PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Biological Significance of Variation in Satellite DNA and Heterochromatin in Newts of the Genus Triturus: An Evolutionary Perspective

H. C. Macgregor and S. K. Sessions

Phil. Trans. R. Soc. Lond. B 1986 **312**, 243-259
doi: 10.1098/rstb.1986.0005

Phil. Trans. R. Soc. Lond. B **312**, 243–259 (1986)
Printed in Great Britain

The biological significance of variation in satellite DNA and heterochromatin in newts of the genus *Triturus*: an evolutionary perspective

By H. C. Macgregor and S. K. Sessions

Department of Zoology, School of Biological Sciences, University of Leicester, Adrian Building, Leicester LE1 7RH, U.K.

The functional and evolutionary significance of highly repetitive, simple sequence (satellite) DNA is analysed by examining available information on the patterns of variation of heterochromatin and cloned satellites among newts (family Salamandridae), and particularly species of the European genus *Triturus*. This information is used to develop a model linking evolutionary changes in satellite DNAs and chromosome structure. In this model, satellites accumulate initially in large tandem blocks around centromeres of some or all of the chromosomes, mainly by repeated chromosomal exchanges in these regions. Centromeric blocks later become broken up and dispersed by small, random chromosome rearrangements in these regions. They are dispersed first to pericentric locations and then gradually more distally into the chromosome arms and telomeres. Dispersal of a particular satellite is accompanied by changes in sequence structure (for example, base substitutions, deletions, etc.) and a corresponding decrease in its detectability at either the molecular or cytological level. On the basis of this model, observed satellites in newt species may be classified as 'old', 'young', or of 'intermediate' phylogenetic age. The functions and effects of satellite DNA and heterochromatin at the cellular and organismal levels are also discussed. It is suggested that satellite DNA may have an impact on cell proliferation through the effect of late-replicating satellite-rich heterochromatin on the duration of S-phase of the cell cycle. It is argued that even small alterations in cell cycle time due to changes in heterochromatin amount may have magnified effects on organismal growth that may be of adaptive significance.

Introduction

This article is about highly repetitive 'satellite' DNA, its possible functions in the genomes of eukaryotes and its significance in development and evolution. The first satellite was isolated from mouse DNA by density gradient centrifugation in 1961 (Kit 1961). Since then, many more satellites have been isolated and characterized, some in the greatest of detail (see Miklos & Gill 1982), and yet they remain a problem. Just two and a half years ago Roger Lewin commented that repeated DNA was 'still in search of a function' (Lewin 1982). The situation remains unchanged, and as more data become available the evidence is harder to sift and the problems take on dimensions that have led some scientists to evade the issues and others to ignore them.

Satellite DNA, as commonly defined, is composed of short, tandemly repeated sequences, each usually less than 1000 nucleotide base pairs (b.p.) in length and present in more than 10^5 copies per haploid genome. These sequences are often concentrated into substantial blocks in certain parts of a chromosome set. Constitutive heterochromatin of the kind that can be

identified on metaphase chromosomes by Giemsa-C-staining or with certain fluorochromes nearly always contains high concentrations of satellite DNA. Therefore, in what follows we shall use the term satellite in the molecular sense and heterochromatin in the cytological sense to refer to the same component of the genome, taking care not to include the much more complex cases where heterochromatin is either facultative, as in the mammalian inactivated X chromosome, or does not contain highly repeated sequences.

In so far as satellite DNA represents a predicament for molecular biologists and cytologists alike, the main problems are these. First, it constitutes a highly variable proportion of the genome in different organisms, ranging from a fraction of 1% to as much as 60%. Secondly, structural analyses of satellite DNAs, and there have now been many, have not so far revealed the nature of their functions. Satellites vary widely in size, sequence and repetitive frequency from one organism to another, and with some notable exceptions their sequences are not highly conserved. Thirdly, arguments for conservation of function based on a few examples of conservation of sequences within and between species have been unconvincing. Fourthly, in no case is there an obvious causal relation between amounts of satellite DNA and morphological change within a group of related animals. It is possible for satellite sequences to be lost from both tissue culture cells and *in vivo* without obvious somatic effects. Lastly, among satellites that are transcribed, it is either hard to assign a cellular function to the transcripts, or the transcriptional event can be shown to be a consequence of read-through from adjacent structural genes (Varley *et al.* 1980; Diaz *et al.* 1981; Diaz & Gall 1985), and its significance will remain obscure until the read-through phenomenon is better understood.

