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Phylogenetic conservation of chromosome numbers in Actinopterygiian fishes

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Abstract

The genomes of ray-finned fishes (Actinopterygii) are well known for their evolutionary dynamism as reflected by drastic alterations in DNA content often via regional and whole-genome duplications, differential patterns of gene silencing or loss, shifts in the insertion-to-deletion ratios of genomic segments, and major re-patternings of chromosomes via non-homologous recombination. In sharp contrast, chromosome numbers in somatic karyotypes have been highly conserved over vast evolutionary timescales – a histogram of available counts is strongly leptokurtic with more than 50% of surveyed species displaying either 48 or 50 chromosomes. Here we employ comparative phylogenetic analyses to examine the evolutionary history of alterations in fish chromosome numbers. The most parsimonious ancestral state for major actinopterygiian clades is 48 chromosomes. When interpreted in a phylogenetic context, chromosome numbers evidence many recent instances of polyploidization in various lineages but there is no clear indication of a singular polyploidization event that has been hypothesized to have immediately preceded the teleost radiation. After factoring out evident polyploidizations, a correlation between chromosome numbers and genome sizes across the Actinopterygii is marginally statistically significant ($p = 0.012$) but exceedingly weak ($R^2 = 0.0096$). Overall, our phylogenetic analysis indicates a mosaic evolutionary pattern in which the forces that govern labile features of fish genomes must operate largely independently of those that operate to conserve chromosome numbers.

Introduction

Recent comparative analyses have shown that ray-finned fishes (Actinopterygii) exhibit rapid genomic changes compared to other vertebrate clades (Robinson-Rechavi et al., 2001; Venkatesh, 2003; Volff, 2005). The sources of this evolutionary dynamism have been hypothesized to include any combination of the following: elevated rates of gene and genome duplication (Wittbrodt, Meyer & Schartl, 1998; Meyer & Schartl, 1999; Robinson-Rechavi & Laudet, 2001;Christoffels et al., 2004) functional sub-partitioning of duplicate genes (Force et al., 1999; Amores et al., 2004); elevated transposon activity (Kawakami, Shima & Kawakami, 2000; Neafsey & Palumbi, 2003; Kawakami & Noda, 2004; Ozouf-Costaz et al., 2004) changes in the insertion-to-deletion ratio of genomic segments (Neafsey & Palumbi, 2003); cytogenetic rearrangements (Arkhipchuk, 1995; de Almeida-Toledo et al., 2002; Postlethwait et al., 2002; Smith et al., 2002; Thomas et al., 2003); and rapid origins and dissolutions of sex chromosomes (Devlin & Nagahama, 2002; Mank, Promislow & Avise, 2006). Causal links have also been suggested between this unusually high genomic variability and the exuberant species diversity of actinopterygiian fishes (Stephens, 1951; Ohno, 1967; Holland et al., 1994; Meyer & Schartl, 1999; Navarro & Barton, 2003a, b; Mank & Avise, in press).

This rapid pace of genomic change in ray-finned fishes might suggest that the overarching chromosomal scaffolding should be highly variable as well, an impression further reinforced by assessments of genome size. In actinopterygiian fishes, haploid C-values span roughly an order of magnitude – from 0.39 picograms (pg) of DNA per cell in the pufferfish (*Fugu rubripes*) to 5.85 pg DNA per cell in the bichir (*Polypterus palmas*) (Hinegardner & Rosen, 1972). This huge span of genomes sizes in actinopterygiian fishes is several-fold greater than those in most other major vertebrate groups (Hinegardner, 1976; Venkatesh, 2003; Gregory, 2005).

Here we examine another feature of actinopterygiian genomes: genetic scaffolding as reflected in chromosome numbers. By interpreting somatic chromosome counts (and genome sizes) in a phylogenetic context provided by a recently published supertree for the Actinopterygii (Mank, Promislow & Avise, 2005), we further document a surprising ultraconservatism in chromosome numbers that contrasts dramatically with the evolutionary dynamisms displayed by numerous other features of actinopterygiian genomes.

