An evolutionary correlate of genome size change in plethodontid salamanders

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SUMMARY

Variation in the amount of nuclear DNA, the C-value, does not correlate with differences in morphological complexity. There are two classes of explanations for this observation, which is known as the 'C-value paradox'. The quantity of DNA may serve a 'nucleotypic' function that is positively selected. Alternatively, large genomes may consist of junk DNA, which increases until it negatively affects fitness. Attempts to resolve the C-value paradox focus on the link between genome size and fitness. This link is usually sought in life history traits, particularly developmental rates. I examined the relationship among two life history traits, egg size and embryonic developmental time and genome size, in 15 species of plethodontid salamanders. Surprisingly, there is no correlation between egg size and developmental time, a relationship included in models of life history evolution. However, genome size is positively correlated with embryonic developmental time, a result that is robust with respect to many sources of variation in the data. Without information on the targets of natural selection it is not possible with these data to distinguish between nucleotypic and junk DNA explanations for the C-value paradox.

1. INTRODUCTION

The amount of DNA per haploid nucleus, referred to as genome size or C-value, spans a range of more than four orders of magnitude in eukaryotes (Cavalier-Smith 1982). Within vertebrates there is also a great range of genome sizes, from 0.4 pg in a pufferfish, *Tetraodon fluviatus* (Hinegardner 1968), to 142 pg in a lungfish, *Protopterus aethiopicus* (Olmo 1983). In general, however, most vertebrate groups exhibit a restricted range of values between 0.7 and 5 pg (Olmo 1983). An exception is the Amphibia. In salamanders, C-values range from 13.7 pg in one population of *Desmognathus ochrophaeus* (Hally *et al*. 1986) to 82.6 pg in *Necturus maculosus* (Olmo 1983). Several families of salamanders, including the Plethodontidae, exhibit a wide range of genome sizes (Morescalchi 1975), indicating that genome size evolution has occurred independently in each of these lineages.

The observation that C-values vary greatly without concomitant variation in organismal complexity has been dubbed the 'C-value paradox'. Explanations for the C-value paradox can be divided into two broad classes based on whether or not the amount of DNA has an adaptive function. Proponents of 'nucleotypic' explanations propose that DNA functions in two ways, via its coding (i.e. genotypic) function and via the quantity of DNA (i.e. 'nucleotype') (Bennett 1971). The amount of DNA may affect features such as nuclear size and structure (nucleoskeletal hypothesis of Cavalier-Smith 1982) or developmental rate (Bennett 1971), and increases in genome size may be adaptive. In contrast, excess DNA may be viewed as 'junk' (Ohno 1970) or 'selfish' DNA (Doolittle & Sapienza 1980; Orgel & Crick 1980) of no positive adaptive value to the organism. Functionless DNA may result from proliferation of elements that replicate autonomously within the genome until the cost to the organism becomes significant (Doolittle & Sapienza 1980; Orgel & Crick 1980). Many examinations of correlates of genome size attempt to explain the wide variation in genome size by evaluating competing explanations for the C-value paradox (e.g. Cavalier-Smith 1982; Sessions & Larson 1987; Pagel & Johnstone 1992).

The relationship between genome size and life history traits, particularly developmental rate, has attracted much attention because it would provide a direct link between genome size and fitness. Such a link is required by all explanations of the 'C-value paradox'. Embryonic developmental time, which is inversely related to developmental rate, is affected by a variety of environmental and organismal factors. Most prominent among environmental factors is the temperature at which an organism develops (Moore 1939). In this study environmental factors are held constant across species, and thus their effects on embryonic developmental time do not confound the analysis. Many organismal traits may also influence embryonic developmental time. Correlations have been hypothesized or demonstrated between developmental time and C-value (Bennett 1971; Pagel & Johnstone 1992), stage at hatching, and egg size (e.g. Steele & Steele 1975; Nussbaum 1985) in a diversity of organisms. A positive correlation between egg size and embryonic developmental time has been incorporated

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into models of life history evolution (Shine 1978; Nussbaum 1985; Sargent *et al*. 1987). However, empirical examination of the relationship within and among species has not produced a consistent pattern (e.g. Steele & Steele 1975; Kaplan 1980, 1989; Beacham *et al*. 1985). Conflicting results could occur because of variation in this relationship across taxa or because the pattern is obscured by changes in a third variable, such as genome size. Egg size was included in this analysis to test whether there is an evolutionary correlation between egg size and embryonic developmental time and adjusts for its effect on the relationship between genome size and developmental time.