It is for these reasons that satellite DNAs have been described by various authors as 'junk', 'selfish' or 'parasitic' (Doolittle & Sapienza 1980; Orgel & Crick 1980). Although these terms are widely familiar by now, there remains some confusion over their precise meaning. We would suggest that the concept of 'junk' DNA be considered as a general hypothesis implying selective neutrality, as opposed to functional or adaptive importance. 'Selfish' or 'parasitic', on the other hand, relates to a *mechanism* by which various kinds of DNA accumulate in the genome. Whereas the 'junk' hypothesis precludes functional significance of the DNA sequences in question, the 'selfish' hypothesis does not.

In the light of such concepts, can we then justify yet another search for satellite DNA function, particularly when the overwhelming bulk of existing evidence supports a neutrality hypothesis? In this respect we join with John & Miklos (1979) who were 'unimpressed in general with the argument that most of it (satellite DNA) constitutes a functionless burden which many eukaryotes must bear'. But why has it proved so difficult and unsatisfactory to correlate function with satellite DNA? Is it, perhaps, that the wrong questions have been asked, and if so then what questions might be more appropriate and promising? In our view, it may be more profitable to direct attention away from structure and function at the strictly molecular level (that is, precise DNA sequences), and focus instead on the role of satellite DNA at the levels of chromosome organization and behaviour, and especially on the physicomechanical effects that the *amount* of satellite DNA, regardless of its sequence, may have through the cell cycle on developing systems. We consider that at these levels there remains wide scope for a continued search for an adaptive role for this material. We have some new evidence from recent studies on newts (*Triturus*, *Notophthalmus* and *Taricha*) and we shall use this as a background for some suggestions that might provide foci for optimistic new ideas with an evolutionary perspective.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Satellites and heterochromatin in newts and salamanders

Over the past five years a considerable amount of information has accumulated about satellite DNA and heterochromatin in certain groups of newts and salamanders. Most of the satellite data have not yet been published, mainly because their significance in the organismal sense is hard to comprehend. In general, satellites have been studied as they have been discovered, rather than as a part of a cohesive programme with regard to phylogeny, morphology or development. We have chosen to concentrate on newts in this article simply because they offer good examples of all the levels of variability that occur in satellites and heterochromatin, and they provide us with a convenient focus for some of the problems.

Newts of the Eurasian genus *Triturus* (family Salamandridae) include at least 12 species and a number of subspecies. The animals are all pond dwellers, widely distributed in England and continental Europe. They are characterized by a biphasic life history, involving aquatic larvae and metamorphosis. The species of *Triturus* form three distinct groups or subgenera, based on adult body size and certain osteological features (Bolkay 1928). *Palaeotriton* includes the smallest newts, *Neotriton* the largest species, and *Mesotriton* a single species of intermediate size. On osteological grounds, *Neotriton* appears to be paedomorphic relative to the smaller newts (see Bolkay 1928). Two species and several subspecies have long been recognized within the *Neotriton* group, *T. marmoratus* (*T. m. marmoratus* and *T. m. pygmaeus*), and *T. cristatus* (*T. c. cristatus*, *T. c. carnifex*, *T. c. dobrogicus* and *T. c. karelinii*). Recent evidence suggests that