Materials and methods

For 1546 vertebrate species, chromosome numbers (per somatic cell) and haploid genome sizes (C-values) were retrieved from the Animal Genome Size Database (Gregory, 2005, online at www.genomesize.com). From histograms of these data for each of several major vertebrate taxa, we computed standard summary statistics, including kurtosis or the sharpness of the distribution peak (Sokal & Rohlf, 1995).

For the actinopterygiian species, we then mapped chromosome numbers onto the supertree topology of Mank, Promislow and Avise (2005), using MacClade 4 (Maddison & Maddison, 2000), and reconstructed putative ancestral states under maximum parsimony criteria (a full phylogeny showing all the species analyzed and their somatic chromosome counts are available in the supplemental materials, which can be found on www.springerlink.com). We inferred putative polyploidization events where terminal or internal nodes showed roughly a two-fold or higher chromosome count than the nearest relative or sister clade.

We also analyzed the relationship between chromosome number and genome size through linear regression, both for all surveyed actinopterygiian species ($n = 615$) and for diploids only (i.e., after removing 78 species identified as evident polyploids). For both analyses, standard correlation coefficients (R^2) and their probabilities (p) were computed.

Results

Chromosome numbers in the 615 species of ray-finned fishes ranged from 22 to 250, but the frequency distribution was strongly leptokurtic (peaked) with a mode at 48 (Figure 1; Table 1). Most actinopterygiians displayed either 48 chromosomes (29.3% of the species surveyed) or 50 chromosomes (25.4%). With the blatant exception of mammals, other vertebrate taxonomic classes (amphibians, reptiles, and birds) exhibited similarly leptokurtic distributions of chromosome counts, albeit with each group having a different mode (Figure 1).

Across all surveyed actinopterygiian species, we uncovered a highly significant association between genome size and chromosome number ($R^2 = 0.26$, $p < 0.001$). However, this relationship was greatly diminished (but remained marginally significant; $R^2 = 0.01$, $p = 0.012$) when evident polyploids were removed from consideration (Figure 2). Approximately 78 such polyploid species were discernible in our survey, and we estimate from the phylogeny that they stem from 7 to 20 separate polyploidization events within the Actinopterygii. Understandably, most of these still-recognizable polyploidization events were concentrated near tips of the supertree, occurring at the genus or species level in all groups except Chondrostei (where all extant acipenseriform taxa appear to be of deeper polyploidy ancestry). Polyploidization events have also been common in Cypriniformes and Salmoniformes, where polyploid lineages clearly are phylogenetically interspersed with diploid lineages.

Figure 3 provides a highly condensed summary (due to space restrictions) of the maximum parsimony reconstruction of chromosomal evolution on the phylogenetic supertree for ray-finned fishes. A complete presentation of this phylogenetic reconstruction is provided in supplemental materials.

