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Evolutionary changes of heterogametic sex in the phylogenetic history of amphibians

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Abstract

A survey of sex-determining systems in amphibians revealed widespread variation in the heterogametic sex and the extent of sex chromosome differentiation. Systems of sex determination have now been resolved in 63 species of salamanders and frogs. Species with ZW female heterogamety, OW female heterogamety, and XY male heterogamety have all been reported. A phylogenetic analysis suggests that the ancestral state for amphibians was female heterogamety. XY/XX sex determination has evolved independently at least seven times, whereas there is only one case in which the data suggest that male heterogamety has subsequently reversed to female heterogamety. Differentiation of heteromorphic sex chromosomes in Amphibia has occured repeatedly. A model of an ancestral two-locus sex-determination mechanism, based on the findings of the phylogenic analysis and the condition found in the primitive frog *Leiopelma hochstetteri*, allows a recessive mutation to result in the eventual acquisition of male heterogamety. Reversal to female heterogamety requires *de novo* evolution of a functional control locus under the model. The model is consistent with all information on sex determination in amphibians, and explains the observed bias in evolution from ZW/ZZ to XY/XX systems.

Introduction

Although generally unrecognized even 10 years ago, the variability of heterogametic sex in amphibians is now apparent and has been noted by several recent authors (Bull, 1983; Schmid, 1983; Duellman and Trueb, 1986). Both male and female heterogametic systems are known, and there is enormous variation in the degree of sex-chromosome heteromorphism. The extent of heterogametic variation and sex-chromosome variation in amphibians (as well as the recent recognition of this variation) is surprising. As late as 1979, Morescalchi (1979) stated that the existence of heterochromosomes in the Anura, as purportedly shown by Singh (1974) or Yadav and Pillai (1976), remained to be convincingly demonstrated. Until 1980, only Morescalchi's (1964) study of *Discoglossus pictus* seemed to adequately demonstrate the existence of a heteromorphic sex-chromosome pair in a frog. For salamanders, Morescalchi (1979) could cite only information personally obtained from J. Kezer, some of which was published by Leon and Kezer (1978), indicating XY sex-chromosome heteromorphisms in some bolitoglossine salamanders. Since 1979, however, our knowledge of sex-determination systems and sex-chromosome heteromorphisms in the Amphibia has grown enormously. Even by 1983, Schmid (1983) listed 25 species of amphibians (5 frogs and 20 salamanders) with heteromorphic sex chromosomes. Now some 9 frog and 34 salamander species are known to have XY/XX, ZW/ZZ or OW/OO heteromorphic sex chromosome systems allowing considerable extension of Schmid's (1983) analysis. In 20 additional species, heterogamety has been verified by indirect genetic means.

Sex-chromosome heteromorphism in amphibians ranges from a lack of any cytologically discernable difference between the sex chromosomes to such extreme cases of chromosomal heteromorphism as have been seen in the hylid frogs *Gastrotheca riobambae* and *Gastrotheca pseustes* (Schmid et al., 1984; Schmid, 1989), various species of salamanders of the genera *Oedipina* and *Necturus* (Sessions, 1984; Sessions and Wiley, 1985), and the primitive frog *Leiopelma hochstetteri* (Green, 1988b). These differences have made it possible to determine the heteromorphic sex by cytogenetic means. In some other species, chromosome banding techniques have allowed less obvious cases of sex-chromosome heteromorphism to be diagnosed (Green, 1988a; Schmid, 1983, 1989; Sessions, 1982). Many species of amphibians, though, do not possess discernably different sex chromosomes. The heterogametic sex in some of these species has been discovered by more laborious means employing the breeding of sex-reversed individuals (Chang and Witschi, 1955, 1956; Panse, 1942; Kawamura and Nishioka, 1977), genetic sex-linkage studies (Elinson, 1981; Wright and Richards, 1983) or, in a few cases, by immunological studies employing H-Y antigen (Wachtel et al., 1975; Zaborski, 1979). In all, the heterogametic sex has been diagnosed in 63 species of amphibians, covering a wide range of taxa, although, unfortunately, no caecilians are included in this group.