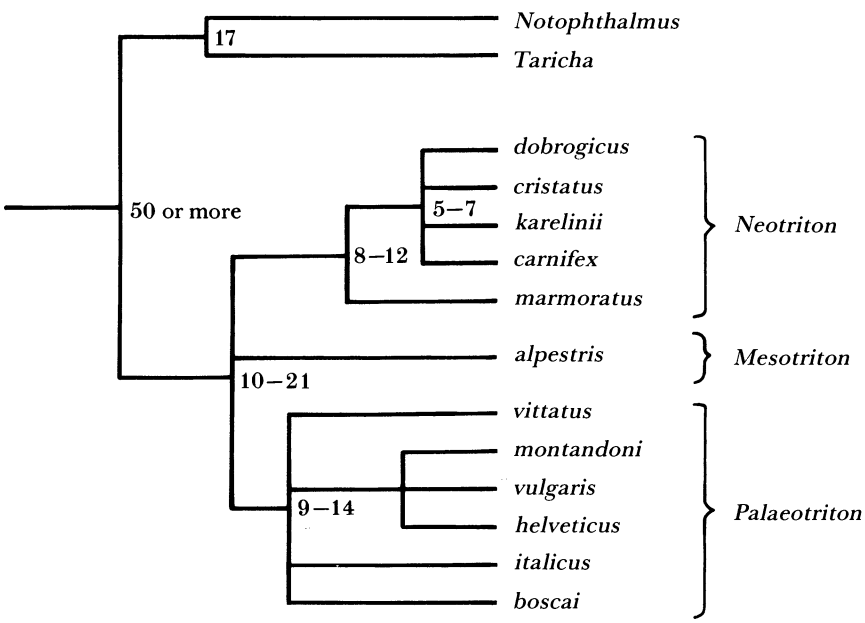

Figure 1. Dendrogram showing possible phylogenetic relationships among species of newts of the genus *Triturus* (after Bolkay 1928). The North American newt genera *Notophthalmus* and *Taricha* are included for reference. Approximate ages of lineages (minimum, or minimum–maximum, in millions of years) are indicated at branch nodes. Divergence times within the genus *Triturus* are based on the electrophoretic data of Frelow *et al.* (1985) and of Kalezic & Hedgecock (1980). The divergence time between *Notophthalmus* and *Taricha* is based on the genetic distance information presented by Ayala (1975). Ages of lineages are estimated from biochemical data assuming a divergence rate of 1.0 unit of Nei's genetic distance per 14 million years (Highton & Larson 1979). The minimum divergence time between *Triturus* and the North American lineage is based on the estimated age of permanent disappearance of the Atlantic connection between the European and North American landmasses (Wake *et al.* 1978).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 1. SEVEN SATELLITE DNA SEQUENCES THAT HAVE BEEN PURIFIED FROM NEWTS BY MOLECULAR CLONING

satellite†	size in base pairs	percentage of genome	present in‡ U.S.A. Nv	U.S.A. Tg	Europe Tc	Tcx	Tk	Tm	Ta	Tvt	Tv	Th	Tb	transcription§ O	O/S	N	N/C	special features‖	location¶
TcS2	330	0.1%	++	++	+++	+++	+++	+++	+++	++	++	++	++		+		+	processed transcript	chromosome 1 and dispersed
Nv2	330	0.09%	+++	+++	+++	+++	?	?	+++	?	+++	?	?		+		+	processed transcript	dispersed
Nv1	222	0.6%	+++	+	?		?	?		?		?	?	+		+		histone-linked	centromeres and histone
TkS1	33	5–10%		?	+	+	+++							+		+		sparsely transcribed	centromeric
TkS2	68	0.5–1%		?	+	+	+++			+				+		+		sparsely transcribed	centromeric
TcS1	390	*ca.* 1%	+	+	++	+++	++	++	++	++	+	+	+	+		+			chromosome 1 and dispersed
Tvm1	313	*ca.* 1%	+	+	++	?	?	?	++	?	+++	?	?	+		+			pericentric
					N				M		P								

† In each case the first letter specifies the genus and the second letter the species from which the satellites were obtained. TcS1 and TcS2, *Triturus carnifex*; Nv1 and Nv2, *Notophthalmus viridescens*; TkS1 and TkS2, *T. karelinii*; Tvm1, *T. vulgaris meridionalis*.

‡ Distribution of each satellite in 11 species of newts; from left to right, 2 from the U.S.A. (*N. viridescens* and *Taricha granulosa*) and 9 from Europe (*T. c. cristatus*, *T. carnifex*, *T. karelinii*, *T. marmoratus*, *T. alpestris*, *T. vittatus*, *T. vulgaris*, *T. helveticus*, and *T. boscai*). N is *Neotriton*, M is *Mesotriton*, and P is *Palaeotriton*. +++, Level to which each satellite binds to whole DNA from the species of origin in a dot hybridization analysis; ++, a lower but none the less distinct level of binding; +, a level of binding that is of the order of 10% or less of that obtained for the species of origin. Blank spaces indicate no detectable binding; a question mark indicates that no data are yet available.

§ O, transcribed in the oocyte only; O/S, transcribed in oocyte and somatic cells; N, transcripts found only in the nucleus; and N/C, transcripts found in nucleus and cytoplasm.