Many correlates of genome size have been investigated in amphibians because of their great range of genome sizes. Correlations have been found between genome size and many cellular traits, including cell size, nuclear volume, and cell proliferation rate (Olmo & Morescalchi 1975; Cavalier-Smith 1982; Roth *et al*. 1990). In addition, correlations have been demonstrated between genome size and organismal level traits, including limb regeneration rate (Sessions & Larson 1987), metabolic rate at high temperature (Licht & Lowcock 1991), and brain organization (Roth *et al*. 1994). Life history traits to which genome size may be related include embryonic developmental time (Horner & Macgregor 1983; Pagel & Johnstone 1992), length of larval period (Goin *et al*. 1968), and metamorphosis (Larson 1984). Many of the correlates of genome size observed in amphibians have also been found in other taxa (Bennett 1971; Grime & Mowforth 1982; Olmo 1983). However, few of these studies were done in an explicitly phylogenetic context. Recent simulation studies have shown that the statistical significance of correlations may be inflated when phylogenetic structure is ignored (Martins & Garland 1991; Díaz-Uriarte & Garland 1996).

The Plethodontidae is the largest salamander family, with more than 250 species in four major lineages (Wake 1993). Phylogenetic analysis of genome size evolution demonstrates that both increases and decreases have occurred in the history of this clade (Larson 1984; Sessions & Larson 1987). Differences in genome size among plethodontids are largely the result of changes in the amount of repetitive DNA (Mizuno & Macgregor 1974). Only one change in chromosome number, a reduction from 2N = 28 to 26, has occurred in the Plethodontidae (Morescalchi 1975). Thus there is no evidence of polyploidization. This is important because the relationship of cellular and organismal traits to genome size may differ depending on whether polyploidization or other mechanisms of genome size change are involved (Bennett 1971). Plethodontids are an ideal group in which to test the hypotheses that egg size and genome size are correlated with embryonic developmental time because of the variation they show in all three traits.

2. METHODS

Data on egg size and embryonic developmental time were collected for 15 species of plethodontids representing the four major lineages (table 1). Eggs of most species were obtained from gravid females collected in the field. Oviposition was induced with luteinizing hormone releasing hormone (Jockusch 1996). Data for developmental time of *Desmognathus quadramaculatus* are from a clutch collected in the field during early cleavage. Eggs were incubated either in 10% Holtfreter's solution or on paper towels moistened with distilled water. All eggs were kept in a constant temperature chamber at 13 °C±1 °C under ambient light cycles. To eliminate the problems associated with geographic variation within species, developmental data from a single population or geographic region were used.

Embryonic developmental time was calculated as the median number of days from oviposition to hatching. The only surviving individual from the clutch of *Oedipina uniformis* was raised at 17.5 °C±1 °C. Because only two other species of bolitoglossines were sampled and this is the lineage with the largest genome sizes, it was important to include *Oedipina* in the data set. Therefore, I calculated a range of estimates for its developmental time at 13 °C by multiplying its developmental time at 17.5 °C (121 days) by the ratios of developmental time at 13 °C to that at 17.5 °C for other plethodontid species (table 1). Three estimates were used in analyses: the minimum, maximum and an intermediate value using the ratio from its sister taxon. Additional analyses omitted *Oedipina* from the data set. This permits examination of whether the results are dependent on the inclusion of *Oedipina* or on the conversion factor used. Developmental times for *D. ochrophaeus* and *D. wrighti* (from Collazo 1990) and *Gyrinophilus porphyriticus* (Collazo & Marks 1994) at 13 °C were taken from the literature.

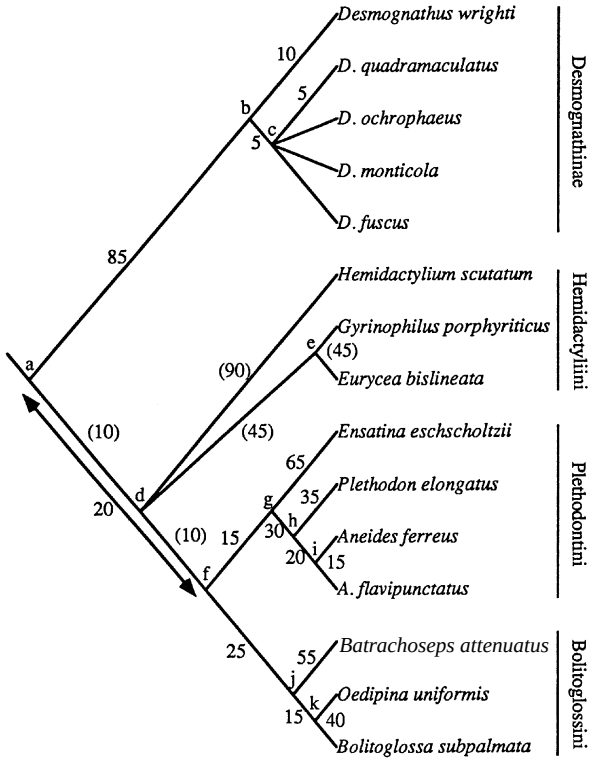

Figure 1. Relationships of the 15 plethodontid species used in independent contrasts analysis. The tree is based on Larson (1984), Sessions & Kezer (1991), Titus (1992), and Wake (1993). Numbers along branches are branch lengths estimated in millions of years. Branch lengths in parentheses indicate divergences for which dates were estimated following the method of Losos (1990). Letters are used to identify nodes in the text and figure 2.