Bull (1983) made a plea for studies of the origins and ancestries of male and female heterogamety in well-known groups of organisms, contending that such studies should be useful in determining biases in the origin and maintenance of particular forms of heterogamety. Although identification of specific examples of male and female heterogamety have continued to accumulate in a wide variety of organisms, there have been no attempts to trace the historv of change in heterogametic sex within any variable group of species. Considering the recent growth in our knowledge of sex determination in amphibians, they would seem to be a particularly useful group for this sort of analysis. The relationship between heterogametic sex in amphibians and their phylogeny has previously not been studied.

Amphibians are ideal for an historical analysis of heterogametic sex not only because of the variability of heterogametic sex in the group, but also because phylogenetic studies of most of the relevant taxa have been published. This allows the occurrence of particular sex-determination systems to be mapped onto a phylogeny of the group. In this paper, we examine the evolution of heterogametic sex among all modern amphibians in order to determine the frequency and direction of changes in this character over a history spanning some 250 million years.

Methods

A search of the literature revealed 63 species of amphibians for which the sex-determining mechanism has been determined (Table 1). Evolutionary changes of heterogametic sex were mapped onto a phylogeny of the amphibians constructed by combining phylogenetic findings from a diversity of studies (Fig. 1 and Table 1).

The major outlines of familial relationships were drawn from recent reviews by Cannatella (1985) and Duellman and Trueb (1986). Evidence of relationships within families was combined from numerous sources (see Fig. 1). Evidence for dichotomous relationships within a few genera and among families of the suborder Neobatrachia are as yet unresolved (Fig. 1). Equivocal relationships were figured as polytomies. Fig. 1 only shows cladogenic relationships; lengths of the branches are arbitrary.

After the phylogenetic tree was constructed, heterogamety character states were mapped to the phylogeny in the most parsimonious arrangement (in other words, in the fewest number of possible changes; Fitch, 1971). We were conservative in mapping character changes in polytomous portions of the tree; only the minimum number of changes possible under any resolution of the polytomy was mapped. Only the polytomy that involved the families of Neobatrachia posed such a problem. Among these families, a resolution of Hylidae and Leptodactylidae as sister groups compared to the Bufonidae and Ranidae would require only a single change in heterogametic sex among these families, whereas any other resolution would require two separate changes (Fig. 1).