‖ Special features of the transcription of each satellite, where known.

¶ Chromosomal locations of each satellite. TcS1 and TcS2 are both found in higher concentrations in the heteromorphic arms of chromosome 1 that are characteristic of newts belonging to the subgenus *Neotriton*. Tvm1 is also present in *T. italicus* (Andronico *et al.* 1981).

many of these subspecies, particularly within the *cristatus* group, probably represent genetically well-defined species (Kalezic & Hedgecock 1980; Bucci-Innocenti *et al.* 1983; Frelow *et al.* 1985; Rafinski & Arntzen 1985) and in this paper they are treated as such. A generalized dendrogram illustrating possible phylogenetic relationships within the genus *Triturus* is shown in figure 1.

Within the genus *Triturus* there is wide variation in the amount of any one specific satellite between species. The satellite TkS1 from *Triturus karelinii* is a case in point (table 1). It is represented in both *T. karelinii* and *T. cristatus*, but is ten times more abundant in *T. karelinii* (Baldwin & Macgregor 1985). There are corresponding differences in the amounts of centromeric heterochromatin in the two species (figures 2*a*, *c*, 7 and 8).

With regard to the actual nucleotide sequences of satellite DNAs, TcS2 isolated from *T. carnifex* (Varley *et al.* 1980) is remarkably similar in both length and sequence to Nv2 from *Notophthalmus viridescens* (K. Mahon and J. G. Gall, personal communication). The two sequences show 80% homology over the first 220 bases, and 50% homology in the remaining 110 (C. Murphy and J. G. Gall, personal communication). The same or a very closely related sequence is present in approximately equal amounts in other species of *Triturus* and in *Taricha* (table 1) (K. Mahon, J. G. Gall, H. C. Macgregor and P. Miller, unpublished). Other satellites from *Notophthalmus* and *Triturus* are present in different amounts and show varying degrees of species specificity (table 1).

All satellites that have been isolated and investigated in *Triturus* and *Notophthalmus* are transcribed, at least on lampbrush chromosomes. The one satellite that seems to have been strongly conserved in sequence evolution, the 330 b.p. Nv2 and TcS2, is transcribed in both oocytes and somatic cells. The RNA transcripts are of sizes equivalent to monomers, dimers and trimers of the sequence itself, and they are found in both nucleus and cytoplasm (Varley *et al.* 1980; L. M. Epstein, K. Mahon and J. G. Gall, personal communication). So although much of the satellite transcription in newts can be accounted for by read-through from adjacent genes (Varley *et al.* 1980; Diaz *et al.* 1981; Diaz & Gall 1985), and this is clearly the case for Nv1 which is read-through after the histone gene clusters (Diaz *et al.* 1981; Diaz & Gall 1985), the phylogenetic conservation and especially the apparently processed transcription of the Nv2–TcS2 sequence does suggest a possible transcriptional function. Indeed, it contrasts sharply with the read-through transcription of Nv1: the transcript in this case being abundant in the germinal vesicle nucleus of the oocyte but absent from the cytoplasm (J. G. Gall, personal communication).

Possible adaptive significance other than transcription

Saltatory replication and unequal sister chromatid exchange, perhaps with added elements of mobility and sequence conversion, are events that would seem to provide all the ingredients that are needed to explain any of the sequence combinations and arrangements that have so far been identified in satellite DNA (see Lewin 1980). Widespread conservatism of certain satellite sequences across major phyletic groups remains a mystery, but, however it may ultimately be explained, the explanation is unlikely to have general significance. Most satellites are highly variable in the phylogenetic sense, and even their degree of variability differs widely between taxonomic groups. Only a few are likely to yield information about the functional significance of precise sequence structure, and these include the most highly conserved sequences and sequences that are more or less sex-limited (Singh *et al.* 1981; Tone *et al.* 1982, 1984).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

At the present time it is possible to extract from past literature certain suggested roles for satellite DNA and, in agreement with recent reviewers (Skinner 1977; John & Miklos 1979), eliminate them from our discussion. Satellite DNA is not a requirement for recognition of homologous chromosome regions at meiotic prophase, and it does not seem to be a significant factor in chromosome pairing (see John & Miklos 1979). It has not yet been shown to generate transcripts that are of definable function in either the translational or the regulatory sense. It is hard to cast aside the strong conservatism and the processed transcription of satellites like Nv2, and such sequences may yet hold some surprises in store; but for the moment, satellite transcription is simply not understood.