Table 1. *Developmental data for 15 species of plethodontids*

(Abbreviations are as follows: MVZ (Museum of Vertebrate Zoology, University of California, Berkeley), ELJ (collection # of E. L. Jockusch), DBW (collection # of D. B. Wake). References for genome size data are (1) Olmo (1974), (2) Sessions (1984), (3) Hally *et al.* (1986), and (4) Licht & Lowcock (1991). Sample sizes are given as number of clutches/number of individuals for both egg size and embryonic developmental time. See text for details on the developmental time in *O. uniformis*. Limb data are from Sessions & Larson (1987).)

species	specimen no. or literature source for developmental data	egg size (mm)	development time: days (13 °C)	development time: ratio 13 °C/17.5 °C	genome size (pg/2N)	reference	*n* (egg size)	*n* (development time)	limb data
Desmognathinae									
Desmognathus fuscus	MVZ 224933, 224935, 224937-38, 224941, 224950, 224953	3.5	84	1.53	35.3	4	7/90	4/18	—
D. monticola	MVZ 224963	4.2	85	—	32.1	2,3,4	1/9	1/2	yes
D. ochrophaeus	Collazo, 1990	2.8	87	—	28.7	2,3	—	—	yes
D. quadramaculatus	ELJ 371; Collazo, 1990	3.9	77	1.54	29.9	2,3	—	1/10	yes
D. wrighti	Collazo, 1990	2.3	79	—	28.1	2,3	—	—	yes
Hemidactyliini									
Eurycea bislineata	ELJ 914, 937 MVZ 225058, 225060, 225062, 225066, 225068, 225071, 225075	2.8	39	1.13	49.8	4	4/20	7/18	yes
Gyrinophilus porphyriticus	Collazo & Marks, 1994	4.0	71	—	44.5	1	—	—	—
Hemidactylium scutatum	ELJ 887, MVZ 225080	2.8	58	1.71	65.2	4	1/5	2/5	—
Plethodontini									
Aneides ferreus	ELJ 338, 364, 366, 367, 373	5.0	135	—	82.5	4	5/94	1/2	yes
A. flavipunctatus	ELJ 1230	5.8	160	—	85.8	4	1/12	1/1	yes
Ensatina eschscholtzii	DBW 4453, 4454, ELJ 1227, MVZ 225003-04, 225006-09, 225011-12, 225023	6.1	125	1.39	70.6	2	10/90	6/15	yes
Plethodon elongatus	DBW 4451, 4452	5.2	124	1.29	61.2	2	2/14	1/1	yes
Bolitoglossini									
Batrachoseps attenuatus	ELJ 558, 562, MVZ 224323-38, 224343-344, 224352-354	4.1	79	1.25	74.0	2	23/191	19/66	—
Bolitoglossa subpalmata	DBW 4517, 4533, 4571-72, 4574 ELJ 365, 368, 369	4.0	234	1.42	137.8	2	6/143	5/13	—
Oedipina uniformis	DBW 4524	4.4		—	89.8	2	1/17	1/1	—
middle			172						
minimum			137						
maximum			206						

Table 2. *Results of regression analyses of embryonic developmental time versus genome size and egg size in plethodontids*
(All regressions were constrained to pass through the origin.)

	multivariate regression				univariate regression	
analysis	R^2	p (total)	p (genome)	p (egg size)	R^2	p (genome)
no hemidactyliines, branch lengths used						
Oedipina						
minimum	0.800	0.0036	0.0013	0.9894	0.800	0.0005
middle	0.755	0.0073	0.0027	0.8776	0.754	0.0011
maximum	0.623	0.0328	0.0132	0.8211	0.620	0.0068
omitted					0.793	0.0013
no hemidactyliines, equal branch lengths						
Oedipina						
minimum	0.802	0.0034	0.0012	0.8530	0.801	0.0005
middle	0.733	0.0098	0.0036	0.9277	0.733	0.0016
maximum	0.572	0.0511	0.0218	0.7913	0.568	0.0119
omitted					0.780	0.0016
hemidactyliines, branch lengths used						
Oedipina						
minimum	0.755	0.0018	0.0006	0.3825	0.732	0.0004
middle	0.710	0.0038	0.0013	0.3825	0.683	0.0009
maximum	0.604	0.0155	0.0058	0.4405	0.575	0.0043
omitted					0.720	0.0010
hemidactyliines, equal branch lengths						
Oedipina						
minimum	0.666	0.0072	0.0023	0.3904	0.636	0.0019
middle	0.595	0.0171	0.0059	0.3728	0.555	0.0054
maximum	0.451	0.0675	0.0264	0.4176	0.407	0.0257
omitted					0.592	0.0056

Egg diameters were measured on recently laid eggs prior to the blastula stage to the nearest 0.1 mm using an ocular micrometer mounted on a dissecting microscope. For *Hemidactylium scutatum*, measurements were taken on formalin-preserved eggs that had not yet gastrulated. Egg diameters for *D. ochrophaeus*, *D. quadramaculatus* and *D. wrighti* (Collazo 1990) and *G. porphyriticus* (Collazo & Marks 1994) are from the literature.

