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Authors

MANK, JUDITH E
PROMISLOW, DANIEL EL
AVISE, JOHN C

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Evolution of alternative sex-determining mechanisms in teleost fishes

JUDITH E. MANK*, DANIEL E. L. PROMISLOW and JOHN C. AVISE†

Department of Genetics, University of Georgia, Athens, GA 30602, USA

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We compiled information from the literature on the taxonomic distributions in extant teleost fishes of alternative sex-determination systems: male-heterogametic (XY) gonochorism, female-heterogametic (ZW) gonochorism, hermaphroditism, unisexuality, and environmental dependency. Then, using recently published molecular phylogenies based on whole-genomic or partial mitochondrial DNA sequences, we inferred the histories and evolutionary transitions between these reproductive modes by employing maximum parsimony and maximum likelihood methods of phylogenetic character mapping. Across a broad teleost phylogeny involving 25 taxonomic orders, a highly patchy distribution of different sex-determination mechanisms was uncovered, implying numerous transitions between alternative modes, but this heterogeneity also precluded definitive statements about ancestral states for most clades. Closer inspection of family-level and genus-level phylogenies within each of four orders further bolstered the conclusion that shifts in sex-determining modes are evolutionarily frequent and involve a variety of distinct ancestral-descendant pathways. For possible reasons discussed herein, the evolutionary lability of sex-determining modes in fishes contrasts strikingly with the evolutionary conservatism of sex determination within both mammals and birds. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, 87, 83–93.

ADDITIONAL KEYWORDS: environmental sex determination – female heterogamety – hermaphroditism – male heterogamety – phylogenetic character mapping – unisexuality.

INTRODUCTION

Rates and patterns of evolutionary transitions between alternative modes of sex determination are poorly understood. Why, for example, have particular sex-determining mechanisms been retained over vast stretches of evolutionary time in some vertebrate lineages but not in others? Extreme conservatism is illustrated by birds and mammals, all extant species of which share, respectively, a monophyletic ZW system of female heterogamety (Fridolfsson *et al.*, 1998), and an XY system of male heterogamety (or its close derivative; Bick & Jackson, 1967). In teleost fishes, by contrast, a wide variety of sex-determining modes (including the involvement of sex chromosomes, autosomal genes, and environmental triggers) implies that at least several evolutionary transitions among repro-

ductive systems have occurred, albeit at uncertain rates and in undetermined directions (Orzack *et al.*, 1980; Maisey, 1996; Ota *et al.*, 2000).

Recent advances in DNA sequencing technology and phylogenetic analysis afford new opportunities to examine the evolutionary histories of sex-determining mechanisms. Here we employed published estimates of teleost phylogeny, based on extensive mtDNA sequences from dozens of taxonomically diverse species (Ishiguro, Miya & Nishida, 2003; Miya *et al.*, 2003; Saitoh *et al.*, 2003), as a phylogenetic backdrop for interpreting current distributions of alternative sex-determination modes (which we compiled from the large but scattered literature). Although results of this exercise in phylogenetic character mapping remained provisional due to uncertainties in clade resolution (and other difficulties to be discussed), they did help to address several questions regarding evolutionary trends of sex determination in teleost fishes, including the following. What are the directions of evolutionary transitions between hermaphroditism and gonochor-

*Corresponding author. E-mail: jemank@uga.edu

†Current address: Department of Ecology and Evolutionary Biology University of California, Irvine Irvine, CA 92697, USA.

rism (separate sexes), between male heterogamety and female heterogamety, and other mechanisms of sex determination? Has phylogenetic inertia constrained evolutionary shifts between alternative modes of sex determination? To what extent, and why, are bony fishes evolutionarily labile in sex-determining mechanisms vis-à-vis birds and mammals?

MATERIAL AND METHODS

We searched the published literature on teleosts for accounts of the following: female (ZW) or male (XY) heterogametic sex chromosomes; male-first (protandrous), female-first (protogynous), or simultaneous hermaphroditism; unisexuality; environmental sex determination. A total of 591 species are included in this summary. As outgroups, we also added information on Acipenseriformes (sturgeons and paddlefish, Actinopterygii), and Rajiformes (Chondrichthyes, cartilaginous fishes). We used a current classification (Nelson, 1994) to reconcile taxonomic discrepancies. A complete list of papers (more than 100 in total) that we used in compiling information on sex determination in teleosts is available as Supplementary Material to this article.

