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The Evolutionary Dynamics of Sex Determination

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REVIEW

There is substantial cytogenetic data indicating that the process of sex determination can evolve relatively rapidly. However, recent molecular studies on the evolution of the regulatory genes that control sex determination in the insect *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, and mammals suggest that, although certain sex determination regulatory genes have evolved relatively rapidly, other sex determination regulatory genes are quite conserved. Thus, studies of the evolution of sex determination, a process that appears to have elements that undergo substantial evolutionary change and others that may be conserved, could provide substantial insights into the kinds of forces that both drive and constrain the evolution of developmental hierarchies.

The past few years have witnessed a marked reemergence of interest in the evolution of developmental processes. The emphasis of most current studies is on whether the mechanisms described in model systems are conserved in other species. This approach has demonstrated that a large number of basic cellular processes are shared across vast phylogenetic distances (*1*, *2*). One developmental process that has seemed exceptional in this regard is sex determination, which appears to have substantial evolutionary plasticity. This evolutionary flexibility is surprising, because the regulation of sexual differentiation does not appear to be genetically any simpler than that of other developmental processes. Indeed, changes in sex determination would appear to face an additional evolutionary obstacle: As discussed below, in species with heteromorphic sex chromosomes, modifications in the control of sex determination often have deleterious side effects. By comparing how a range of animal species confront these problems, insight is being gained into the constraints on how sex determination mechanisms evolve.

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Classical View: Sex Determination Evolves Rapidly

Cytogenetic studies during the first half of this century showed that there are variations in sex chromosome systems among animal species, even those that are closely related, suggesting that sex chromosomes may evolve rapidly (*3*, *4*). Moreover, subsequent genetic studies showed that sex determination can be radically different in species whose chromosomal complements are apparently identical, thus further widening the possible variations in sex determination mechanisms (Table 1).

Such cytogenetic studies even identified species in which there are intraspecific variations in the mechanism of sex determination. For example, in the "standard" strains of *Musca domestica*, the housefly, sex determination is controlled by a masculinizing Y-linked gene (*M*). These strains are thus XY:XX. However, in other natural populations of this species, the chromosomes of males and females are indistinguishable. It has been genetically demonstrated that in males of those strains, *M* is autosomal (*5*). Finally, in still other populations, the autosomal *M* factor is homozygous in both males and females**.** Unisexuality is avoided because females carry a dominant female-determining gene (F^D), which is able to override the presence of *M* [reviewed in (*6*)]. Similarly, in natural populations of the wood lemming *Myopus schisticolor*, there are both normal males (XY) and females (XX) as well as females with a Y chromosome (X*Y females). Generally, in mammals, maleness is determined by the presence of the Y-linked gene *Sex-determining region Y* (*Sry*) (see below). In *Myopus*, however, although the Y chromosome carried by these X*Y females contains a normal *Sry* gene, they develop as females because the X* chromosome is able to overcome the masculinizing effect of the Y (*7*). Because close relatives of these exceptional species do not have similar polymorphisms, these observations provide additional evidence that sex determination can sometimes change rapidly.

These kinds of observations led to the view that the genetic systems that control sex determination, taken as a whole, may lack the "respectable antiquity" of the genetic machinery involved in other basic developmental processes (including specification of

segment identity, key genes controlling the development of the eyes and appendages, and other body parts) that current evidence suggests appeared before the Cambrian (530 million years ago), that is, before arthropods, nematodes, and chordates diverged (*1*). Until quite recently, data from molecular genetic studies of sex determination reinforced such a view. For example, the primary signals and most downstream genes involved in somatic sex determination in the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* are unrelated [reviewed in (*8*); we will not consider here the peculiarities of sex determination in the germ lines of these species (*8*)]. Although data for mammals are fragmentary, the few mammalian sex determination genes molecularly characterized to date are also different from those found in the two invertebrate model species (*8*, *9*) (Fig. 1).