All genome size measurements were taken from four literature sources (table 1; Olmo 1974; Sessions 1984 (most of these data are also reported in Sessions & Larson (1987)); Hally *et al.* 1986; Licht & Lowcock 1991). To adjust for the different values assigned to the same standard, all values were restandardized based on a value of 6.20 pg/diploid nucleus for *Xenopus laevis* using the value for *X. laevis* reported in each source. Because genome size may vary within species, I only used data from populations phylogenetically or geographically close to the population in which I measured life history traits.

The phylogeny of the Plethodontidae (figure 1) is based on the literature (Larson 1984; Sessions & Kezer 1991; Titus 1992; Wake 1993). Two polytomies are included: relationships within *Desmognathus* are unresolved (Titus 1992) as is the position of *Hemidactylium* (Wake 1993). Branch length estimates in millions of years since divergence are based on molecular data (Larson *et al.* 1981; Larson 1984; Sessions & Larson 1987; Larson & Wilson 1989; Titus 1992; M. García-París, personal communication). No estimates for divergence dates are available for the three hemidactyliines. To include them in analyses using branch lengths, the divergences were assumed to have occurred halfway through the available time, as suggested by Losos (1990).

Standard statistical analyses cannot be done directly on species values because they are not independent data points (Felsenstein 1985). To overcome this problem I used independent contrasts, which compute standardized differences in trait values between nodes on a phylogeny (Felsenstein 1985). This method was first used by Sessions & Larson (1987) to examine phenotypic correlates of genome size. Independent contrasts were calculated using the program Comparative Analysis by Independent Contrasts (CAIC; Purvis & Rambaut 1995). Genome size was specified as the main variate in all analyses. Initially I did multiple regressions through the origin of developmental time on egg size and genome size. Regression through the origin is appropriate because the direction of subtraction is arbitrary when computing a contrast between two taxa (Garland *et al.* 1991). Regression coefficients were tested for significant difference from 0 with t-tests, and regressions through the origin were recalculated excluding variables with non-significant coefficients.

To standardize independent contrasts so that they can be analysed using standard statistics, branch lengths in units of variance of expected change are required (Felsenstein 1985). A significant correlation between the absolute value of standardized contrasts and the associated standard deviation of expected change (i.e. square-root of branch lengths) indicates that contrasts were not properly standardized (Garland *et al.* 1991). Using this procedure, there was evidence that egg size was not properly standardized ($r = -0.56$, $p = 0.08$ for the data set including hemidactyliines). Log_{10} transformation of branch lengths led to adequate standardization ($r = -0.26$, $p = 0.46$). Therefore, $\log_{10}$ transformed branch lengths were used to calculate standardized contrasts in egg size.

Robustness of the results with respect to variation from several sources of uncertainty was examined. Four analyses were done on each of four data sets, which differed only in the developmental time for *Oedipina*. The analyses included all combinations of including or excluding hemidactyliines and

assuming equal branch lengths or using divergence times. The effects of alternate resolutions of the polytomy involving *Hemidactylium* (figure 1, node d) were examined. Treatment of polytomies in CAIC follows the method of Pagel (1992). Taxa included in a polytomy are resolved into two subclades based on whether their value for the main variate falls above or below the mean for the clade. Then a single contrast is calculated between the two subgroups (Purvis & Rambaut 1995). In effect, this procedure selects only one possible topology. An alternate way to treat polytomies is to analyse the data using some or all possible trees (Losos 1994). Therefore, contrasts were calculated for the three possible trees using both equal branch lengths and branch lengths in estimated divergence times, and the middle developmental time for *Oedipina*. The effects of alternate resolution within *Desmognathus* were not tested because contrasts in this clade lie close to the origin and thus have little effect on the regression.

Nine of the species included in this study were also used by Sessions & Larson (1987) to measure rates of growth and differentiation in regenerating limbs (table 1). Although this constitutes a small sample, I tested for an evolutionary correlation between these developmental traits of adult organisms and embryonic developmental time using the complete phylogeny with branch length information.

3. RESULTS

Data on genome size, egg size and embryonic developmental time are presented in table 1. Genome size of the included species nearly spans the range found within plethodontids (Sessions & Kezer 1991). Average egg diameter ranged from 2.3 mm in *D. wrighti* to 6.1 mm in *Ensatina eschscholtzii*. Embryonic developmental time ranged from 39 d in *Eurycea bislineata*, the shortest recorded developmental time at 13 °C in plethodontids, to 234 d in *Bolitoglossa subpalmata*. Ratios of developmental time at 13 °C to that at 17.5 °C are not conserved phylogenetically.