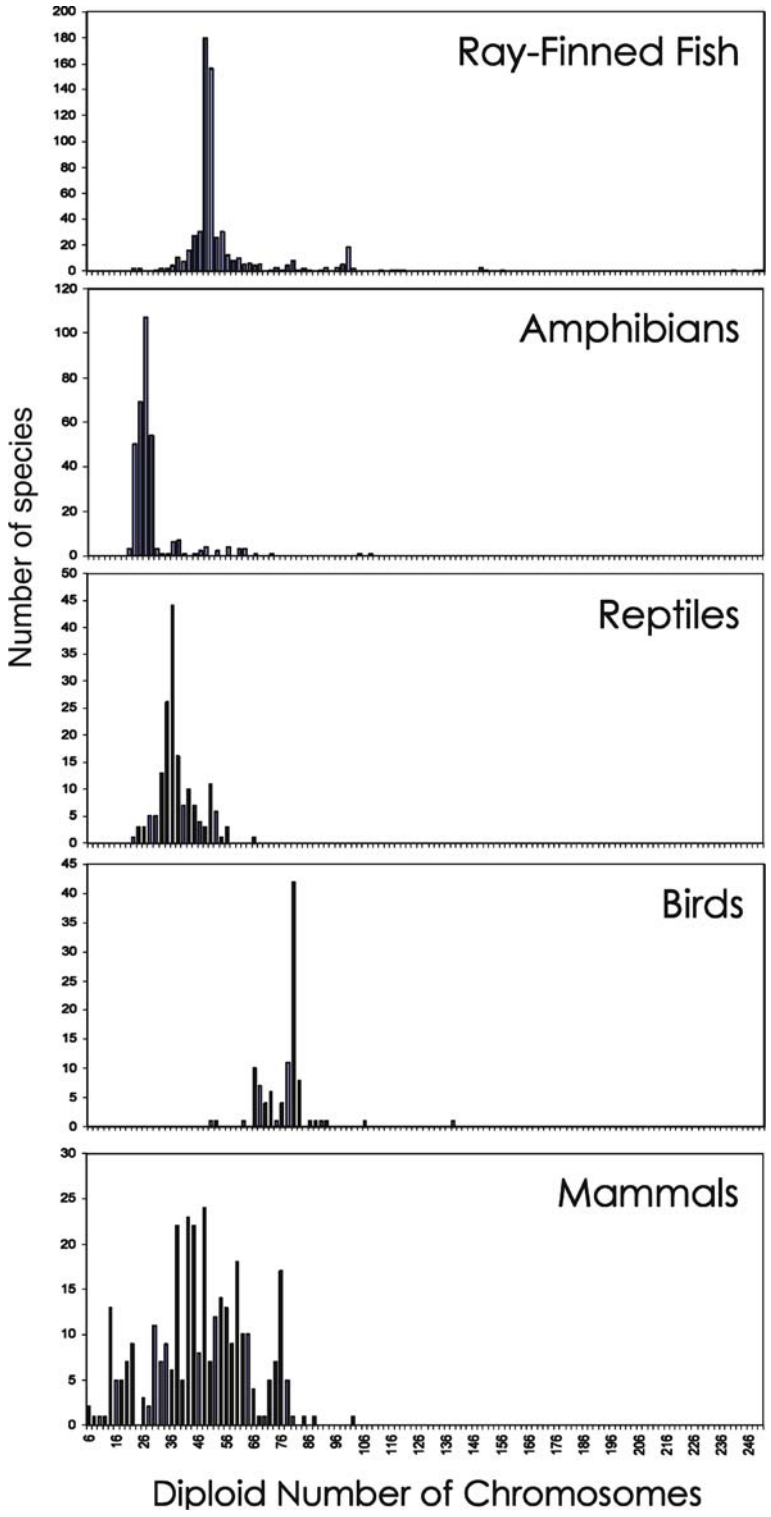

Figure 1. Histogram of diploid chromosome numbers for several taxonomic groups of vertebrates (data taken from Gregory, 2005). See also Table 1.

Table 1. Summary statistics for chromosome numbers in major vertebrate groups

Clade (*n*)	2*N* Range	Mean	Mode	Variance	Kurtosis
Actinopterygii (615)	22–250	55	48	22	30.27
diploid species (537)	22–78	48	48	6	6.69
Amphibia (326)	20–108	28	26	11	22.91
Reptilia (170)	22–66	38	36	8	1.39
Aves (104)	50–138	76	80	10	14.99
Mammalia (327)	6–102	46	48	18	−0.32

Despite numerous small departures from the modal number, 48 chromosomes per somatic cell is the most common extant condition as well as the most parsimonious ancestral state for Teleostei and several major subclades therein (Figure 3). Interestingly, most acanthopterygiian lineages display 48 chromosomes, whereas most ostariophysiian lineages exhibit 50 chromosomes (although 48 remains the most parsimonious ancestral count for the basal Ostariophysi). Overall, the Ostariophysi also exhibit far more variation in chromosome numbers than do the Acanthopterygii, with several lineages exhibiting small or modest reductions, expansions, and also polyploid deviations from the probable ancestral state.