However, recent studies indicate that the sex determination genes found in mammals and flies are fairly old (data for nematodes are scant). Moreover, in spite of the profound differences in their primary sex determination mechanisms, it is an open possibility that some of the downstream sex determination regulatory genes in flies and nematodes are functioning similarly.

Evidence for Evolutionary Conservation: Levels of Analysis

Before examining the data in detail, it is important to understand the limitations of the different types of molecular genetic evidence for functional similarity between genes of two species. The least important evidence is the finding of related genes. Even if two genes are orthologous (homologous genes, common by descent to different species) and their products still perform the same biochemical reactions today, their contexts of action may be so different in distant organisms that their biological functions may be unrelated. A further level of analysis is to indirectly assay for conservation of the biological function of orthologous genes: Knowledge of how a gene works in one species is used to design tests for functional conservation in a second species. For sex determination, the simplest assay is to establish whether a gene that produces sex-specific products in one species shows a similar sex-specific expression pattern in another species.

Finally, a third level of analysis involves direct tests for functional conservation. Ideally, this analysis would be accomplished by demonstrating that the genes act similarly in homologous genetic hierarchies. Because such data are difficult to obtain outside of model genetic organisms, a popular shortcut has been to ask whether a particular mutation in one species is complemented by the orthologous gene from the second species. Although positive results in such experiments are tantalizing, it should not be overlooked that this evidence is still indirect and is not as definitive as tests for complementation of a mutation in a species by introducing a candidate cloned gene from the same species. The difference is that, in the interspecific experiment, we are providing the gene with a context in which to act that is potentially different from that in which it is found in its species of origin. It is possible that the product of the gene has the ability to fulfill a biological role in the recipient species unrelated to what this protein does in the donor species, provided that its original function and the function to be complemented are biochemically similar. A second potential problem appears when the rescue of the mutant phenotype is accomplished by introducing not one but multiple copies of the gene or by inducing high levels of its expression. The concern here is that a related, but not truly homologous, protein might have some ability to carry out the function in question and thus if expressed at a high enough level might spuriously complement the mutant. All these caveats have to be considered when pondering the evidence for conservation that we present in the next section.

Conservation of the Sex Determination Hierarchies

Studies on the evolution of molecularly characterized sex determination genes from vertebrates and flies are beginning to reveal how these genes evolved. The primary sex determination gene *Sry* is found on the Y chromosome in all mammals analyzed, including marsupials [with the single exception of a mole rat species that

Table 1. A simplified summary of the variability of sex chromosomes and sex determination mechanisms in the order Diptera (*3*) as an example of the diversity of sex determination mechanisms within a species group. Sex chromosome constitution of females is indicated first. H, homomorphic chromosomes.

Suborder	Infraorder	Family	Genus	Sex chromosomes	Sex determination mechanisms
Nematocera	Tipulomorpha	Tipulidae	*Tipula*	XX/XY, H	
			Pales	XX/XY	Male-determining dominant factor
	Bibionomorpha	Sciaridae	*Sciara*	XX/X0 (somatic)	Genotype of the mother; X:A balance
		Cecidomyiidae	*Mayetiola*	$X_1X_1X_2X_2/X_1X_20$	Genotype of the mother, X:A balance?
	Culicomorpha	Culicidae	*Culex*	H	Male-determining dominant factor
			Anopheles	H	Male-determining dominant factor
			Aedes	XX/XY	Male-determining dominant factor
		Simuliidae	*Eusimulium*	H	Male-determining dominant factor
		Chironomidae	*Chironomus*	H	Male-determining dominant factor (variable location)
			Polypedilum	ZW/ZZ	
Brachycera	Tabanomorpha	Tabanidae		XX/XY	
		Stratiomyidae		XX/XY	
Cyclorrhapha	Muscomorpha Aschiza	Phoridae	*Megaselia*	H	Male-determining dominant factor (variable location)
	Schizophora Acalyptratae	Tephritidae	*Ceratitis*	XX/XV	Male-determining dominant factor
			Anastrepha (exceptions)	$X_1X_1X_2X_2/X_1X_2Y$ ZW/ZZ	
		Drosophilidae	*Drosophila*	XX/XY (exceptions: X0, X_1X_2Y, XY_1Y_2)	X:A balance
	Schizophora Calyptratae	Muscidae	*Musca*	XX/XY, H	Several (see text)
		Calliphoridae	*Calliphora*	XX/XY, H	Male-determining dominant factor
			Chrysomya	XX/XY, H	Genotype of the mother
			Lucilia	XX/XY	Male-determining dominant factor