We explored two evolutionary levels. First, to examine broad histories of sex determination using the comparative approach (e.g. Harvey & Pagel, 1991; Martins, 1996), we assembled an informal supertree of teleost families. This composite tree represented an amalgamation of published phylogenies based on whole-genome or partial-genome mtDNA sequences (or in one case on morphological data) for representative species (Table 1). When multiple published phylogenies were available, we chose the tree topology

that was based on the largest number of informative characters and that maximized phylogenetic resolution. To increase the sample size of taxa included, we grafted monophyletic clades onto subtree skeletons from lower-level published phylogenies (Sillen-Tullberg, 1988; Donoghue, 1989; Janz & Nylin, 1998; Weiblen, Oyama & Donoghue, 2000). We then mapped sex-determination modes onto the terminal nodes in each phylogeny and, for clarity as well as to minimize bias, pruned clades with unknown mechanisms of sex determination. At this broad level of analysis, we used the maximum parsimony approach of MacClade (Madison & Madison, 2000) to estimate minimum numbers of origins and evolutionary transitions between alternative sex-determination modes.

In a second, more detailed level of examination, we focussed on four clades that were information-rich and polymorphic regarding sex determination modes, and that were sufficiently characterized to merit comparative phylogenetic analysis. For these lower-level phylogenies, we used both the maximum parsimony approach of MacClade 4 (Madison & Madison, 2000) and the maximum likelihood approach of DISCRETE (Pagel, 1994, 1997) to reconstruct ancestral states, and to better understand finer-scale evolutionary patterns of sex determination. We used DISCRETE to assess statistical support for these inferred states, and to calculate their relative likelihoods at each ancestral node in a given tree (Schluter *et al.*, 1997). Although Pagel's (1994, 1997) model can incorporate different branch lengths (ages of clades) into the analysis, the absence of sufficient temporal information in the reconciled molecular trees forced us to consider only the cladogenetic aspect of tree structure. Thus, in DISCRETE, all

Table 1. Published phylogenies, and their data matrices, used to construct teleost composite tree

Clade	Published phylogenies	Data matrix
(1) Teleostei	Ishiguro <i>et al.</i> (2003) Miya <i>et al.</i> (2003) Saitoh <i>et al.</i> (2003)	Complete mitochondrial genome Complete mitochondrial genome Complete mitochondrial genome
(2) Anguillidae	Inoue <i>et al.</i> 2001 Obermiller & Pfeiler 2003	Complete mitochondrial genome Mitochondrial 12S and 16S rRNA
(3) Cypriniformes	Liu <i>et al.</i> 2002 Perdices & Doadrio 2001	Mitochondrial control region Mitochondrial ATP syn 6,8, cyt <i>b</i>
(4) Basal neoteleosts	Elmerot <i>et al.</i> 2002 Miya <i>et al.</i> (2003) Ota <i>et al.</i> 2000	Complete mitochondrial genome Complete mitochondrial genome Mitochondrial cyt <i>b</i>
(5) Gobioidae	Akihito <i>et al.</i> 2002 Thacker 2003 Wang <i>et al.</i> 2001 Pezold (1993)	Mitochondrial cyt <i>b</i> Mitochondrial ND1, ND2, COI Mitochondrial 12S rRNA, tRNA Morphology

branch lengths were coded as equal to one (see e.g. Weiblen *et al.*, 2000).

RESULTS

BROAD-SCALE PHYLOGENETIC CHARACTER MAPPING

Figure 1 shows the condensed ordinal-level composite tree, with sex-determining modes cataloged within each clade. Fifteen teleost orders contained sex-

chromosome gonochorist species and no known hermaphroditic species. The reverse was never true, because all orders with hermaphroditic species also contained members with sex chromosomes. Species representing both sex-chromosome-based and hermaphroditic reproductive modes have been recorded in nine teleost orders. With regard to gonochoristic teleosts displaying sex chromosomes, the incidence of male heterogamety (appearing in 22 teleost orders)