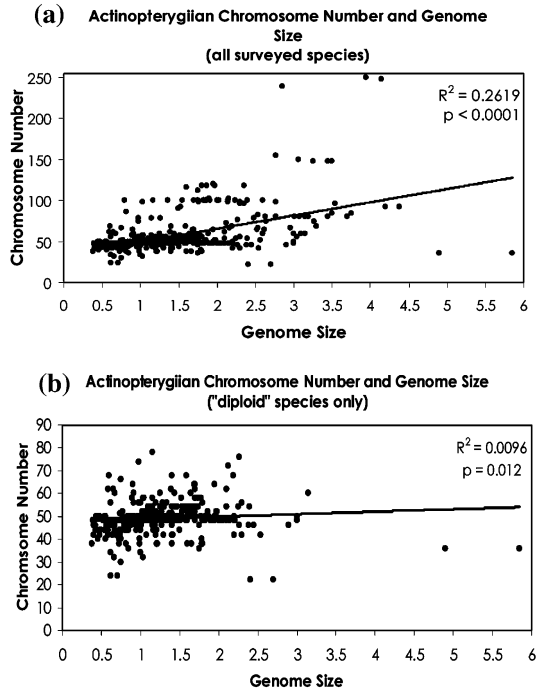

Figure 2. Scatterplot of the relationship between genome size (as measured by haploid C-value) and somatic chromosome number. Panel (a) shows the relationship for all 615 surveyed species of Actinopterygii; panel (b) shows the relationship for 537 "diploid" species of Actinopterygii, after removal of putatively polyploid taxa.

The precise ancestral chromosome number at the base of the full actinopterygiian clade could not be reconstructed with confidence, due primarily to variability in this trait among ancient Chrondrostei. However, that original ancestral condition was probably less than 48 chromosomes, according to the parsimony analysis.

Discussion

Our analysis has added a phylogenetic perspective to several interesting patterns previously reported in fish chromosomal evolution. First, somatic chromosome numbers are indeed remarkably stable in acanthopterygians, and this evolutionary conservatism contrasts with the striking diversity of actinopterygiian lineages in terms of genome size, composition, and synteny. Thus, dramatic evolutionary changes in these latter genomic features have been accomplished within a relatively steadfast framework of genomic scaffolding as reflected in chromosome numbers. Second, this evolutionary conservatism in chromosome numbers holds despite the evident capacity of actinopterygiian lineages to accommodate large karyotypic alterations via occasional polyploidization events. Third, chromosome numbers in actinopterygiian species are centered at 48 and 50 per somatic cell, with the frequency distribution being strongly leptokurtic. Maximum parsimony reconstruction suggests that the somatic cell count in ancestral teleosts was