Results

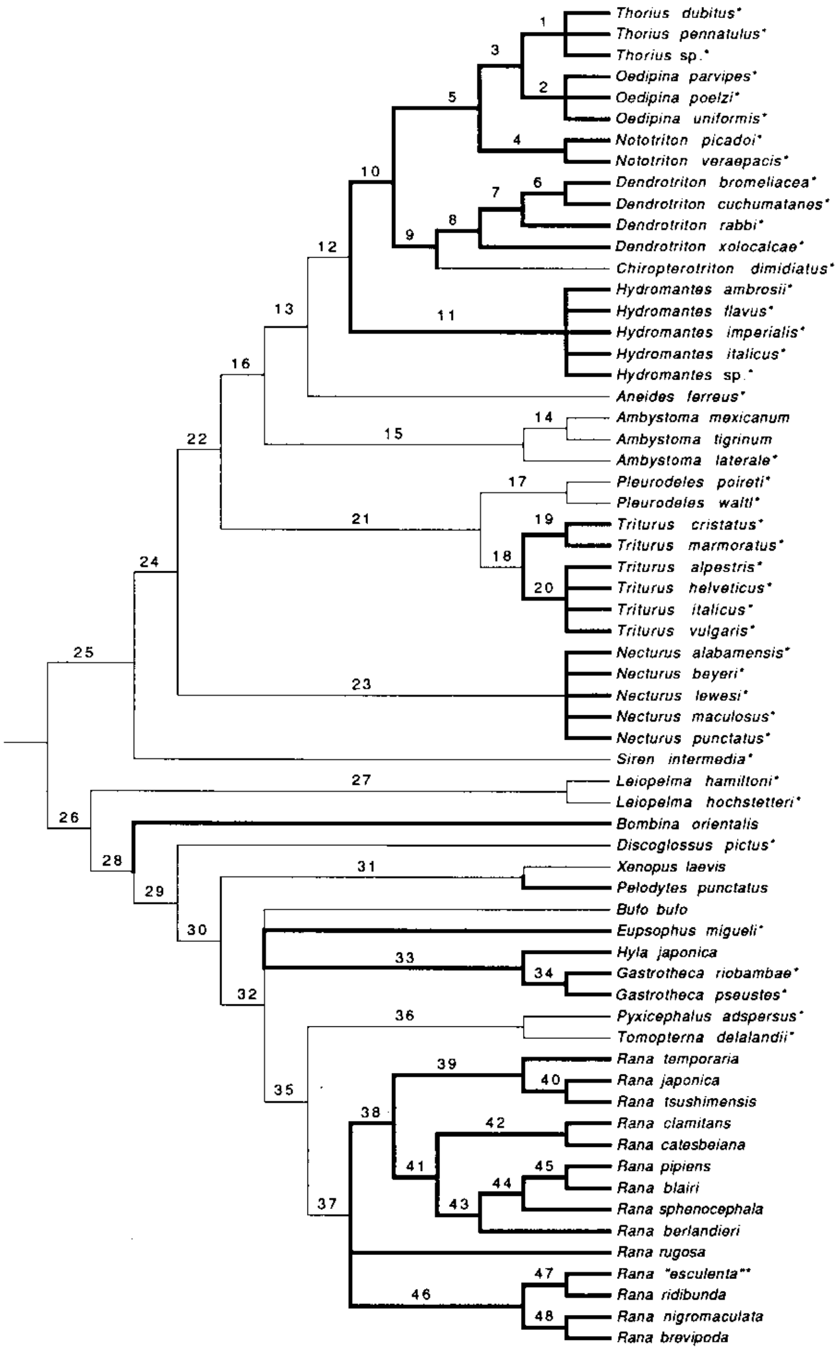
The most parsimonious explanation for the evolution of sex-determining mechanisms in amphibians (Fig. 1) indicates that female heterogamety is the ancestral condition for amphibians and that male heterogamety has subsequently arisen at least seven times independently. Male heterogamety, reported from 48 of 63 species, has been more commonly found among amphibians than female heterogamety, but this appears largely to be a result of uneven sampling among lineages. For instance, both the bolitoglossine salamanders and frogs of the genus *Rana* have been relatively extensively sampled and appear to be nearly fixed for male heterogamety (Fig. 1 and Table 1). In contrast, other similarly speciose groups, such as salamanders in the tribe Plethodontini and toads of the genus *Bufo*, are each represented in the analysis by only a single species in which female heterogamety has been determined. However, our analysis did reveal a significant bias toward the evolution

Table 1. Sex-determining mechanisms of amphibians. Methods of determination of the heterogametic sex are indicated in parenthesis following the mechanism: C (cytological), G (genetic), and I (immunologic).