Multiple regression of genome size and egg size on developmental time was always significant, and tests of the significance of the multiple regression coefficients showed that this was due to the relationship between genome size and developmental time (table 2; figure 2). Egg size, however, had no effect on developmental time. Therefore, egg size was eliminated from the analysis, and univariate regressions of developmental time on genome size were calculated (table 2).

The strength of the correlation between genome size and developmental time is always greatest for the minimum estimated developmental time for *Oedipina* and least for the maximum estimate (table 2). When hemidactyliines are excluded, the results are similar regardless of whether branch length information is used or not. However, when hemidactyliines are included, use of branch length information has a much greater effect (table 2).

The evolutionary correlation between genome size and developmental time was not greatly affected by the position of *H. scutatum*. Correlation coefficients ranged from 0.621 to 0.667 ($p < 0.002$) when branch lengths were used, and from 0.559 to 0.589 ($p < 0.004$) when branch lengths were assumed to be equal. The topology that produced the highest correlation differed in these two sets of analyses.

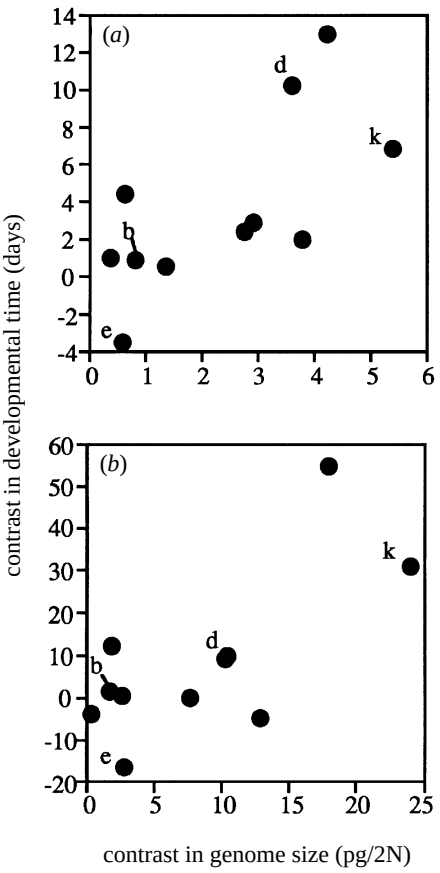

Figure 2. Standardized independent contrasts in embryonic developmental time versus standardized independent contrasts in genome size. Hemidactyliines were included in these analyses. (*a*) Branch length information used, (*b*) branch lengths assumed to be equal. Letters indicate nodes from figure 1. For coefficients of determination and significance of regression through the origin, see table 2.

Growth rate of regenerating limbs is not correlated with embryonic developmental time ($R^2 = 0.23$, $p = 0.23$). However, there was a negative correlation between differentiation rate in regenerating limbs and developmental time ($R^2 = 0.44$, $p = 0.07$), both of which are related to genome size. As developmental time is inversely proportional to developmental rate, the developmental rates of embryos and adult limbs (during regeneration) are positively correlated.

4. DISCUSSION

Data from 15 species of plethodontid salamanders do not support the prediction that egg size and embryonic developmental time are positively correlated, even when other influences on embryonic development time, such as temperature and genome size, are controlled for. Interspecific correlations between egg size and embryonic developmental time have been found in some (Berrill 1935; Steele & Steele 1975) but not all (graphs in Steele & Steele 1975) invertebrate taxa. As phylogenies become available for these groups, these correlations should be re-examined in a modern comparative framework. A number of fish and amphibian species have been examined for intraspecific correlations between egg size and time to hatching, but significant correlations were never found (e.g. Kaplan

1980, 1989; Beacham *et al.* 1985). Thus the lack of correlation between egg size and embryonic developmental time in plethodontids is concordant with data on intraspecific variation in other vertebrates.

To understand the evolutionary significance of changes in egg size, it is necessary to know the relationship of variation in egg size to other traits. Current evidence from vertebrates indicates that the assumption of a positive correlation between egg size and embryonic developmental time, which has been incorporated into models of life history evolution (e.g. Shine 1978; Nussbaum 1985; Sargent *et al.* 1987), is unjustified. However, variation in egg size may be significant for other aspects of life history evolution, such as maternal fecundity (Smith & Fretwell 1974; Kaplan & Salthe 1979), pattern of early development (Raff 1987) and offspring phenotype (Parichy & Kaplan 1995).

The conclusion that evolutionary changes in genome size and embryonic developmental time are positively correlated in plethodontid salamanders is robust with respect to sources of uncertainty in the data set. Developmental time of a single taxon, *Oedipina*, has a large influence on the overall correlation because the contrast in genome size with its sister taxon is among the largest even after standardization (figure 2, node k). As the regression is constrained to pass through the origin, nodes with the greatest contrasts will have the most influence on the slope of the regression line. However, the significant correlation is not dependent on inclusion of this clutch. Exclusion of *Oedipina* also yielded high estimates of the correlation coefficient (table 2).