Heterochromatin and recombination

Heterochromatin that is rich in satellite DNA is often correlated with certain patterns of meiotic crossing over (John & Miklos 1979), and this relationship may apply to *Triturus* in the same sense as to other organisms that have been investigated in more detail. Among the small newts, for example, *T. helveticus* has the least amount of heterochromatin in the simplest banding pattern (figures 3, 7 and 8), and it has the most distally localized chiasmata. *T. vulgaris*, on the other hand, has a relatively large amount of heterochromatin in a more complex banding pattern (figures 3, 7 and 8), and most of its chiasmata are interstitial (Callan 1942). Also, within the *cristatus* group of species, *T. karelinii* has a significantly higher chiasma frequency than other species and has the most satellite-rich centromeric heterochromatin. However, discussion along these lines must await a better understanding of the molecular mechanisms that regulate distributions and frequencies of chiasmata.

Centromeric location of heterochromatin

The common centromeric location and quantitative variation within and between karyotypes are features that have to be taken into account when assessing the possible adaptive roles of heterochromatin. Is centromeric location primarily a consequence of the juxtaposition of centromeres at telophase and the frequent association of these regions at other stages of the cell cycle, providing ample opportunities for chromatid and chromosome exchanges? Do different amounts of centromeric heterochromatin on different chromosomes perhaps indicate an effect of heterochromatin on some aspect of chromosome behaviour? Vig (1982), for example, has shown that in several species of mammals there is a good relationship between the order in which chromatids separate at anaphase and amounts of centromeric heterochromatin. The chromosomes with the least heterochromatin separate first, those with the most separate last. Vig suggests that the ordered sequence of centromere separation may reflect their attachment positions on the nuclear envelope at interphase. If this were the case then the idea should undoubtedly be linked with the studies of Bennett (1983) and Heslop-Harrison & Bennett (1984). These workers have argued convincingly that chromosome order within the nucleus may have important implications for development, in that chromosomes that exert major effects on nuclear behaviour may occupy special positions in a nucleus, and changing the spatial relationships of chromosomes with respect to each other and the nuclear envelope may affect cell differentiation and gene activity.

In newts of the genus *Triturus*, considerable quantitative variation in centromeric hetero-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