Taxon	Mechanism	References
Order Caudata		
Family Ambystomatidae		
Ambystoma laterale	ZW (C,)	Sessions, 1982
A. mexicanum	ZW (G, I)	Humphrey, 1942, 1945, 1957; Zaborski, 1979
A. tigrinum	ZW (G)	Humphrey, 1942, 1945, 1957
Family Plethodontidae		
Tribe Bolitoglossini		
Chiropterotriton dimidiatus	ZW (C)	Sessions, 1984
Dendrotriton bromeliacea	XY (C)	Leon and Kezer, 1978
D. cuchumatanes	XY (C)	Leon and Kezer, 1978
D. rabbi	XY (C)	Leon and Kezer, 1978
D. xolocalcae	XY (C)	Sessions, 1984
Hydromantes ambrosii	XY (C)	Nardi et al., 1986
H. flavus	XY (C)	Nardi et al., 1986
H. imperialis	XY (C)	Sessions, 1984; Nardi et al., 1986
H. italicus	XY (C)	Sessions, 1984; Nardi et al., 1986
H. sp.	XY (C)	Nardi et al., 1986
Nototriton picadoi	XY (C)	Leon and Kezer, 1978; Sessions, 1984
N. veraepacis	XY (C)	Sessions, 1984
Oedipina parvipes	XY (C)	Sessions, 1984
P. poelzi	XY (C)	Leon and Kezer, 1978; Sessions, 1984
O. uniformis	XY (C)	Leon and Kezer, 1978
Thorius dubitus	XY (C)	Leon and Kezer, 1978
T. pennatulus	XY (C)	Leon and Kezer, 1978
T. sp	XY (C)	Sessions, 1984
Tribe Plethodontini		
Aneides ferreus	ZW (C)	Kezer and Sessions, 1979
Family Proteidae		
Necturus alabamensis	XY (C)	Sessions and Wiley, 1985
N. beyeri	XY (C)	Sessions and Wiley, 1985
N. lewesi	XY (C)	Sessions and Wiley, 1985
N. maculosus	XY (C)	Sessions, 1980; Sessions and Wiley, 1985
N. punctatus	XY (C)	Sessions and Wiley, 1985
Family Salamandridae		
Pleurodeles poiretti	ZW (C, G)	Gallien, 1954; Lacroix, 1970
P. waltl	ZW (C, G, I)	Gallien, 1954; Lacroix, 1986a, b; Zaborski, 1979
Triturus alpestris	XY (C, G)	Mancino et al., 1977;
T. cristatus	XY (C)	Schmid, 1983
T. helveticus	XY (C)	Schmid et al., 1979
T. italicus	XY (C)	Mancino et al., 1977
T. marmoratus	XY (C)	Schmid, 1983
T. vulgaris	XY (C, G)	Mancino et al., 1979
Family Sirenidae		
Siren intermedia	Z~~X~~W (C)	Leon and Kezer, 1974

Table 1 (continued)

Taxon	Mechanism	References
Order Anura		
Family Bombinatoridae		
Bombina orientalis	XY (G)	Kawamura and Nishioka, 1977
Family Bufonidae		
Bufo bufo	ZW (G)	Panse, 1942
Family Discoglossidae		
Discoglossus pictus	ZW (C)	Morescalchi. 1964
Family Hylidae		
Gastrotheca riobambae	XY (C)	Schmid, 1983
Gastrotheca pseustes	XY (C)	Schmid, 1989
Hyla japonica	XY (G)	Kawamura and Nishioka, 1977
Family Leiopelmatidae		
Leiopelma hamiltoni	ZW (C)	Green, 1988a
L. hochstetteri	OW (C)	Green, 1988b
Family Leptodactylidae		
Eupsophus migueli	XY (C)	Itura and Veloso, 1981
Family Pelodytidae		
Pelodytes punctatus	XY (1)	Zaborski, 1979
Family Pipidae		
Xenopus laevis	ZW (G, I)	Chang and Witschi, 1955, 1956; Gallien, 1953; Zaborski, 1979
Family Ranidae		
Pyxicephalus adspersus	ZW (C)	Engel and Schmid, 1981; Schmid, 1980b; Schmid and Bachman, 1981
Rana berlandieri	XY (G)	Wright et al., 1983
R. blairi	XY (G)	Wright et al., 1983
R. brevipoda	XY (G)	Kawamura and Nishioka, 1977
R. catesbeiana	XY (G)	Elinson, 1981
R clamitans	XY (G)	Elinson, 1981, 1983
R. "esculenta"	XY (C)	Schempp and Schmid, 1981; Witschi, 1923
R. japonica	XY (G)	Moriwaki, 1959; Kawamura and Yokota, 1959; Kawamura and Nishioka, 1977
R. nigromaculata	XY (G)	Kawamura, 1939; Kawamura and Nishioka, 1977
R. pipiens	XY (G, I)	Richards and Nace, 1978; Wachtel et al., 1975; Wright and Richards, 1983
R. rugosa	XY (G)	Kawamura and Nishioka, 1977
R. ridibunda	XY (G, I)	Kawamura and Yokota, 1959; Zaborski, 1979
R. sphenocephala	XY (G)	Wright et al., 1983
R. temporaria	XY (G)	Schempp and Schmid, 1981; Witschi, 1923
R. tsushimensis	XY (G)	Kawamura and Nishioka, 1977
Tomopterna delalandii	ZW (C)	Schmid, 1980b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Ambystoma laterale*
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Pleurodeles waltl*
Triturus cristatus*
Triturus marmoratus*
Triturus alpestris*
Triturus helveticus*
Triturus italicus*
Triturus vulgaris*
Necturus alabamensis*
Necturus beyeri*
Necturus lewesi*
Necturus maculosus*
Necturus punctatus*
Siren intermedia*
Leiopelma hamiltoni*
Leiopelma hochstetteri*
Bombina orientalis
Discoglossus pictus*
Xenopus laevis
Pelodytes punctatus
Bufo bufo
Eupsophus migueli*
Hyla japonica
Gastrotheca riobambae*
Gastrotheca pseustes*
Pyxicephalus adspersus*
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of male heterogamety from female heterogamety. If the analysis is correct, changes from ZW to XY have occurred at least seven times, whereas there is only one case of a change in the reverse direction.

