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Cladogenetic correlates of genomic expansions in the recent evolution of actinopterygiian fishes

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Genomic expansions via regional gene duplications and polyploidization events have been implicated as catalysts for rapid cladogenetic speciation in some fish taxa, but any general relationships between genome sizes and patterns of evolutionary radiation remain poorly characterized. Here we examine empirical correlations between genome size and species richness (number of extant species within a given clade) both across Actinopterygii (ray-finned fishes) and within several large actinopterygiian clades. We conducted the analyses both without and with correction (by independent contrasts) for phylogenetic effects. Across the full suite of 461 surveyed genera, relatively small but significant positive correlations were present between species richness and evolutionary increases in *C*-value. Although many variables (including ecological and behavioural factors) clearly can influence speciation rates, the current results are consistent with the notion that genomic architecture may play a role in species proliferation as well.

Keywords: gene duplication; speciation; phylogeny; *C*-value paradox; ray-finned fishes

1. INTRODUCTION

Large-scale genomic expansions or whole-genome duplication events have been documented in early vertebrate evolution (Friedman & Hughes 2001; Ohno 1970; Wang & Gu 2000), near the base of the phylogenetic tree of teleost fishes (Christoffels *et al.* 2004; Meyer & Schartl 1999; Robinson-Rechavi *et al.* 2001; Wittbrodt *et al.* 1998), and near the basal roots of several major teleostean clades [such as salmonids (Allendorf & Thorgaard 1984), catastomids (Ferris 1984; Uyeno & Smith 1972), acipenserids (Vasil'ev 1999) and some cyprinids (Larhammar & Risinger 1994)]. Such genomic enlargements have been hypothesized as key factors that enable or perhaps even drive diversification in various vertebrate groups (Holland *et al.* 1994; Meyer & Malaga-Trillo 1999; Navarro & Barton 2003*a*,*b*; Ohno 1970; Stephens 1951). Indeed, plausible theories that causally link genomic expansions to evolutionary radiations (Force *et al.* 1999; Lynch & Conery 2000; Taylor *et al.* 2001*a*) have led to a widespread notion that such enlargements routinely accelerate speciation processes (Hoegg *et al.* 2004; Taylor *et al.* 2001*b*). However, little comparative work has explicitly tested for the hypothesized correlations between genome dynamics and cladogenetic patterns.

Genomic architecture in collaboration with ecological or other factors could affect speciation rates via several mechanisms. First, following a genomic expansion event (e.g. by aneuploidy or polyploidization), newly duplicated loci may evolve new functions, as exemplified by the emergence of antifreeze proteins in extreme cold-water fishes (Cheng & Chen 1999). Duplicated loci that evolve new structural, catalytic, or regulatory roles (Dulai *et al.* 1999; Manzanares *et al.* 2000; Nanda *et al.* 2003) may permit a taxonomic group to exploit new habitats and thereby adaptively radiate. Second, most duplicated loci become mutationally silenced over time (Grauer & Li 2000), but these too may promote speciation by fostering chromosomal re-patternings via illicit recombination of non-homologous gene regions (Lynch 2002; Navarro & Barton 2003*a*,*b*). Third, reciprocal silencing of complimentary duplicate genes (or their regulatory regions) in separate populations is potentially another major source of genomic divergence conducive to the emergence of genetic incompatibilities (Lynch & Conery 2000; Lynch & Force 2000). Finally, some appreciable genomic expansions may be due to repetitive transposable elements, and these too may alter gene expression patterns or otherwise alter genomic profiles in ways that promote speciation events (Brosius 1999; Capy 1997; McDonald 1990, 1995, 1998). In theory, any or all of these factors could increase cladogenetic rates in lineages that experience salient genomic expansions. This is the working hypothesis tested here, using comparative phylogenetic methods on fishes.

Among the vertebrates, ray-finned fishes display exceptionally high variation in genome size (Hinegardner 1976; Venkatesh 2003). In contrast to mammals, birds and reptiles, where in each case genome sizes collectively span only about a two-fold range, fish genomes vary in DNA content (*C*-values) by more than an order of magnitude: e.g. from the compact genome of the pufferfish (*Fugu rubripes*) with 0.39 picograms (pg) of DNA per cell, to the huge genome of the armoured catfish (*Corydoras aeneus*) with 4.4 pg DNA per cell (Hinegardner & Rosen 1972). Such wide variation in genome size in a well-known taxonomic group with more than 20 000 described extant species makes fishes excellent candidates for examining empirical relationships between genome dynamics and evolutionary radiations.