lacks a Y chromosome (*9*, *10*)], suggesting that the *Sry*-based system is at least 130 million years old. Although there are no sex-specific *Sry*-related sequences in birds or reptiles (*11*), an autosomal gene involved in sex determination, *SRY-box related–9* (*Sox9*), is highly conserved from mammals to fish and shows sex-specific expression in male gonads of both mammals and birds (*12*). *Sox9*, which encodes a DNA-binding protein of the same family as SRY, could be part of an ancestral sex-determining machinery, now under the control of *Sry* only in mammals (*12*) (Fig. 1C).

With respect to somatic sex determination in *Drosophila*, *Sex-lethal* (*Sxl*), the gene at the top of the hierarchy, three genes that function downstream of *Sxl* [*transformer* (*tra), tra-2*, and *doublesex* (*dsx*); see Fig. 1A], and *sisterless-a* (*sis-a*), a gene involved in *Sxl* activation, are probably acting similarly in other *Drosophila* species. These genes have been cloned in other drosophilids, including (for all five genes) the distant relative *D. virilis* (*13–17*) (the *melanogaster-virilis* split occurred about 60 million years ago). The structures and functions of these genes in *D. virilis* and *D. melanogaster* appear to be equivalent. For *tra* and *tra-2*, the *D. virilis* genes are able to rescue the respective mutations in *D. melanogaster*, whereas rescue by *D. virilis sis-a* is partial. A single difference has been reported in drosophilids: Although SXL protein is found only in *D. melanogaster* females, an SXL isoform is present in males as well as females of *D. virilis* and some other closely related species (*13*). The available data suggest, however, that the ancestral state in drosophilids is the absence of SXL protein in males, and in these exceptional species SXL expression in males may be irrelevant to sex determination (*13*).

Data from outside the Drosophilidae family with respect to *Sxl, dsx*, and *tra-2* are also accumulating. These three genes are conserved at the nucleotide level, a requirement that *tra*, one the fastest evolving *Drosophila* genes known (*15*), does not meet. One interesting result is that, in those nondrosophilid dipterans in which *Sxl* expression has been examined, *Sxl* transcripts appear to be identical in males and females, suggesting that *Sxl* may not have a role in somatic sex determination in these species (*18*, *19*). Indeed, in the phorid *Megaselia scalaris*, *Sxl* transcripts are found in adult flies only in ovaries and testes (*19*). Several genes related to *tra-2* have been cloned from humans and mice (*20*, *21*). One of these genes rescues the *tra-2* mutant phenotypes in transgenic flies (*20*). It is not known whether these *tra-2* orthologs function in mammalian sex determination.