Discussion

Identification of sex-determination systems in Amphibia

All species of amphibians studied to date exhibit heterogametic sex determination. Polyfactorial sex determination, environmental sex determination, and arrhenotoky are unreported as standard means of sex determination in amphibians. Although several hundred species of amphibians have been karyotyped (Morescalchi, 1973; Duellman and Trueb, 1986), morphological divergence of sex chromosomes is usually minor in amphibians and their sex chromosomes rarely can be identified using traditional cytogenetic techniques (Morescalchi, 1971, 1973, 1975, 1979; Schmid, 1980a). The advent of chromosome banding methods and their refinement for application with amphibian chromosomes has increased the ability to detect sex chromosome heteromorphisms (Schmid, 1983, 1989; Green, 1988). However, identification of the heterogametic sex in some amphibians has been possible only by the use of sex-reversal experiments (Panse, 1942; Chang and Witschi, 1955; Schmid, 1983).

Although multiple reports of heterogametic sex have been consistent for most species, both male and female heterogamety have been reported for *Triturus cristatus* and *T. marmoratus*. The reports of female heterogamety in these species were based on reports of morphological asymmetry of lampbrush bivalent I, together with a lack of chiasmata between two supposedly heteromorphic segments in females (Callan and Lloyd, 1956; 1960; Mancino and Nardi, 1971; Mancino et al., 1972). However, Mancino et al. (1973) reported that both male and female C-banded karyotypes have a Giemsa-positive segment that corresponds to the "heteromorphic" region on lampbrush bivalent I, and Mancino et al. (1977) concluded that this region has the same morphology and behavior at meiosis in both sexes. Additional banding studies by Schmid (1983) have revealed that these two species exhibit male