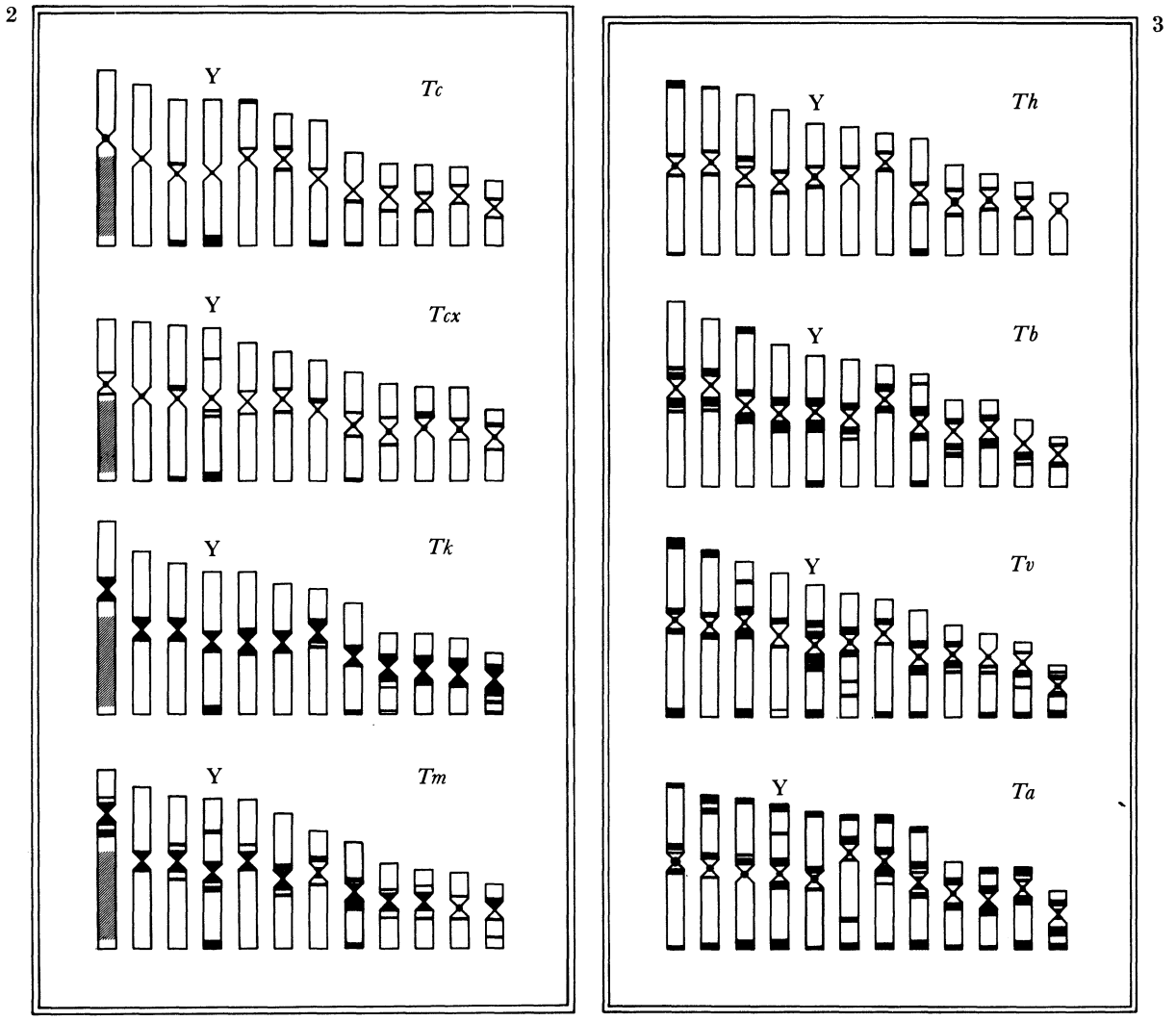

FIGURE 2. Idiograms of four species of the subgenus *Neotriton*. Dark regions indicate C-band heterochromatin. The heterochromatic long arm of chromosome 1 is indicated by diagonal hatching. The Y chromosome (chromosome 4) is identified in each case, and differs from the X in having telomeric heterochromatin. *Tc*, *T. cristatus*; *Tcx*, *T. carnifex*; *Tk*, *T. karelinii*; *Tm*, *T. marmoratus*.

FIGURE 3. Idiograms of *T. alpestris* (*Mesotriton*), and three species of *Palaeotriton*. Dark regions indicate C-band heterochromatin. Note that the Y chromosome of *T. helveticus* lacks the telomeric heterochromatin seen in the other species. Note that the Y chromosome in *Palaeotriton* (*Th*, *Tb*, *Tv*) is number 5. *Th*, *T. helveticus*; *Tb*, *T. boscai*; *Tv*, *T. vulgaris*; *Ta*, *T. alpestris*.

chromatin is observed between species, and there is no consistent correlation between the amount of centromeric heterochromatin and chromosome size. In *T. karelinii*, for example, each chromosome has similar absolute amounts of centromeric heterochromatin (rich in satellite TkS1) so that the relative amount of this material increases with decreasing chromosome size (figure 4*a*). *T. carnifex*, on the other hand, shows greater differentiation between chromosomes in heterochromatin amount (figure 4*b*).