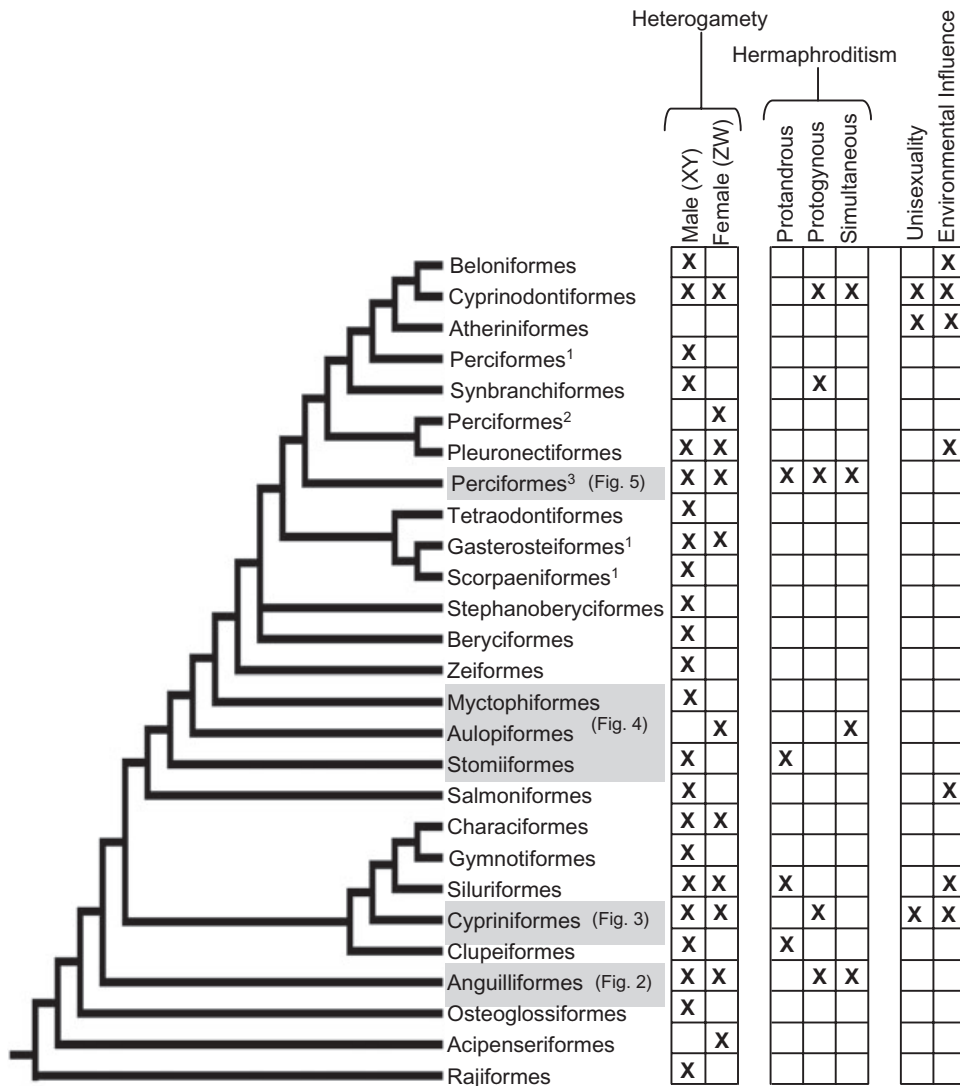


Figure 1. Condensed, ordinal-level composite phylogeny for teleosts displaying known sex-determining mechanisms. Shown is a cladogram, adjacent to which are indicated reproductive modes recovered from the published literature. A single mark in a given row denotes that all examined species within that taxon display the indicated mode; two or more checkmarks indicate that various species within that taxon display alternative reproductive modes. Polyphyletic clades within the current taxonomy are marked and indicated to the side. Shaded boxes indicate clades that are examined in greater detail in Figs 2–5. Polyphyletic orders are indicated on the cladogram, and are as follows: Perciformes¹, Gobiescoidei and Blennioidei; Perciformes, Caragnidae; Perciformes, Gobioidi; Gasterosteiformes, Gasterosteioidi; Scorpaeniformes, Cottoidei.

was approximately double that of female heterogamy (10 orders). In permutation tests (Harvey & Pagel, 1991; Manley, 1991), isolated monophyletic incidences of XY, ZW, and hermaphroditic reproductive modes showed no significant departures from random-combination expectations (given their frequencies) across the teleost phylogeny (all $P > 0.1$). We found reports of environmental controls on sex differentiation in seven teleost orders, six of which also included species with chromosomal sex determination. Unisexual taxa were found in three taxonomic orders representing at least two widely separated branches in the teleost tree.

Thus, the phylomap in Figure 1 suggested strongly that particular reproductive modes had polyphyletic origins. For example, maximum-parsimony reconstructions of ancestral states implied that hermaphroditism probably emerged at least nine times during teleost evolution, and that female heterogamy may have evolved independently ten times or more (plus at least once in the outgroup Acipenseriformes). In contrast, our formal analysis indicated just one origin for male heterogamy in teleosts, but we think this is probably a gross underestimate. Maximum parsimony earmarked XY systems as basal in the tree because they appeared at face value to be by far the most common and widespread mode of sex determination in teleosts (Fig. 1). However, appearances in this case may be deceiving due to a reporting bias in the literature. Ohno (1967) argued that autosomal sex determination is a likely precursor to heterogametic sex chromosomes, but autosomal modes are almost impossible to confirm from cytological evidence (the usual basis for identifying sex chromosomes). Indeed, this lack of certainty forced us to omit 'autosomal' sex determination from current analyses, and also to appreciate that instances of sex-chromosome heterogamy in teleosts might be underestimated considerably from current karyotypic evidence.