Involvement of *dsx* in sex determination may be ancient. A *dsx*

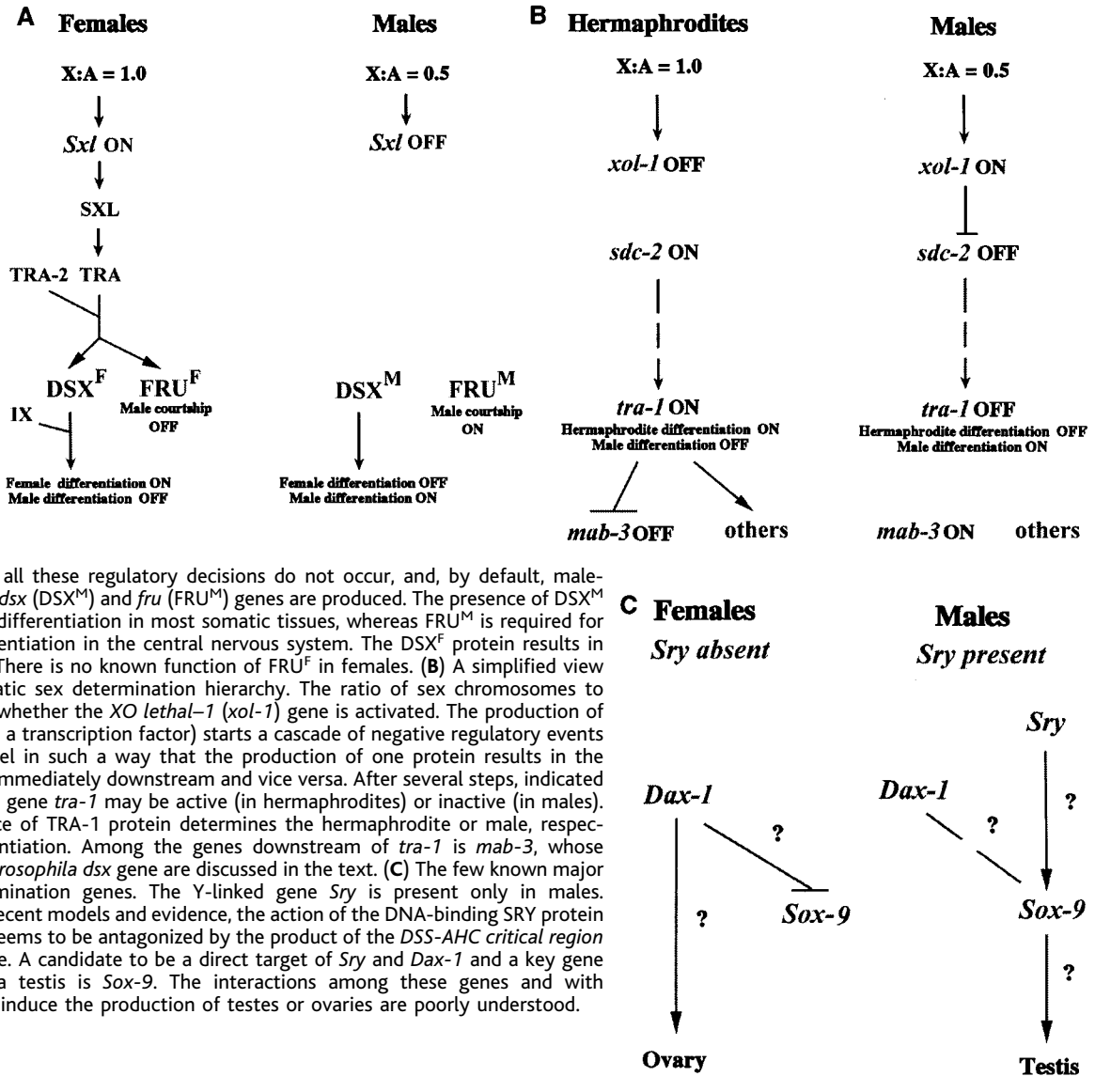

Fig. 1. Main features of the sex determination systems in model species (*8*, *9*). (**A**) A simplified view of the somatic sex determination hierarchy in *D. melanogaster*. The ratio of X chromosomes to autosomes (X:A) determines whether *Sxl* is activated. The SXL protein acts as a splicing factor on the RNA produced by the *tra* gene, resulting in the production of active TRA protein in females. TRA, together with TRA-2 (the product of the gene *tra-2*), determines the female-specific splicing of the *dsx* (DSX_F) and *fruitless* (*fru*) (FRU^F) RNAs. In the absence of SXL, all these regulatory decisions do not occur, and, by default, male-specific products of the *dsx* (DSX^M) and *fru* (FRU^M) genes are produced. The presence of DSX^M protein results in male differentiation in most somatic tissues, whereas FRU^M is required for aspects of sexual differentiation in the central nervous system. The DSX^F protein results in female differentiation. There is no known function of FRU^F in females. (**B**) A simplified view of the *C. elegans* somatic sex determination hierarchy. The ratio of sex chromosomes to autosomes determines whether the *XO lethal–1* (*xol-1*) gene is activated. The production of XOL-1 protein (which is a transcription factor) starts a cascade of negative regulatory events at the transcription level in such a way that the production of one protein results in the absence of the protein immediately downstream and vice versa. After several steps, indicated by the dashed lines, the gene *tra-1* may be active (in hermaphrodites) or inactive (in males). The presence or absence of TRA-1 protein determines the hermaphrodite or male, respectively, mode of differentiation. Among the genes downstream of *tra-1* is *mab-3*, whose relationships with the *Drosophila dsx* gene are discussed in the text. (**C**) The few known major mammalian sex determination genes. The Y-linked gene *Sry* is present only in males. According to the most recent models and evidence, the action of the DNA-binding SRY protein on downstream genes seems to be antagonized by the product of the *DSS-AHC critical region on the X-1* (*Dax-1*) gene. A candidate to be a direct target of *Sry* and *Dax-1* and a key gene in the production of a testis is *Sox-9*. The interactions among these genes and with downstream targets to induce the production of testes or ovaries are poorly understood.