Fig. 1. Phylogenetic tree of amphibian species for which the sex determination system is known. The thin branches indicate lineages with female heterogamety; the thick branches indicate lineages with male heterogamety. The taxa with asterisks have heteromorphic sex chromosomes. Numbers on branches indicate references in support of the tree as follows: 1–5) Wake and Elias, 1983; 6–8) Lynch and Wake, 1975; 9) Wake and Elias, 1983; 10) Elias and Wake, 1983; 11) Wake et al., 1978; 12) Wake 1966; 13) Wake, 1966; 14) Shaffer, 1984; 15) Tihen, 1969; 16) Duellman and Trueb, 1986; 17) Thorn, 1968; 18–20) Bucci-Innocenti et al., 1983; 21) Wake and Ozeti, 1969; 22–23) Duellman and Trueb, 1986; 24) Estes, 1981; 25–27) Duellman and Trueb, 1986; 28–32) Cannatella, 1985; 33) Duellman and Trueb, 1986; 34) Duellman and Hillis, 1987; 35) Dubois, 1981; 36) Clarke, 1981; 37) Wallace et al., 1973; 38) Case, 1978; 39) Wallace et al., 1973; 40) Kawamura and Nishioka, 1977; 41–42) Hillis and Davis, 1986; 43–45) Hillis et al., 1983; 46) Wallace et al., 1973; 47) Uzzell et al., 1977; 48) Kawamura and Nishioka, 1978.

heterogamety, although citations of the earlier reports in support of female heterogamety persist (e.g., Duellman and Trueb, 1986). This example suggests that other reports of sex-chromosome differentiation based on limited samples (e. g., the report of heteromorphic chromosomes in female *Siren intermedia*; Leon and Kezer, 1974) should be verified.

Early reports of the unbanded karyotype of the salamander, *Necturus maculosus* (Seto et al., 1964; Morescalchi, 1975), failed to identify heteromorphic sex chromosomes, although King (1912) had long before described the occurrence of a heteromorphic spermatogenic bivalent. Using banding techniques, Sessions (1980) clearly demonstrated the XY heteromorphic sex chromosomes of this species, and later (Sessions and Wiley, 1985) showed that XY heteromorphism occurs in all species of *Necturus*. Thus, even reports that failed to find sex chromosomes in amphibians may be open to reconsideration. Earlier authors not only did not have access to present chromosome banding methods but may have been working under the paradigm that amphibians did not have heteromorphic sex chromosomes.

Other difficulties may exist among some early reports of sex chromosomes in frogs. The first report of heteromorphic sex chromosomes in a frog, *Hyla japonica* (Yosida 1957), was considered inconclusive by Morescalchi (1964), but heterogamety in males of this species was later confirmed by Kawamura and Nishioka (1977) using sex-reversal experiments. Morescalchi's (1964) report of ZW sex chromosomes in *Discoglossus pictus* remained virtually the only record of sex chromosome heteromorphism in frogs until the early 1980s. Morescalchi (1964) described a small length difference between the members of a pair of telocentric chromosomes in the female but did not indicate how many frogs he examined nor where they came from. *Discoglossus* has been subject to considerable revision recently (Lanza et al., 1984; Busack, 1986) and a number of new species have been erected. Thus, it is not known exactly which population Morescalchi (1964) actually examined. Frogs from Spain, *D. jeaneae*, recently separated from *D. pictus* by Busack (1986), show no sexual chromosome heteromorphism (Green, unpublished).

Although the phylogenetic analysis suggests that female heterogamety is the primitive condition for amphibians, the heterogametic sex has not yet been determined for a number of basal amphibian lineages (e. g., caecilians, the frog *Ascaphus*, and the salamander families Dicamptodontidae, Cryptobranchidae, and Hyonbiidae). It is conceivable that determination of the heterogametic sex in these groups could change the analysis. Therefore, cytogenetic and genetic studies of sex determination in these groups is needed to test our findings.

Evolution of sex-chromosome heteromorphism

Clear examples of closely related species with and without differentiated sex-chromosomes have been observed in other genera besides *Discoglossus*. In the leptodactylid frog *Eupsophus migueli*, metacentric Y and telocentric X chromosomes were found in males (Iturra and Veloso, 1981), but the more widespread *E. roseus* is without this heteromorphism. Even more similar karyotypes were found shared by the frogs *Leiopelma hamiltoni* and *L. archeyi* (Green, 1988; Green and Sharbel, 1988), which are sister species. However, chromosome bands revealed a heterochromatin difference in a pair of chromosomes in female *L. hamiltoni* that was not discernable in *L. archeyi* (Green and Sharbel, 1988). Examples such as these can be useful in examining the evolution of sex-chromosome heteromorphisms.