FINE-SCALE PHYLOGENETIC CHARACTER MAPPING

Finer-scale character mapping in each of four teleost clades painted a clearer picture of the polyphyletic origins of all the sex-determining mechanisms studied here. In each case, results from maximum parsimony were identical to those in maximum likelihood reconstructions. For example, all reconstructed ancestral states that received only poor or marginal support under maximum likelihood were also equivocal under maximum parsimony, and vice versa. Thus, for clarity, and also because parsimony can better deal with polytomies in trees, only results from maximum parsimony are presented here.

In various cases of the four fine-scale analyses, instances of multiple independent origins for her-

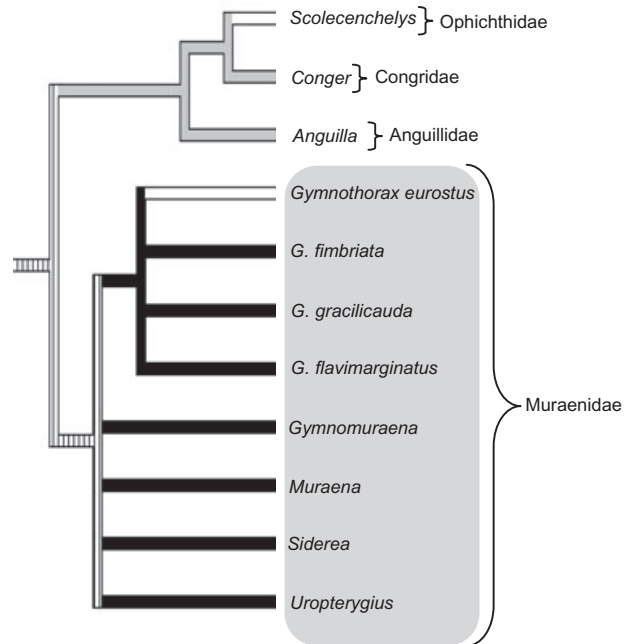


Figure 2. Molecular phylogeny for Anguilliformes. Genera often associated with marine reef habitats are shaded. Phylogenetically reconstructed ancestral modes of sex determination, shown along branches, are indicated as follows: black lineages, hermaphroditism; white lineages, male heterogamy (XY); grey lineages, female heterogamy (ZW). Hatched branches indicate equivocal ancestral states.

maphroditism, female and male heterogamy, and/or unisexuality could be inferred. For example, the anguilliform phylogeny (Fig. 2) illustrated polyphyletic origins for male heterogamy, with XY lineages apparently having emerged from both hermaphroditic and female-heterogametic lines. All of the hermaphroditic species in this phylogeny were contained in reef-dwelling members of the Muraenidae.

The cypriniform phylogeny (Fig. 3) likewise implied polyphyletic origins for at least some sex-determining modes, although the great diversity in reproductive systems in this group made it impossible to estimate, with confidence, ancestral states above the genus level. If it were assumed, for example, that hermaphroditism was the ancestral condition, then female heterogamy and unisexuality would each have arisen at least twice; and if it were assumed that female heterogamy was ancestral, then hermaphroditism and unisexuality would each have originated at least twice. Also evidenced in this case was the strong likelihood that male heterogamy (in *Cyprinus*) indeed arose from some other ancestral sex-determining mode.

The basal neoteleosts (Fig. 4) further illustrated the repeated origin (but often short evolutionary persis-

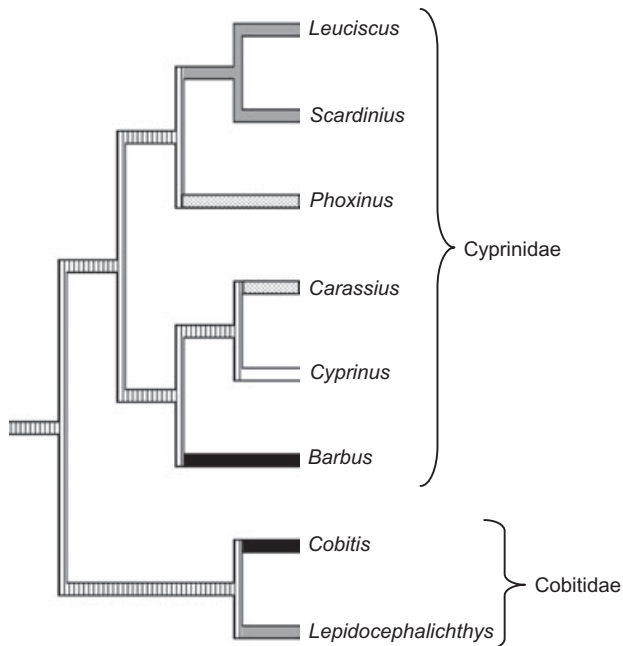


Figure 3. Molecular phylogeny for Cypriniformes. Dotted branches signify unisexuality (*Phoxinus* and *Carrasius*). See Fig. 2 for a description of pattern codes for modes of sex determination along other branches in the phylogeny.