homolog, very similar to the *D. melanogaster* gene in terms of structure and sex-specific expression, is present in the tephritid *Bactrocera tryoni* (Queenslad fruit fly) (*22*). In a much more distantly related fly, the phorid *Megaselia scalaris*, sex-specific *dsx* RNAs have been detected (*19*). Finally, the gene *male abnormal–3* (*mab-3*), which is one of the last genes in the sex determination hierarchy in *C. elegans* males (Fig. 1B), contains *dsx*-related zinc finger domains. Most interestingly, a *Drosophila dsx* cDNA clone that expresses the male-specific isoform of this protein (DSX^M; see Fig. 1A) is able to rescue the *C. elegans mab-3* mutant phenotypes as does a *mab-3* transgene (when either of them is introduced in several copies under a heat-shock–inducible promoter), whereas a transgene encoding the female *dsx* isoform (DSX^F; Fig. 1A) does not rescue the *mab-3* phenotype (*23*). Thus, *dsx*-related functions in sex determination may have been conserved across the hundreds of millions of years that separate flies from worms.

Although the data are limited, these observations provide some suggestions as to how the genetic hierarchies controlling sex evolved. First, it appears that at least some parts of the regulatory network that controls sex determination are changing quite slowly. The most complete information, that for flies, suggests that sex determination has been controlled by exactly the same hierarchy for at least 60 million years and part of that hierarchy (*dsx* and *tra-2)* may well have been involved in sex determination much longer. The same is true for vertebrates, where *Sry* has been involved in sex for at least 130 million years and *Sox9* probably much longer than that. Second, although those genes in the upper part of the hierarchies (*Sry* and *Sxl*) have become involved in sex determination only relatively recently, at least some of the genes downstream (*Sox9* and *dsx*) appear to have been involved in this process for much longer times.

Rhythm of Change of Sex Determination

There are a number of theoretical and experimental studies of how sex determination mutations may become fixed in natural populations. Three main points can be deduced from these studies: (i) Not all changes are equally likely. The probability of each change is highly dependent on the genetic architecture that underlies sex determination. (ii) In many species, the primary sex determination mechanism is tied to sexually dimorphic sex chromosomes. These dimorphisms are frequently associated with marked differences in the gene content of the (X/Y or Z/W) sex chromosomes, with one of those chromosomes losing all or nearly all its genes [a process known as "chromosome degeneration" (*24*)]. As we will see below, the presence of heteromorphic chromosomes greatly influences the likelihood of sex determination changes. (iii) There are situations where sex determination transitions may be advantageous.

