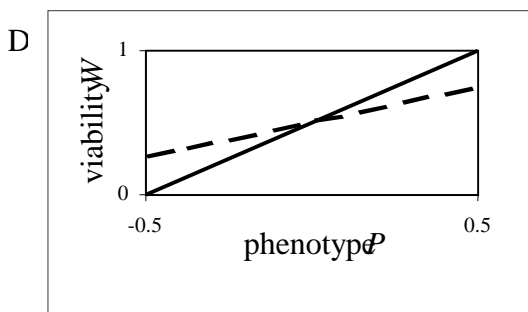
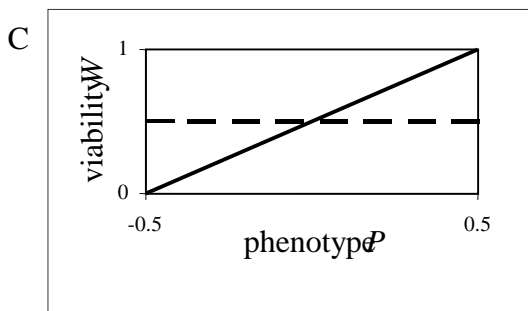
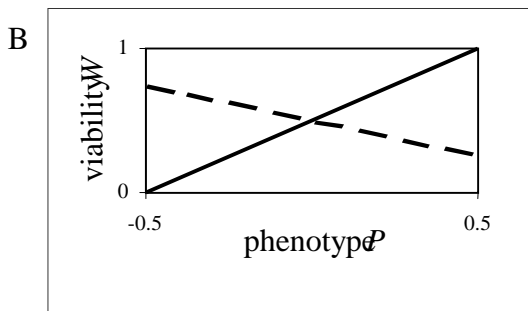
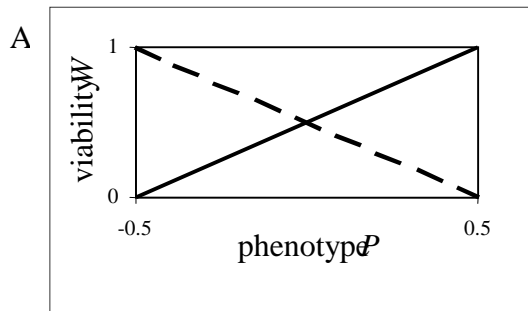


Figure 7.6