tence) of hermaphroditic lineages, as well as the emergence of female heterogamety from a probable ancestral mode of male heterogamety in the aulopi-forms. Finally, the phylogeny for Gobioidae (Fig. 5) again illustrated multiple evolutionary shifts between different reproductive modes, as well as a clustering of hermaphroditic lineages in reef-dwelling taxa.

DISCUSSION

Results from our analysis are provisional for several reasons. First, teleost phylogeny is far from fully resolved, and the inclusion of many more taxonomic families as well as additional classes of molecular (or other) information should help to clarify further the evolutionary histories of sex-determination modes. For example, the mtDNA-based phylogenies supported long-standing suspicions of polyphyly for several teleost orders, including the Scorpaeniformes, Gasterosteiformes, and Perciformes (Johnson, 1993; Nelson, 1994). Also, even whole-genome mtDNA sequences failed to resolve the exact positions of several key groups within the huge order Perciformes, and without this information a complete analysis of teleost phylogeny retains considerable uncertainty and bias (Sanderson, Purvis & Henze, 1998; Weiblen *et al.*, 2000).

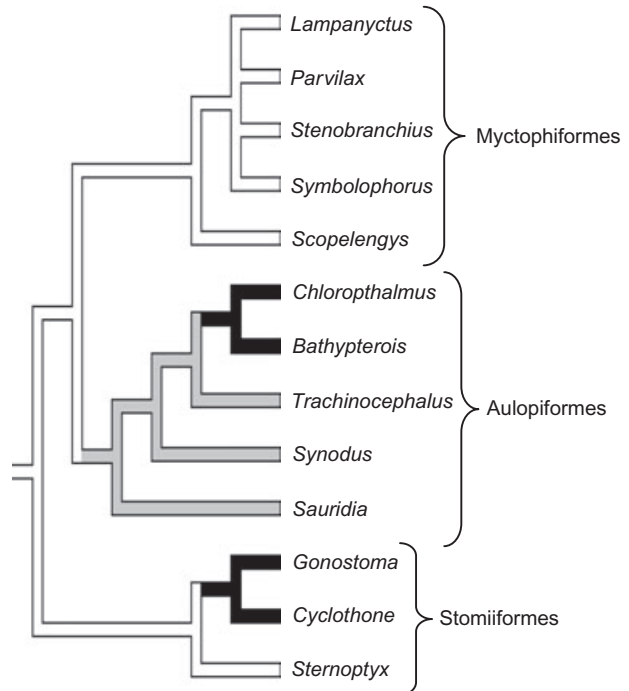


Figure 4. Molecular phylogeny for basal Neoteleosts. See Fig. 2 for a description of pattern codes for modes of sex determination along branches in the phylogeny.

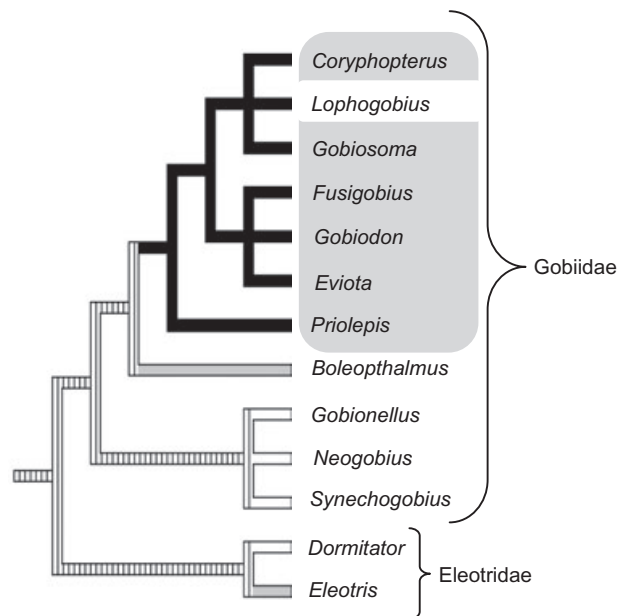


Figure 5. Molecular phylogeny for the suborder Gobioidae. Shaded genera are typically associated with reefs. See Figs 2 and 3 for descriptions of pattern codes for modes of sex determination along branches in the phylogeny.