Because complete phylogenetic information (i.e. topology and branch length) was available for only some of the taxa for which trait data were available, I was faced with a dilemma: to omit some taxa or to incorporate them in the absence of knowledge of branch lengths and phylogenetic resolution. Omitting taxa with unknown branch lengths was undesirable because all hemidactyliines would have been omitted. Hemidactyliines appear to differ from other plethodontids in the relationship of genome size to developmental time (table 1). However, because simulation studies have shown that the use of incorrect branch lengths affects the results, it was also undesirable to ignore branch lengths (Martins & Garland 1991; Díaz-Uriarte & Garland 1996). To explore the effects of these alternatives, I analysed the data in four ways: with or without hemidactyliines, and with or without branch lengths.

While hemidactyliines have the fastest developmental times, their genome sizes are intermediate between those of desmognathines and other plethodontids. However, the correlation between genome size and developmental time is not altered greatly by the inclusion of hemidactyliines. When branch length information is included, all changes in genome size are accompanied by a change in developmental time in the same direction, with one exception: the contrast between two hemidactyliines, *E. bislineata* and *G. porphyriticus* (figure 2, node e). Although the inclusion of hemidactyliines results in one negative contrast in developmental time, the second node added (figure 2, node d) has a large positive contrast in both genome size and developmental time.

Correlation coefficients were generally similar for the data set without hemidactyliines, regardless of whether branch lengths estimated in units of time or equal branch lengths were used. However, when hemidactyliines were included, branch lengths had a stronger effect on the correlation between genome size and embryonic development time. Branch lengths play two roles in the calculation of independent contrasts. First, they are used to weight the tip values when estimating nodal values. Second, they are used to standardize the contrasts (Felsenstein 1985). Their effect in this analysis appears to be a result of the different estimates of nodal values because of the differential weighting, not a result of lack of standardization. This is seen from the increase in the number of pairs of contrasts with opposite signs when branch lengths are set equal (figure 2). Only a change in the estimated nodal values can change the sign.

The conclusion that genome size and the duration of life history stages are correlated is in accordance with conclusions based on a non-phylogenetic approach (Goin *et al.* 1968; Oeldorf *et al.* 1978; Horner & Macgregor 1983; Camper *et al.* 1993) and one previous phylogenetically based analysis (Pagel & Johnstone 1992). It is consistent with most explanations for the C-value paradox, but fails to discriminate among them. One potentially important variable that has not been controlled for in this or any other analysis is stage at hatching. If individuals passed through identical ontogenetic trajectories but hatched at different points along that trajectory, then it would be straightforward to conduct the analysis using time to a homologous stage as the measure of developmental time rather than time to hatching. However, in this case, as will normally be the case, the ontogenetic trajectories of these species differ. Extensive ontogenetic repatterning has occurred in direct-developing bolitoglossines, including loss of larval features (Wake & Larson 1987). At no point in their development do they pass through a stage closely resembling extant plethodontid larvae. Direct development has evolved independently within the desmognathines (Collazo & Marks 1994). In this analysis there are two contrasts between a clade that is direct-developing and one that has larval development: across the basal node within desmognathines (figure 1, node b) and across the node joining hemidactyliines and the plethodontine–bolitoglossine clade (figure 1, node d). If the contrasts at these two nodes were outliers, it would suggest that the relationship between developmental time and genome size depends on the developmental mode. Figure 2 shows that neither of these points stands out. Thus, there is no evidence that a radical alteration in development, the evolution of direct development, has affected the relationship of genome size and developmental time.

Genome size is evolutionarily correlated with the rates of two developmental processes, embryonic development and differentiation of regenerating limbs (Sessions & Larson 1987) in the Plethodontidae.

However, it is probably not correlated with the duration of a third developmental phase, the larval period, as proposed for other amphibians by Goin *et al.* (1968). In desmognathines, larval period ranges from one to four years (Bruce 1989), with little variation in genome size. In *H. scutatum*, which has a much larger genome size, metamorphosis occurs in six weeks (Blanchard 1923), whereas larval life may exceed three years in *G. porphyriticus* and two years in *E. bislineata* (Bishop 1941). The relationship between genome size and duration of metamorphosis has not been examined in plethodontids. However, the largest genome sizes have evolved in lineages lacking metamorphosis, a pattern repeated in other salamander clades. This suggests that metamorphosis may constrain genome size evolution (Larson 1984).

Pagel & Johnstone (1992) present the only other analysis of the relationship between genome size and embryonic developmental time in which phylogenetic relationships were taken into account. Using data from the literature on 24 salamander species, including seven plethodontids, they found a significant positive correlation between genome size and time to hatching, and also a significant positive correlation between the residuals of these variables after removal of potentially confounding effects of nuclear and cytoplasmic volume. However, their results are flawed by ignoring developmental temperature, which greatly affects the developmental rate of amphibians (Moore 1939). Temperatures at which the developmental times were measured are not reported (Pagel & Johnstone 1992); however, the data for plethodontids clearly span a wide temperature range.