2. MATERIAL AND METHODS

From recent compendiums (Brainerd *et al.* 2001; Gregory 2001; Hardie & Herbert 2003; Hinegardner & Rosen 1972),

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we assembled a database on haploid genome sizes (pg DNA per cell) in 823 surveyed species of actinopterygiian fishes representing 461 genera. We then averaged the *C*-values within each genus, omitting from our calculations the few cases where polyploidy occurred as an intraspecific polymorphism. We also recorded the number of extant species for each genus from the current standard taxonomy (Eschmeyer 1998; Froese & Pauly 2004; Nelson 1994).

To examine whether species richness per genus varied with regard to genome size, we employed least squares regression to calculate correlation coefficients (r) and test their significance (p). An ongoing debate about whether phylogeny should be explicitly accommodated (Felsenstein 1985; Harvey & Pagel 1991) or ignored (Harvey & Rambaut 1998; Price 1997; Ricklefs 1996) in comparative evolutionary studies has not yet been resolved, so we present analyses from both types of investigations, as follows.

First, we treated all 461 surveyed genera as independent observations, i.e. without regard to their phylogenetic associations. Second, to correct for phylogeny, we used a recently constructed supertree for Actinopterygii (Mank *et al.* 2005), which itself was based primarily on extensive recently published phylogenetic data for various groups of teleost fishes. This phylogenetic cladogram was analysed by independent contrasts (Felsenstein 1985; Grafen 1990) as implemented for measures of species richness in the software package macroCAIC (Agapow & Isaac 2002). This method attempts to correct for phylogenetic non-independence among data points by confining attention to trait comparisons across each bifurcating node in an underlying phylogeny, thereby yielding sets of independent data points or 'contrasts' (Martins 1996).

In our analyses, soft polytomies were coded as such, and altogether the dataset yielded 189 independent contrasts that we used to test for significant associations, employing linear regression (Harvey & Pagel 1991; Pagel 1993; Purvis & Rambaut 1995). These contrasts proved to be scattered across the supertree (rather than concentrated in particular sets of related genera), as evidenced in part by the fact that 121 of the contrasts (64%) were above the taxonomic level of family. Raw *C*-value contrasts were square-root-transformed to reduce skew (Quinn & Keough 2002). To prevent a few outlying observations from unduly influencing the regression relationships, we removed two genera (*Haplochromis* and *Barbus*) that were each more than seven standard deviations from the mean species count.

We also conducted comparable analyses on several large actinopterygiian clades for each of which 20 or more data points were available. These involved the superorders Ostariophysi and Atherinomorpha, and the taxonomic orders Tetraodontiformes and Pleuronectiformes. These sub-clade analyses were performed in identical fashion to those described above for the full Actinopterygii.

3. RESULTS

Haploid genome sizes among the surveyed taxa ranged from 0.39 pg/cell (pufferfish genus *Chelonodon*) to 3.57 pg/cell (sturgeon genus *Acipenser*), with values showing a roughly normal distribution around a mean of 1.19 pg/cell. This distribution is similar to previous reports for fishes (Hardie & Herbert 2003; Hinegardner & Rosen 1972).

Across the full suite of more than 450 actinopterygiian genera surveyed, a statistically significant positive correlation emerged between average genome size and number of species in a genus (figure 1). This relationship held both for the raw data ($n=461$, $r=0.15$, $p<0.001$; figure 1*a*), and for the independent-contrast data corrected for phylogeny ($n=189$, $r=0.20$, $p=0.002$; figure 1*b*).