Second, current understanding of the genes and mechanistic pathways involved in sex determination are grossly inadequate. Thus, although broad categories of sex determination are clearly polyphyletic in teleosts, at least some of their component aspects may yet prove to be monophyletic. Finally, karyotyping is another area in which better information is needed. Often, fish chromosomes are notoriously small and numerous, so further refinements in cytological techniques may help to distinguish, for example, true autosomal modes of sex determination from those that involve now-cryptic sex chromosomes.

Despite these limitations, several striking patterns emerged from our phylogenetic character mapping analyses. Most salient was that most if not all of the broad categories of sex determination in teleosts had multiple evolutionary origins.

ALTERNATIVE REPRODUCTIVE MODES

Environmental dependence

Influences of environmental factors, primarily temperature, on sexual differentiation are known in seven teleost orders, six of which (the exception being Atheriniformes) also contain species with documented chromosomal sex determination. In the atheriniforms, temperature exposures during development are known to interact with genetic factors to determine an individual's sex (Lagomarsino & Conover, 1993). The restricted phylogenetic distributions of temperature influences on sex in fishes suggest that this phenomenon may have evolved several times, secondarily, as an auxiliary mechanism that can modify or even override chromosomal sex determination. This could be advantageous, for example, if ecological conditions sometimes favour particular families that can temporarily alter their sex ratios (Trivers & Willard, 1973; Conover & Heins, 1987). Another possibility, however, is that environmental influences are simply harder to detect (because they normally involve experimental manipulation).

Unisexuality

This phylogenetic analysis merely adds support to what was already well-known about unisexuality in fish (and other vertebrates), namely that the phenomenon is polyphyletic, evolutionarily ephemeral, and generally confined in extant lineages to the outermost tips of branches in the vertebrate phylogenetic tree (Avisé, Quattro & Vrijenhoek, 1992; Dawley & Bogart, 1989). Indeed, prior evidence for the polyphyletic origins of unisexuality was even more *prima facie*: across diverse vertebrate taxa, each of the approximately 70 known unisexual biotypes originated via one or more independent hybridization events between closely

related sexual species (Dawley & Bogart, 1989; Avisé *et al.*, 1992).

Sex chromosomes

Most teleost lineages for which data are available include representatives with known chromosomal sex determination (Fig. 1). Arkhipchuk (1995) estimated that sex-chromosome systems exist in about 10–50% of all fish species (although not all of these are evident in the gross karyotypic inspections conducted to date). Whatever the exact tally, due to the many apparent evolutionary transitions among sex-determination modes (as well as missing data for some teleost orders), we cannot conclude definitively that sex chromosomes were the ancestral mode of sex determination in teleosts.

Nor within the XY vs. ZW dichotomy can we determine the ancestral teleost condition with certainty. Although the majority of extant teleost lineages include species with male heterogamety (Fig. 1), this does not necessarily indicate that the XY mode is plesiomorphic for the group for the following reasons: male and female heterogamety were both dispersed throughout much of the teleost tree; both XY and ZW systems are evident in some non-teleost fishes, including the outgroups employed in this study (Fig. 1); many other fish species (both teleost and non-teleost) do not have well demarcated sex chromosomes (Harvey *et al.*, 2002; Schwartz & Maddock, 2002), and at least some of these probably lack them entirely (Devlin & Nagahama, 2002); in at least one of our finer-focus appraisals (involving Cypriniformes), male heterogamety appeared to have evolved secondarily from some other ancestral sex-determination mode. All of these lines of evidence indicate that sex chromosomes of both the XY and ZW type can arise repeatedly and perhaps interconvert quite readily (but see below) during evolution.

Autosomal sex determination is also thought to be common in teleosts, and has been proposed as the probable ancestral condition prior to the initial evolution of well-differentiated sex chromosomes (Ohno, 1967; Traut & Winking, 2001). If this is indeed correct (and not merely an artefact of poor karyotypic resolution), then it must be the case that this state commonly gives evolutionary rise to sex-determination systems with a clear XY or ZW basis. This would also provide an alternative model (other than direct XY \leftrightarrow ZW interconversion) for the recurrent origin of the XY or ZW systems along the teleost evolutionary tree. In summary, the various phylogenies we examined indicate that teleost lineages may switch back and forth readily between male and female heterogamety over evolutionary time, but we could not determine from these analyses whether or not these changes are interspersed with intervals devoid of bona fide sex chromosomes.