Pagel & Johnstone (1992) support the junk DNA hypothesis and argue against the nucleoskeletal view of Cavalier-Smith (1982). Their results and the results of this study are consistent with another nucleotypic explanation, that genome size is causally related to embryonic developmental time, a trait on which selection can act directly. What is needed to distinguish among explanations of the C-value paradox is an examination of the evolutionary causes of genome size change. A necessary first step in this is identifying evolutionary correlations. Subsequent work must decipher whether genome size or any trait causally related to it was the target of selection in lineages in which genome size has evolved.

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REFERENCES

Beacham, T. D., Withler, F. C. & Morley, R. B. 1985 Effect of egg size on incubation time and alevin and fry size in chum salmon (*Oncorhynchus keta*) and coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool.* **63**, 847–850.

Bennett, M. D. 1971 The duration of meiosis. *Proc. R. Soc. Lond.* B **178**, 259–275.

Berrill, N. J. 1935 Studies in tunicate development part III—differential retardation and acceleration. *Phil. Trans. R. Soc. Lond.* B **225**, 255–326.

Bishop, S. C. 1941 The salamanders of New York. *New York State Mus. Bull.* **324**, 1–365.

Blanchard, F. N. 1923 The life history of the four-toed salamander. *Am. Nat.* **57**, 262–268.

Bruce, R. C. 1989 Life history of the salamander *Desmognathus monticola*, with a comparison of the larval periods of *D. monticola* and *D. ochrophaeus*. *Herpetologica* **45**, 144–155.

Camper, J. D., Ruedas, L. A., Bickham, J. W. & Dixon, J. R. 1993 The relationship of genome size with developmental rates and reproductive strategies in five families of neotropical bufonoid frogs. *Life Sci. Adv. Genet.* **12**, 79–87.

Cavalier-Smith, T. 1982 Skeletal DNA and the evolution of genome size. *A. Rev. Biophys. Bioengng* **11**, 273–302.

Collazo, A. 1990 Development and evolution in the salamander family Plethodontidae. Ph.D. dissertation, University of California, Berkeley, California.

Collazo, A. & Marks, S. B. 1994 Development of *Gyrinophilus porphyriticus*: identification of the ancestral developmental pattern in the salamander family Plethodontidae. *J. Exp. Zool.* **268**, 239–258.

Díaz-Uriarte, R. & Garland, T. Jr 1996 Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviation from Brownian motion. *Syst. Biol.* **45**, 27–47.

Doolittle, W. F. & Sapienza, C. 1980 Selfish genes, the phenotype paradigm and genome evolution. *Nature*, *Lond.* **284**, 601–603.

Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.

Garland, T. Jr, Harvey, P. H. & Ives, A. R. 1991 Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18–32.

Goin, O. B., Goin, C. J. & Bachmann, K. 1968 DNA and amphibian life history. *Copeia* **1968**, 532–540.

Grime, J. P. & Mowforth, M. A. 1982 Variation in genome size—an ecological interpretation. *Nature*, *Lond.* **299**, 151–153.

Hally, M. K., Rasch, E. M., Mainwaring, H. R. & Bruce, R. C. 1986 Cytophotometric evidence of variation in genome size of desmognathine salamanders. *Histochemistry* **85**, 185–192.

Hinegardner, R. 1968 Evolution of cellular DNA content in teleost fishes. *Am. Nat.* **102**, 517–523.

Horner, H. A. & Macgregor, H. C. 1983 C-value and cell volume: their significance in the evolution and development of amphibians. *J. Cell Sci.* **63**, 135–146.

Jockusch, E. L. 1996 Techniques for obtaining and raising plethodontid salamander eggs. *Int. J. Devl Biol.* **40**, 911–912.

Kaplan, R. H. 1980 The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). *Evolution* **34**, 51–64.

Kaplan, R. H. 1989 Ovum size plasticity and maternal effects on the early development of the frog, *Bombina orientalis* Boulenger, in a field population in Korea. *Funct. Ecol.* **3**, 597–604.