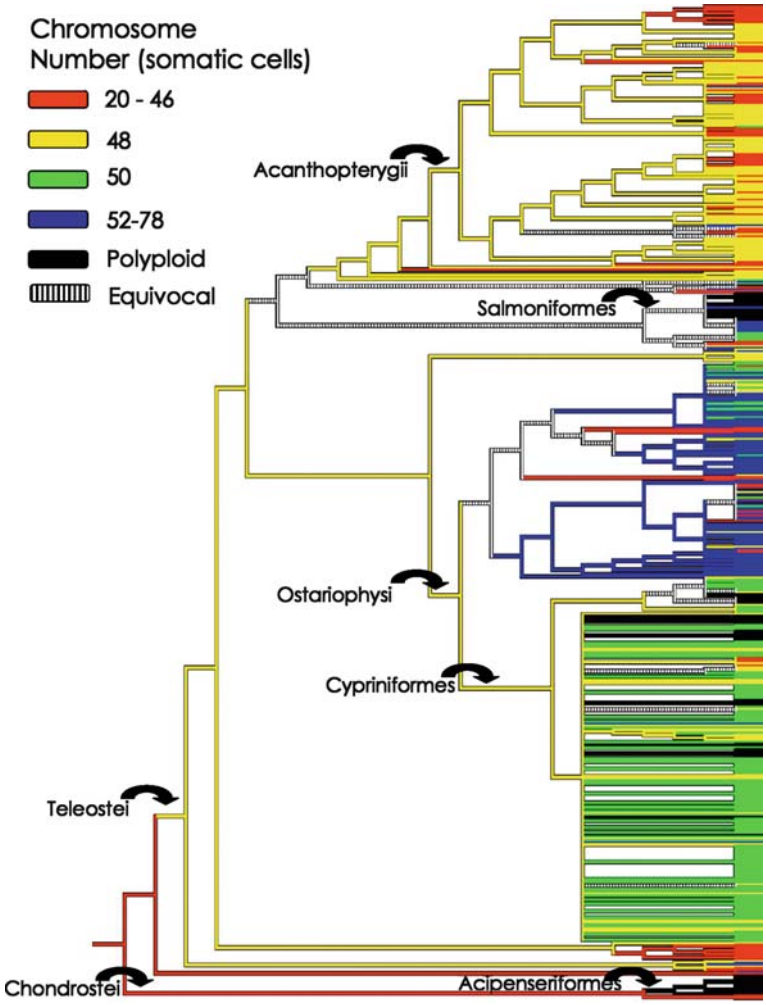

Figure 3. Actinopterygiian phylogeny showing chromosome numbers in extant taxa as well as results of a maximum parsimony reconstruction of ancestral character states. Clades mentioned in the text are indicated.

probably 48, but also that numerous small permutations from this or a similar number have occurred throughout the teleost clade.

Our reconstructions also agree with previous assertions that ray-finned fishes exhibit a high tolerance for polyploidy (Uyeno & Smith, 1972; Allendorf & Thorgaard, 1984; Ferris, 1984; Larhammar & Risinger, 1994; Vasil'ev, 1999; Christoffels et al., 2004). Based on current C-value comparisons, about 7–20 polyploidization events were inferred in the present phylogenetic analysis. These were usually most evident on recent twigs of the phylogenetic tree, where the evolutionary footprints of sudden large shifts in chromosomal numbers are expected to be best preserved. We found no discernable phylogenetic evidence for a previously proposed whole-genome duplication at the root of the teleosts (Wittbrodt, Meyer & Schartl, 1998; Meyer & Malaga-Trillo, 1999; Meyer & Schartl, 1999; Christoffels et al., 2004). However, this observation carries a significant caveat: over time, genome dynamics including large-scale deletions and chromosome re-patternings could likely have erased most direct karyotypic evidence for ancient genomic doublings.

Our analysis lacks the karyotypic resolution to determine how polyploidization, changes in genome size, and other forms of genomic dynamism manifest cytologically. Although the current literature lacks sufficient data to permit a comparative analysis of detailed cytogenetics across the Actinoptergyii, preliminary analyses based on available

genome sequence data (Thomas et al., 2003) suggest that synteny is not well conserved in this group. This suggests that there may be a great deal of cryptic cytological diversity at finer karyotypic levels.

We have no compelling explanation for the general conservation of chromosome numbers in actinopterygian fishes. An *ad hoc* (but unenlightening) possibility is that phylogenetic inertia generally has inhibited changes in chromosomal numbers (Blomberg & Garland, 2002), especially since considerable modifications in genome size in fishes can evidently occur largely independent of changes in chromosome counts (Figure 2). But this merely begs the question of why such phylogenetic inertia might exist for this but not many other genomic features. Perhaps there are cytokinetic constraints of some sort on shifts in chromosome numbers in fishes. But then why would such constraints appear to apply with much less force to some other, younger vertebrate clades (e.g., mammals; Figure 1)?

Even more perplexing is why actinopterygiian fishes display a highly leptokurtic distribution of chromosome counts centered at 48–50 chromosomes per somatic cell. If one speculates that this outcome reflects something inherently important about the absolute number and distribution of chromosomes (or perhaps associated genetic factors such as chromosomal break-points or total recombination potential), then one must also be prepared to explain why various other vertebrate groups show leptokurtic distributions centered on very different chromosome numbers (26, 30, and 80 in amphibia, reptiles, and birds, respectively; Figure 1; Table 1).

If definitive answers to these and related conundrums are eventually to emerge, comparative genome analyses on larger comparative scales may be required, perhaps coupled with novel lines of thought about the possible evolutionary forces that shape chromosomal dynamics. Novel insights about other aspects of vertebrate genomes (e.g., regarding molecular mechanisms of rampant DNA loss in pufferfish; Neafsey & Palumbi, 2003) have already emerged from massive sequencing efforts (Aparicio et al., 2002; Jaillon et al., 2004), so perhaps the evolutionary patterns that we have summarized here will someday be understood also in terms of evolutionary mechanism and process.

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