The notion that sex chromosomes arise repeatedly from autosomes gains some support from evidence that the X-Y and Z-W chromosome pairs are not homologous (Graves & Shetty, 2001), and also that teleost sex chromosomes of the same general heterogametic mode are polyphyletic (Solari, 1994; Woram *et al.*, 2003). It is thus improper to assume that recurrent karyotypes displaying the same heterogametic mechanism are necessarily homologous in all teleosts, especially across large evolutionary distances. Further molecular-genetic characterizations (e.g. as per Froschauer *et al.*, 2002) will be required to distinguish genuine homology from analogy.

We prefer not to speculate about the endless variety of eco-biological factors that might influence the evolution of alternative sex-determination modes, except to note one recurring trend (not previously appreciated, to our knowledge) from this literature review: sex chromosomes were often associated with diadromous species (e.g. catadromous Anguillidae and anadromous Salmonidae). In such species, individuals congregate on spawning grounds, such that a given individual is unlikely to encounter a shortage of potential mates. Thus, we might speculate that by virtue of conferring a relatively equitable sex ratio in such a breeding population, chromosomal sex determination could enhance mean individual fitness under a negative frequency-dependent scenario (as per Fisher, 1930). More generally, perhaps the phylogenetic approach that we have taken in this study will be useful in identifying additional such relationships that are worthy of further functional investigation.

Hermaphroditism

This reproductive mode is scattered throughout much of the teleost phylogeny (Fig. 1), but appeared to be embedded within clades otherwise characterized by gonochorist species with chromosomal sex determination (Figs 2–5). Thus, extant hermaphroditism in teleosts is most likely polyphyletic and derived, and no single hermaphroditic lineage seems to be evolutionarily ancient.

Hermaphroditism appears to be particularly common in reef-dwelling fishes, as illustrated in this study by the Muraenidae eels (Fig. 2) and Gobiinae gobies (Fig. 5). Like most reef fish, these eels and gobies have a pelagic larval phase that promotes spatial gene flow (Robertson, 2001) but also entails high risks in successful larval settlement and subsequent mate acquisition. Reef fishes tend to have inherently patchy habitat distributions, and many (such as moray eels) have low population densities. Many reef fishes (including gobies) show evidence that fitness advantages to functioning as a male (or as a female) change during life according to an individual's body size or ecological circumstance (St. Mary, 1994, 1996). All of

these factors, which are generally consistent with previous predictive theory (Ghiselin, 1969; Smith, 1975), might have contributed to selection pressures recurrently promoting evolution of hermaphroditism in various reef fishes. However, this alone would not account for why hermaphroditism seems to be typically evolutionarily short-lived.

Furthermore, the emergence of hermaphroditism in the basal Neoteleosts (Fig. 4) seems hard to rationalize using the above logic. Although hermaphroditic Aulopiformes (*Chlorophthalmus* and *Bathypterois*) and Stomiiformes (*Cyclothone* and *Gonostoma*) are deep-water species in which hermaphroditism might be favoured for reasons of low population density (for example), other deep-water representatives of these same orders are sex-chromosome gonochorists (Fig. 4). Likewise, it is unclear what, if any, distinguishing ecologies might apply to hermaphroditic cypriniform genera compared with their gonochoristic relatives (Fig. 3). Phylogenetic analyses of the sort conducted here cannot by themselves address the idiosyncrasies of why particular lineages evolved one or another mode of sex determination; for that, focussed ecological and other biological studies are required. Phylogenetic character mapping can merely help to identify particular lineages in which such analyses might meaningfully be focussed.

PHYLOGENETIC CONSTRAINT VERSUS EVOLUTIONARY LABILITY

Various hypotheses might be advanced for why sex determination appears evolutionarily labile in fishes but conserved in mammals and birds. Perhaps fish lineages that are polymorphic for sex-determining systems are simply much older and thus have had more evolutionary time to experience shifts between modes. Our analysis demonstrates quite strongly, however, that this is unlikely to be the case. Although ray-finned fishes (Actinopterygii) originated in the Devonian and thus are collectively older than birds and mammals (which originated about 150 and 200 million years ago, respectively; Vaughan, 1986; Feduccia, 1996), the earliest known teleosts are from 235 million-year-old fossils (Maisey, 1996), and are thus not greatly older than birds or mammals. More telling is the fact that far more recent teleost clades (e.g. within an order, family, or sometimes genus) often show a greater diversity of sex-determining mechanisms than do the monotypic classes of mammals and birds. The cypriniforms, for example, show enormous diversity in sex-determining mechanisms (Fig. 3), but their earliest fossils date only to the Eocene (Carol, 1988). Some shifts between reproductive modes in teleosts are known to have occurred even in contemporary time, as for example transi-

tions from gonochorism to unisexuality in the genus *Poecilia* (Avise *et al.*, 1992).