Kaplan, R. H. & Salthe, S. N. 1979 The allometry of

reproduction: an empirical view in salamanders. *Am. Nat.* **113**, 671–689.
Larson, A. 1984 Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. *Evol. Biol.* **17**, 119–217.
Larson, A., Wake, D. B., Maxson, L. R. & Highton, R. 1981 A molecular phylogenetic perspective on the origins of morphological novelties in the salamanders of the tribe Plethodontini (Amphibia, Plethodontidae). *Evolution* **35**, 405–422.
Larson, A. & Wilson, A. C. 1989 Patterns of ribosomal RNA evolution in salamanders. *Molec. Biol. Evol.* **6**, 131–154.
Licht, L. E. & Lowcock, L. A. 1991 Genome size and metabolic rate in salamanders. *Comp. Biochem. Physiol.* **100B**, 83–92.
Losos, J. B. 1990 Ecomorphology, performance capability and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* **60**, 369–388.
Losos, J. B. 1994 An approach to the analysis of comparative data when a phylogeny is unavailable or incomplete. *Syst. Biol.* **43**, 117–123.
Martins, E. P. & Garland, T. Jr 1991 Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution* **45**, 534–557.
Mizuno, S. & Macgregor, H. C. 1974 Chromosomes, DNA sequences, and evolution in salamanders of the genus *Plethodon*. *Chromosoma*, *Berl.* **48**, 239–296.
Moore, J. A. 1939 Temperature tolerance and rate of development in the eggs of amphibia. *Ecology* **20**, 459–478.
Morescalchi, A. 1975 Chromosome evolution in the caudate amphibia. *Evol. Biol.* **8**, 339–387.
Nussbaum, R. A. 1985 The evolution of parental care in salamanders. *Misc. Publ. Mus. Zool. Univ. Mich.* **169**, 1–50.
Oeldorf, E., Nishioka, M. & Bachmann, K. 1978 Nuclear DNA amounts and developmental rate in holarctic anura. *Z. Zool. Syst. Evol.-forsch.* **16**, 216–224.
Ohno, S. 1970 *Evolution by gene duplication.* New York: Springer-Verlag.
Olmo, E. 1974 Further data on the genome size of the urodeles. *Boll. Zool.* **41**, 29–33.
Olmo, E. 1983 Nucleotype and cell size in vertebrates: a review. *Bas. Appl. Histochem.* **27**, 227–256.
Olmo, E. & Morescalchi, A.. 1975 Evolution of the genome and cell sizes in salamanders. *Experientia* **31**, 804–806.
Orgel, L. E. & Crick, F. H. C. 1980 Selfish DNA: the ultimate parasite. *Nature*, *Lond.* **284**, 604–607.
Pagel, M. D. 1992 A method for the analysis of comparative data. *J. Theor. Biol.* **156**, 431–442.
Pagel, M. & Johnstone, R. A. 1992 Variation across species in the size of the nuclear genome supports the junk-DNA explanation for the C-value paradox. *Proc. R. Soc. Lond.* B **249**, 119–124.
Parichy, D. M. & Kaplan, R. H. 1995 Maternal investment and developmental plasticity: functional consequences for locomotor performance of hatchling frog larvae. *Funct. Ecol.* **9**, 606–617.
Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC); an Apple Macintosh application for analysing comparative data version 2.0.0. *Computer Appl. Biosci.* **11**, 247–251.
Raff, R. A. 1987 Constraint, flexibility, and phylogenetic history in the evolution of direct development in sea urchins. *Devl Biol.* **119**, 6–19.
Roth, G., Blanke, J. & Wake, D. B. 1994 Cell size predicts morphological complexity in the brains of frogs and salamanders. *Proc. Natn. Acad. Sci. USA* **91**, 4796–4800.
Roth, G., Rottluff, B., Grunwald., W., Hanken, J. & Linke, R. 1990 Miniaturization in plethodontid salamanders (Caudata: Plethodontidae) and its consequences for the brain and visual system. *Biol. J. Linn. Soc.* **40**, 165–190.
Sargent, R. C., Taylor, P. D. & Gross, M. R. 1987 Parental care and the evolution of egg size in fishes. *Am. Nat.* **129**, 32–46.
Sessions, S. K. 1984 Cytogenetics and evolution in salamanders. Ph.D. dissertation, University of California, Berkeley, California.
Sessions, S. K. & Larson, A. 1987 Developmental correlates of genome size in plethodontid salamanders and their implications for genome evolution. *Evolution* **41**, 1239–1251.
Sessions, S. K. & Kezer, J. 1991 Evolutionary cytogenetics of bolitoglossine salamanders (family Plethodontidae). In *Amphibian cytogenetics and evolution* (ed. D. M. Green & S. K. Sessions), pp. 89–130. San Diego: Academic Press.
Shine, R. 1978 Propagule size and parental care: the 'safe harbor' hypothesis. *J. Theor. Biol.* **75**, 417–424.
Smith, C. S. & Fretwell, S. D. 1974 The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499–506.
Steele, D. H. & Steele, V. J. 1975 Egg size and duration of embryonic development in Crustacea. *Int. Rev. Gesamten Hydrobiol.* **60**, 711–715.
Titus, T. A. 1992 A phylogenetic analysis of the Desmognathinae (Caudata: Plethodontidae): evolutionary patterns inferred from mitochondrial DNA sequences. Ph.D. dissertation, University of Kansas, Lawrence, Kansas.
Wake, D. B. 1993 Phylogenetic and taxonomic issues relating to salamanders of the family Plethodontidae. *Herpetologica* **49**, 229–237.
Wake, D. B. & Larson, A. 1987 Multidimensional analysis of an evolving lineage. *Science*, *Wash.* **238**, 42–48.

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