There are several important intrinsic factors that constrain transitions in the genetic hierarchies controlling sex determination. First, consider regulatory changes within a sex. One often overlooked point is that changes at the top of the hierarchies will be in general easier to accommodate than changes at the bottom, because it is more likely that the former will have no deleterious effects. For example, in simple cases such as when sex is dominantly determined by a single gene at the top of the hierarchy, any gene that takes control of the expression of such a gene will cause a shift in sex determination. The variants found in *Musca* may be examples of this kind of transition. Even in more complex cases, it has been suggested that changes at the top of the hierarchies should be more likely to occur (*25*). These theoretical expectations are in good agreement with the data indicating that genes at the top of the hierarchies have been coopted for sex determination relatively recently (see above). A second factor is the pleiotropic effects of the genes involved in sex determination. Pleiotropy, defined from a molecular perspective as multiple effects of a gene on several independent targets or biochemical pathways, may act as a powerful force against evolutionary change (*26*). Therefore, genes with a single function in sex determination will be easier to replace than genes with multiple functions. In particular, genes at the bottom of the hierarchies, which directly control the expression of many other target genes, may have multiple effects, thus being difficult to substitute. A third factor is that, at least in some species, one sex is produced by default, whereas the production of the other requires the activity of a genetic hierarchy. This may create biases, because in the sex where the hierarchy is active, each gene in the hierarchy is a potential target for altering the sex determination mechanism. On the other hand, in the sex produced by default, only the terminal effectors may be altered, and, as just noted, those changes are unlikely.

When one considers dominant mutations that transform the phenotype of individuals of one sex into the other, the situation is further complicated, because the probability of change depends on whether heteromorphic chromosomes are present. Consider the simplest possible transition between systems—a species in which a dominant sex-determining mutation arises on an autosome and that already has a pair of heteromorphic (XY) sex chromosomes. Transition to a new sex chromosome system involving the autosomal pair on which the new sex-determining mutation arose will be potentially difficult to achieve: (i) If the new mutation is a dominant female-determining gene, two types of deleterious effects may occur. First, YY individuals will appear as offspring of XY females. Because YY individuals will be inviable or have a very low fitness, XY females will have a handicap when competing with XX females. Second, indirect effects may occur if there are specific genes on the Y chromosome that have been conserved because of their effects in males and the activity of such Y chromosome genes interferes with the development of functional XY females. (ii) If the new mutation is a dominant male-determining gene, the absence of necessary Y chromosome genes in XX males may keep them from being fully functional.

In the absence of heteromorphic sex chromosomes, these problems are not encountered, and so there is a higher probability of transitions in the sex determination system. Moreover, chromosomal degeneration may be avoided indefinitely, provided that the master gene that controls sex is changing often or can be transferred from one chromosome to another [such transfer may occur by successive translocations or when, as suggested for *Megaselia scalaris*, among other species, the sex-determining gene behaves as a transposable element (*27*)]. The fortuitous fact that these systems are changing constantly may allow them, at least temporarily, to avoid the degenerative process. We can conclude that the relationship between the dynamics of sex determination and chromosome degeneration is bidirectional. The limitation of a chromosome to one sex is what triggers degeneration, but once this process has started, degeneration itself diminishes the probability of subsequent change in the sex determination system.