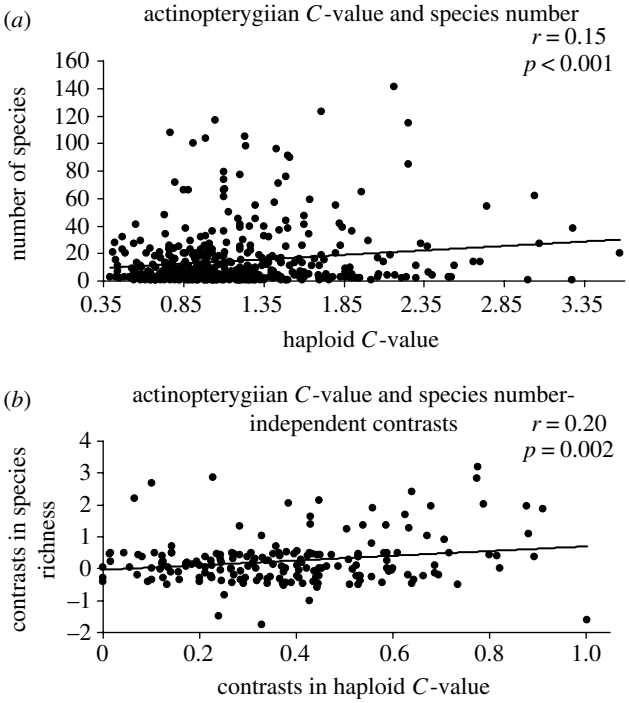

Figure 1. Correlational relationship between genome size and species richness in all surveyed actinopterygiian genera. (*a*) Phylogenetically uncorrected. (*b*) Phylogenetically corrected by independent contrasts. In both analyses, *C*-value is measured in picograms DNA per haploid cell; contrasts in *C*-value (*b*) are square-root transformed. The trend line in both regressions is shown.

In the finer-scale analysis of taxonomic superorders and orders, several patterns appeared (table 1). Ostariophysi showed a marginally significant positive correlation between genome size and generic species richness in the phylogenetically uncorrected analysis ($n=179$, $r=0.11$, $p=0.07$) as well in the analysis via independent contrasts ($n=41$, $r=0.29$, $p=0.03$). Atherinomorpha showed a stronger positive correlation in both the uncorrected analysis ($n=24$, $r=0.55$, $p=0.002$; figure 2*a*) and in the phylogenetically corrected version ($n=20$, $r=0.54$, $p=0.006$; figure 2*b*), although statistical significance in this latter analysis relies quite heavily on what might arguably be viewed as an outlier data point. Pleuronectiformes exhibited a positive correlation in the phylogenetically uncorrected analysis ($n=23$, $r=0.48$, $p=0.009$), but independent contrasts failed to recover a significant relationship ($n=8$, $r=0.26$, $p=0.27$). Finally, Tetraodontiformes showed a negative correlation ($n=26$, $r=0.54$, $p=0.002$; figure 2*c*) that proved to be statistically significant in the uncorrected analysis but not so when analysed by independent contrasts ($n=11$, $r=0.30$, $p=0.18$; figure 2*d*).

4. DISCUSSION

The notion that genomic expansions might contribute to speciation was introduced long before the modern molecular era (Haldane 1933; Ohno 1970; Stephens

Table 1. Summary of statistical regressions between genome size and species richness for Actinopterygii and various subclades.

clade	# species surveyed	# genera surveyed	mean *C*-value[a] (SD)	correction for phylogeny?[b] (# ind. con.)[c]	correlative trend[d]	*r*	*p*
Actinopterygii	823	461	1.19 (0.50)	no	+	0.15	0.001
				yes (189)	+	0.20	0.002
Ostariophysi	350	179	1.41 (0.49)	no	+	0.11	0.07
				yes (41)	+	0.29	0.03
Pleuronectiformes	28	23	0.75 (0.14)	no	+	0.48	0.009
				yes (8)	n.s.	0.26	0.27
Tetraodontiformes	41	25	0.62 (0.18)	no	−	0.54	0.002
				yes (11)	n.s.	0.30	0.18
Atherinomorpha	68	26	1.03 (0.25)	no	+	0.55	0.002
				yes (20)	+	0.54	0.006

[a] pg DNA per haploid cell.
[b] Correction by independent contrasts.
[c] Number of independent contrasts.
[d] Positive correlations indicate statistically significant situations in which clades with larger genomes have relatively more extant species; negative correlations are cases in which clades with smaller genomes contain more extant species; n.s. means a non-significant association.