By dint of their obligate heterozygosity in one sex, sex chromosomes are subject to more rapid evolutionary change than autosomes (Charlesworth et al., 1987). A number of mechanisms have been proposed to explain their divergence (Rice, 1987), the most widely accepted being "Muller's Ratchet" (Felsenstein, 1974; Charlesworth, 1978), which is proposed as a device to promote degeneracy of the Y or W chromosome. The progress of Y-chromosome degeneracy is amply illustrated in amphibians by the bolitoglossine salamanders and the genus *Necturus* (Leon and Kezer, 1978; Sessions, 1984; Sessions and Wiley, 1985). The mechanism, however, requires that the two sex chromosomes already be genetically divergent enough so that crossing-over between them is suppressed and so may be inadequate to explain the origination of this genetic divergence in the first place.

Frogs of the genus *Rana* have male heterogamety without differentiated sex chromosomes. The one species in which sex chromosomes can be distinguished, *R. esculenta* (Schempp and Schmid, 1981) is actually a hybrid (Berger, 1977) and so probably reflects a karyotypic difference between the two parental species, *R. lessonae* and *R. ridibunda*. In *Rana*, there is little genetic difference between sex-determining chromosomes, because genetic linkage studies (Wright et al., 1983; Elinson, 1983) indicate that the sex-determining genes are embedded among many other functional loci. From this initial condition, differentiation probably commences, in many cases, by accumulation of nonfunctional loci and heterochromatin in the W or Y chromosome. This can be seen in *Leiopelma hamiltoni* relative to *L. archeyi* (Green 1988a; Green and Sharbel, 1988) where the slightly differentiated W chromosome of *L. hamiltoni* has more heterochromatin associated with the centromere than is seen in the presumed homologue in *L. archeyi*.

Although it is expected that the development of sex chromosome differentiation may hamper change in the heterogametic system of a species (Bull and Charnov, 1985), our data can offer little to confirm or deny this hypothesis. In part, this is due to the nature of the data themselves as we have relatively little information on the heterogametic systems of species without sex-chromosome heteromorphism.

Changes in heterogametic sex

Heterogametic sex appears to be a relatively labile character among amphibians. Yet, in spite of this, changes in heterogametic sex are strongly correlated with phylogenetic history (Fig. 1), which may now provide a basis for using heterogametic sex as a phylogenetic character in amphibian systematics. If changes in both directions were equally probable and the amount of time available for each type of change were equal, then the probability of at least seven changes from ZW to XY out of eight changes in heterogametic sex is $p < 0.0352$. If ZW is the ancestral state (as indicated in the phylogenetic analysis), then there has been more time available for ZW to XY changes, but the rarity of XY to ZW changes within clades with male heterogamety is noteworthy. This bias, if real, indicates either a selective advantage for male over female heterogamety in amphibians, or else a genetic or cytogenetic constraint in reverting to female heterogamety once male heterogamety has evolved.

The peculiar OW/OO sex-chromosome system in *Leiopelma hochstetteri* (Green, 1988b) is particularly intriguing as it rules out many potential mechanisms of sex determination in this species. Many models have been proposed to explain sex determination in genetic terms (Bull and Charnov, 1977; Bull, 1983; Page et al., 1987). As there is no Z chromosome in *L. hochstetteri*, any model requiring a genetic contribution from the Z, or X, chromosome can be ruled out. This precludes any recessive-X systems relying upon dosage effects to determine sex, as occur in *Drosophila* fruit flies (Baker and Lindsley, 1983) or *Caenorabditis* nematodes (Madl and Herman, 1979). *L. hochstetteri* must have a dominant-W system in which a gene on the W chromosome specifies femaleness, just as mammals have dominant-Y systems specifying maleness.