Besides all the internal factors (genetic architecture and presence of heteromorphic chromosomes), there are also external factors that influence the probability of fixation of sex determination variants. For example, Bull and Charnov (*28*) have considered various cases of multifactorial genetically determined sex determination (when two, or a few, genes may be alternatively used to determine sex, as in *Musca*). In general, when the individuals that carry a particular mutation have the same fitness as the individuals without the mutation, the different systems may coexist in stable equilibria with fixations following a typical neutral dynamics. A transition to a new sex determination system will occur even more frequently when one of the mutations is itself selectively advan-

tageous or is physically close to a favorable mutation (this later effect is known as "hitchhiking"). For example, it has been suggested that the mutation *M* of *Musca* has been favored because it is associated with DDT resistance (*29*). Another possible advantage of sex determination mutations is related to the fact that they often cause modifications of the sex ratio. Although producing offspring with a 1:1 sex ratio is usually advantageous, in some situations other sex ratios are preferable, and then alternative sex determination systems may have an advantage [see (*4*, *30*) for a discussion].

Concerning the selective pressure on sex determination genes, an observation that has generated considerable discussion is that some of the sex determination genes evolve at a fast pace, including *Sry* in mammals (*31*), *tra* in *Drosophila* (*15*, *32*), and *tra-1* and *tra-2* in *Caenorhabditis* (*33*). It has been proposed that the reason for this rapid evolution could be positive selective pressure on these genes (*31*, *32*). For *Sry*, when sequences of certain species are compared, there is an excess of nonsynonymous substitutions, a result suggesting positive selection (*31*, *34*). However, the evidence is inconclusive. The excess of nonsynonymous changes is concentrated in the terminal regions of the protein, away from the evolutionarily conserved DNA-binding high mobility group box (*34*). Finally, no excess of nonsynonymous changes has been found in a group of closely related wallaby species (*35*). On the basis of these results, it has been suggested that the patterns of nucleotide substitutions could be explained by (i) lack of constraints on the evolution of the terminal regions and (ii) occasional selection for genes other than *Sry*, plus hitchhiking effects on *Sry* due to the lack of recombination in the Y chromosome (*35*, *36*). The evidence for selection in the *Drosophila tra* gene is based on the fact that the region containing *tra* has a low level of polymorphism and a high level of diverged sites when compared with other genes (*32*). Again, the problem is whether it is *tra* itself that is being selected or whether it is a physically close gene that causes this effect. In any case, if it is demonstrated that these genes are under positive selective pressure, it would be most interesting to understand why sex determination genes, once established as such, might be the targets for such selection.

Dynamic Changes in Genetic Control of Developmental Processes

Two points emerge from the comparative studies of sex determination that may be relevant to other developmental mechanisms. First, it may be that mutations affecting the sex determination genes, including those with marked effects, are intrinsically less damaging for the fitness of the carriers than are many mutations affecting other developmental processes. This may be so because, in some cases, the biochemical collapse of the system that actively induces one sex simply causes a totally normal individual of the other (default) sex to appear. It is worth considering whether this kind of situation may arise in developmental processes other than sex determination. Obvious candidates are those cases where two, or more, morphs (showing variation in size, development of weapons such as horns or mandibles, presence or absence of wings, differences in behavior, and so forth) occur among individuals of the same sex in a particular species. If the different morphs are produced by regulatory changes that involve a single gene, then rapid evolutionary changes in the genetic control of such systems are also to be expected.

A second aspect of why sex determination is sometimes able to evolve rapidly may have even broader implications. As we have seen, some changes are tolerated because the genetic architecture of the system allows certain alterations in part of the hierarchy without deleterious effects. Changes appear to be most tolerated at the top of the sex determination hierarchies. Thus, it is worth considering whether other developmental processes may be evolving in the same way, that is, keeping constant the genes that are the terminal effectors of the process (downstream in their respective hierarchies), while the regulatory genes at the top are changing relatively often. It is worth noting that these types of changes are relatively easy to detect in the case of sex determination, where cytogenetics, or very simple classical genetics, has been used to screen a large number of species, looking for those exceptional cases where changes actually occurred. However, our prediction is that molecular studies of other developmental systems may eventually detect a similar evolutionary dynamic. If this is so, the "peculiar flexibility" of the evolution of sex determination may, with further data, also become apparent as a feature of other developmental processes.

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