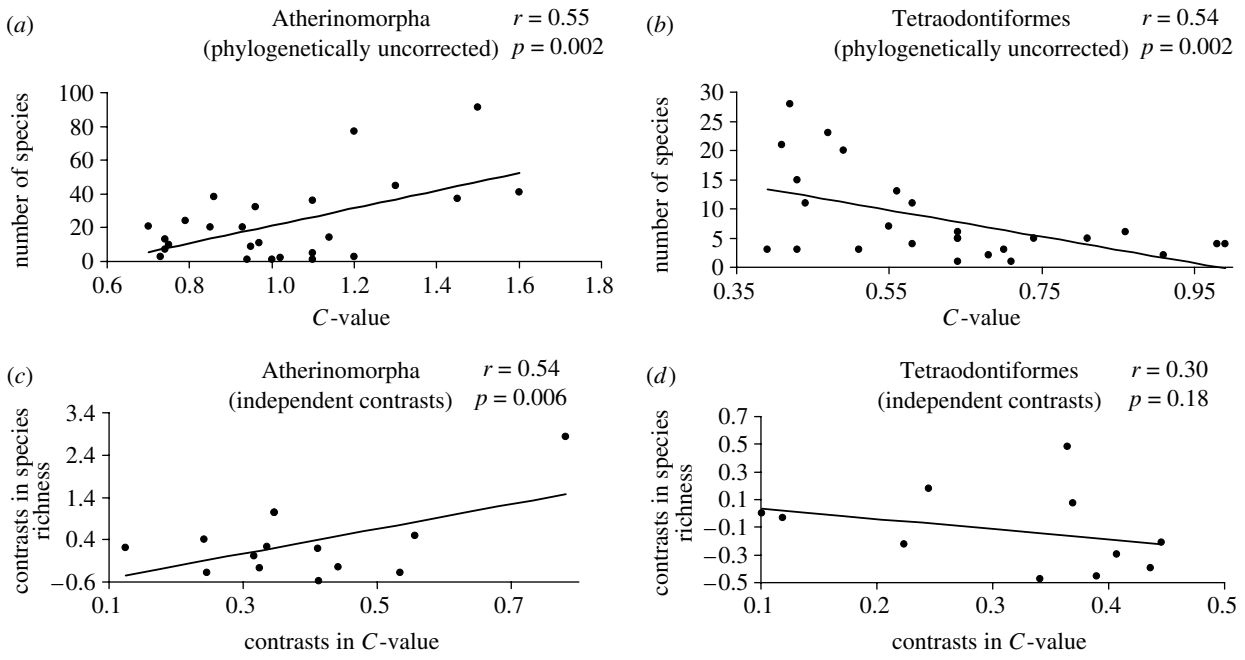

Figure 2. Examples of empirical regressions between genome sizes and species numbers in actinopterygiian subclades (see legend to figure 1 for further explanation). (*a*) and (*b*) Atherinomorpha (phylogenetically uncorrected and corrected, respectively). (*c*) and (*d*) Tetraodontiformes (phylogenetically uncorrected and corrected, respectively). *C*-value is measured in picograms DNA per haploid cell; contrasts in *C*-value (*b* and *d*) are square root transformed.

1951), but interest in the topic has been rekindled with the recent explosion of genome-level data (Christoffels *et al.* 2004; Meyer & Schartl 1999; Wittbrodt *et al.* 1998). For example, it now appears likely that the initial evolutionary radiation of teleosts was immediately preceded by large-scale or whole-genome duplication events (Amores *et al.* 1998; Hoegg *et al.* 2004; Taylor *et al.* 2003). Apart from polyploidizations, regionalized duplications of both extensive (Postlethwait *et al.* 2002; Smith *et al.* 2002) and more limited (Amores *et al.* 1998; Nanda *et al.* 2003) genomic sections have been documented in several groups of fishes, as have genomic expansions due to activities of repetitive element families (Nogare *et al.* 2002; Volff *et al.* 2001*a*,*b*).

Compared to most other vertebrate groups, the genomes of ray-finned fishes are evolutionarily labile in DNA content, apparently expanding and contracting rather quickly via extensive duplications and losses of genetic material (Neafsey & Palumbi 2003; Robinson-Rechavi & Laudet 2001). Despite long-standing suspicions that genomic expansions may often be associated with bursts of cladogenesis, this study is the first to our knowledge to assess this possibility empirically across multiple clades in a large taxonomic group of animals. We addressed net changes in genome content only, because the particular mechanistic reasons for alterations in genome size are not yet well understood in most fish genera.