In mammals, a testis-determining factor, or TDF, gene occurs on the Y-chromosome and directs development of the embryonic bipotential gonad into a testis (Jacobs and Strong, 1959; Page et al., 1987). A potential TDF gene (designated ZFY, for zinc finger Y) has recently been cloned from humans (Page et al., 1987). A sequence homologous to this also occurs on the X-chromosome of eutherian mammals, as well as on autosomes throughout the amniotes (Bull et al., 1988; Page et al., 1987). A dominant-W system (as seen in *L. hochstetteri*) could operate in one of two ways. Perhaps the W-chromosome contains an ovary-determining factor analogous to the mammalian TDF. Or, conversely, perhaps the W-chromosome contains a regulator gene suppressing expression of a TDF-like sequence located on a pair of autosomes. If the second model is correct, it may help explain the multiple origination of male heterogametic XY systems from female heterogamety in amphibians, and the rarity of reversals to female heterogamety. If we suppose that an ancestral amphibian sex-determination system consisted of TDF genes on a pair of autosomes (allele T in Table 2) and a TDF-suppressor gene located on the W, a mutation at one of the TDF loci could give rise to a

Table 2. Sex of various genotypes upon the introduction of a recessive, inactive TDF (testis determining factor) allele into a population with WZ/ZZ female heterogamety, assuming that the W-chromosome carries a TDF-suppressor gene not present on the Z-chromosome. T indicates a chromosome with an active TDF allele, o indicates the homologous chromosome with a mutant TDF.

	WZ	ZZ
TT	female	male
To	female	male
oo	female	female

non-functional allele (allele o in Table 2). In the heterozygous state, this recessive allele would have no effects, but in the homozygous state, it would have the effect of changing the sex of the individual from male to female (Table 2). This model of change is largely the same in effect as a mechanism proposed by Bull and Charnov (1977) to explain changes in heterogametic sex. However, this model is a restriction of the Bull and Charnov model requiring only a simple loss, by mutation, of genetic effect in a functional gene rather than the *de novo* advent of a new regulatory sequence. We apply this model specifically to amphibians where it can particularly explain the preponderance of changes from WZ heterogamety to XY heterogamety in amphibian evolution.

As a recessive, the new, non-functional TDF allele would be selectively neutral in the heterozygous state and so could easily rise in frequency within the population following its inception due to stochastic processes. Change from female to male heterogamety would occur by fixation of the Z chromosome, which is possible because ZZ individuals heterozygous at the TDF locus (To) would be male, and those homozygous for the new non-functional allele (oo) would be female (Table 2). Bull and Charnov (1977) showed that the endpoints in this system (i. e., male and female heterogamety) are connected by a continuous series of stable equilibrium frequencies, but that stochastic processes of finite populations or fortuitous linkage to selected genes would result in eventual fixation of one of the endpoints unless intermediate genotypes have a higher fitness. However, any major mutation at the TDF locus would produce a non-functional allele, so the potential for male heterogamety is constantly introduced into lineages with female heterogamety. In contrast, once male heterogamety becomes fixed in the population, a control system for the TDF locus would have to evolve *de novo* in order to reintroduce the potential for female heterogamety. The net result would be a drive toward XY male heterogamety from ZW female heterogamety. The TDF-bearing chromosome would become a neo-Y-chromosome and the mutant TDF-bearing chromosome would become a neo-X-chromosome. Both of these new sex chromosomes would contain similar DNA sequences, as has been found in mammalian sex chromosomes (Page et al., 1987; Weissenbach, 1987).

This mechanism is specific for the change from WZ/ZZ to XY/XX, as has occurred repeatedly through the phylogenetic history of amphibians. Its specific details cannot be construed as a general model for heterogametic change throughout the animal kingdom. It may, however, prove useful to explain the known facts concerning the distribution of different heterogametic systems and the genetic mechanisms of sex-determination in amphibians and, possibly, other tetrapods.

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