Another hypothesis (one that we favour) appeals to developmental and genomic peculiarities of fishes. Developmentally, testes and ovaries in teleosts (as well as ovotestes in some species; Atz, 1964) all derive from the same precursor tissue, and can rather flexibly differentiate at various life stages within an individual. This differs from the situation in most other vertebrates including birds and mammals, in which gonadal differentiation activates early in development and generally is irreversible (Hoar, 1969).

This relative developmental plasticity in fishes carries implications with regard to sex determination. First, gonadal development and sexual differentiation are somewhat decoupled. Second, in fish lacking genetically hardwired sex chromosomes, sexual differentiation and even sexual alterations can take place late in development (e.g. even well after sexual maturity in sequential hermaphrodites). This opens wider windows of opportunity for environmental pressures such as social status (Robertson, 1972), ecological conditions (Conover, 1984; Francis, 1992), and population composition (Warner & Hoffman, 1980) to play proximate roles in sex determination, and also, via the selection pressures they impose, to influence the genetic evolution of underlying sex-determining mechanisms. Furthermore, negative selection against intersex individuals is more likely to be diminished or absent. Unlike most other vertebrates, where mixed signals in sexual differentiation often result in low reproductive fitness or even sterility (Armstrong, 1964; Taber, 1964), many fish are less prone to suffer reproductive losses when distinctions between the sexes are less than definitive. For example, in some teleosts an ovotestis produces both sperm and ova, and some individuals may have higher fitness than gonochorists (Smith, 1975). This may help to explain why hermaphroditic lineages are not uncommon in teleosts.

In some important regards, fish also display more rapid genomic evolution than most other vertebrates. Notably, a fast pace of genic and genomic duplication (Robinson-Rechavi & Laudet, 2001; Robinson-Rechavi *et al.*, 2001), both recent (Allendorf & Thorgaard, 1984; Ferris, 1984; Vandepoele *et al.*, 2004) and ancient (Amores *et al.*, 1998; Taylor *et al.*, 2003), has been documented in the ray-finned fishes. Such duplications initially provide redundancy and thereby open the potential for replicates of genes and gene pathways to evolve new, often related functions (Ohno, 1970). Thus, the increased evolutionary potential afforded by regional and genome-wide duplications probably creates additional evolutionary flexibility in sex-determination pathways.

Wilkin's (2002) retrograde model of pathway evolution predicts that emergent genes at the apex of a sex-determining hierarchy can requisition downstream components in the pathway. In this way, even if the underlying basis of a sex-determination mode is conserved, that mode nonetheless can become altered by a new set of conditions. For example, any apical sex-determining genes that come under the control of inducible promoters can in theory put sex determination under social and environmental influence. Constitutive promoters, in contrast, presumably inhibit alterations in sex determination, but when recombination is curtailed on chromosomes carrying different male and female alleles at loci encoding such promoters, sex chromosomes may evolve (Ohno, 1967; Charlesworth, 1991; Rice, 1994). Also, female and male heterogamety can interconvert in evolution when emergent upstream sex-determining switches are closely linked to a gene of high adaptive value (Bull & Charnov, 1977), or when new upstream switches emerge in systems with large sex-ratio biases (Wilkins, 1995; Ogata *et al.*, 2003).

Some evidence does exist for this type of evolution in the sex-determining pathways of teleosts. Locus DMRT1 is typically a downstream component in sex-determination cascades, and has homologies throughout the animal kingdom, from invertebrates (Baker & Ridge, 1980; Shen & Hodgkin, 1988) to mammals (Raymond *et al.*, 2000). In fishes, DMRT1 has been recognized as a conserved module in the male sex-determination pathway in heterogametic species including *Oncorhynchus mykiss* (Marchand *et al.*, 2000) and *Oryzias latipes* (Brunner *et al.*, 2001), and in hermaphrodites including *Acanthopagrus schlegelii* (He *et al.*, 2003) and *Monopterus albus* (Huang *et al.*, 2002). Duplicate copies of DMRT1 may also assume a role of apical initiator in sex determination in some species such as *O. latipes*, where DMY (a recent duplicate of the DMRT1 gene; Lutfalla *et al.*, 2003) is thought to be the initiating switch in male sex determination (Matsuda *et al.*, 2003; Nanda *et al.*, 2003). In the future, further elucidation of such mechanisms, especially when integrated with phylogenetic considerations, should offer much additional insight into the evolution of sex-determination modes in teleosts.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:

Appendix S1. Citations used in the construction of the teleost sex determination database used in this study.

This material is available as part of the online article from <http://www.blackwell-synergy.com>