The current analysis provides some support for the oft-hypothesized link between genome dynamics and cladogenesis. The presence of a statistically significant trend, despite numerous confounding variables (enumerated below), suggests that appreciable genome expansions have indeed been a factor associated with accelerated speciations in ray-finned fishes.

(a) *Qualifications*

Several sources of biological and statistical noise are nearly inevitable in the type of comparative phylogenetic analyses employed here. First, differential extinction rates across clades could have masked the postulated relationship between genome size and speciation rate in extant clades. Older clades might be most susceptible to this problem because extinctions would tend to accumulate over time following any bursts of cladogenesis. We attempted to minimize such extinction effects by focusing on genera rather than higher taxonomic levels. In other words, because discernable consequences of genomic expansions on cladogenesis might be evolutionarily ephemeral, they might best be examined in recent clades where their historical footprints should remain most evident. Two additional reasons motivated our focus on genus-level species richness: many more comparisons are available at this level than at higher echelons of the taxonomic hierarchy; and the mean half-life of duplicate genes (i.e. before they are silenced by mutations) is about four million years in animals (Lynch 2002; Lynch & Conery 2000; Lynch & Force 2000), so evolutionary radiations promoted by gene duplications might be expected to proceed within the general time-frame associated with congeneric divergences in many vertebrate groups (Avise *et al.* 1998; Johns & Avise 1998).

Second, taxonomic biases could have introduced noise into our analysis. Suppose, for example, that genomic enlargements tend to spur exceptionally large evolutionary alterations in organismal morphology or behaviour. Then, a rapidly speciating clade might have been split by systematists into more genera than a slowly speciating clade, and thereby show fewer (rather than more) extant species per genus on average. We took existing generic assignments at face value, so these or other kinds of taxonomic artifacts would not have been recognized or accommodated in our analyses.

Third, our comparative analyses were based strictly on cladogram structure and did not include information on branch lengths or evolutionary time-scales. Unfortunately, neither fossil records nor molecular data for Actinopterygii are as yet adequate to date all relevant nodes in the supertree that provided the phylogenetic framework for this report. This is another reason why our indicators of relative speciation rates across genera might be inaccurate.

Our fourth reservation is a general caveat that applies to all evolutionary studies of this ilk. The comparative method can only identify trait associations, so mechanisms (e.g. ecological, genetic, or physiological) underlying any correlations remain unspecified. Indeed, the possibility cannot be ruled out that evolutionary variables are correlated merely because they are both influenced by third-party factors (although in the current case it seems difficult to imagine what factor could promote cladogenetic rates and genome size variation jointly but without involving at least some causal links between the two).

Finally, another potential confounding factor is that salient genomic contractions (like salient genomic expansions) might also accelerate cladogenesis, if for example they tend to foster regulatory changes or cytogenetic rearrangements that promote genetic incompatibilities between populations (Lynch & Force 2000; Venkatesh 2003). In the current study, the negative correlation between genome size and species richness in Tetraodontiformes (figure 2*c*,*d*) is consistent with this possibility. This taxonomic order includes species that by virtue of extensive recent deletions of non-functional DNA (Neafsey & Palumbi 2003) display some of the smallest genomes known for any vertebrate taxa (Aparicio *et al.* 2002).

(b) *Genome dynamics and cladogenesis*

Despite the several reasons (discussed above) for pessimism in detecting any general correlation between changes in genome size and apparent speciation rates, our comparative evolutionary analysis of recently evolved fish taxa nonetheless was able to detect a statistically significant relationship between these two variables. If not spurious, this correlation could be reflective of any of several causal mechanisms by which changes in genome size might translate into increased probabilities of cladogenesis, such as via alterations of gene expression patterns (Brosius 1999; Capy 1997; McDonald 1998) or via the reciprocal silencing of redundant duplications at different locations in the genome (Lynch & Conery 2000; Lynch & Force 2000). Dissections of such casual processes will require case-by-case functional genomic analyses of particular actinopterygiian taxa.

Speciation is a multifaceted phenomenon (Coyne & Orr 2004), and genomic dynamism is only one plausible category in a complex nexus of causative agents that also includes many ecological and behavioural considerations. Given the diversity of factors impinging on cladogenetic patterns, the current documentation of a significant association between genomic expansion and increased cladogenesis across many piscine genera, as well as within several larger subclades of Actinopterygii, seems to us quite surprising.

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