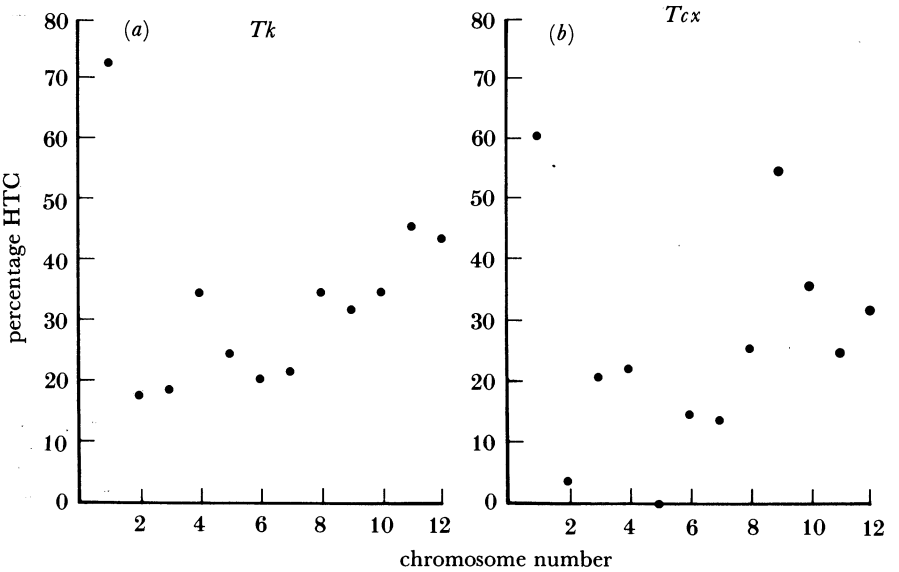

FIGURE 4. Graphs showing the relative amount (percentage) of heterochromatin per chromosome in two species of the *cristatus* group. In *T. karelinii* (*a*), each chromosome has the same amount of heterochromatin, thus the relative amount of heterochromatin increases with decreasing chromosome size; in *T. carnifex* (*b*), the distribution of heterochromatin among chromosomes is more variable.

HETEROCHROMATIN, CELL PROLIFERATION AND ORGANISMAL GROWTH

The degree to which natural selection can act on quantitative variation in satellite DNA depends on the phenotypic significance of this variation. In our view, satellite DNA is most likely to be of adaptive significance through its potential effect on rates of cell proliferation and growth in organismal development.

Evidence from a wide variety of amphibians, including newts and salamanders, suggests that growth rate is strongly influenced by genome size (Oeldorf *et al.* 1978; Horner & Macgregor 1983; Sessions 1985): the larger the genome the slower the growth rate. It seems reasonable to suppose that this relationship is partly a consequence of the damping effect that nuclear DNA has on its own replication during S-phase of the cell cycle (see Callan 1972). Large amounts of nuclear DNA apparently prolong all phases of the cell cycle, resulting in reduced rates of cell proliferation (Wallace & Maden 1976; Horner & Macgregor 1983). If the addition of satellite DNA (and heterochromatin) adds substantially to genome size, then it may be involved in some of these effects.

However, heterochromatin that contains satellite DNA has replicative properties that may have an important effect on the duration of S-phase of the cell cycle even in the absence of genome size changes. A number of common observations from a variety of organisms, including newts, are of crucial significance here. The most important of these is that heterochromatin replicates asynchronously with respect to the rest of the genome during S-phase of the cell cycle. In most cases that have been examined, replication of heterochromatin occurs late in S-phase, although its replication rate may be higher than that of euchromatin (Collins 1978; Schmid 1980). The combined effects of the replicative characteristics of heterochromatin on cell cycle time are bound to be complex, involving interplay between several variable parameters: the overall duration of S-phase, the amount of heterochromatin relative to genome size, the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

timing of initiation and termination of heterochromatin replication relative to other components of the genome, and the specific rate of heterochromatin replication (satellite DNA synthesis). We can express these parameters and their relationships to cell cycle time in mathematical terms as follows: α_e = initiation of replication of euchromatin; α_h = initiation of replication of heterochromatin; β_e = termination of replication of euchromatin; β_h = termination of replication of heterochromatin; R_h = rate of replication of heterochromatin; H = total amount of heterochromatin; S_e = replication time for euchromatin; S_h = replication time for heterochromatin; S_t = total duration of S-phase of the cell cycle;

$$S_t = S_e + S_h - (\beta_e - \alpha_h).$$

If we set $\alpha_e = 0$ (that is, the start of S-phase), then the duration of S-phase is simply equal to the termination of heterochromatin replication (β_h) and the above relation reduces to

$$S_t = S_h + \alpha_h.$$

Since the replication time of heterochromatin (S_h) is determined by the amount of heterochromatin and its rate of synthesis,

$$S_t = (H/R_h) + \alpha_h.$$

While admittedly simplistic, this expression demonstrates that in a genome that contains heterochromatin with the above replicative properties, the length of S-phase is determined by only three parameters: (i) the amount of heterochromatin; (ii) its initiation time relative to the start of S-phase; (iii) its rate of replication. This relationship is illustrated in figure 5.

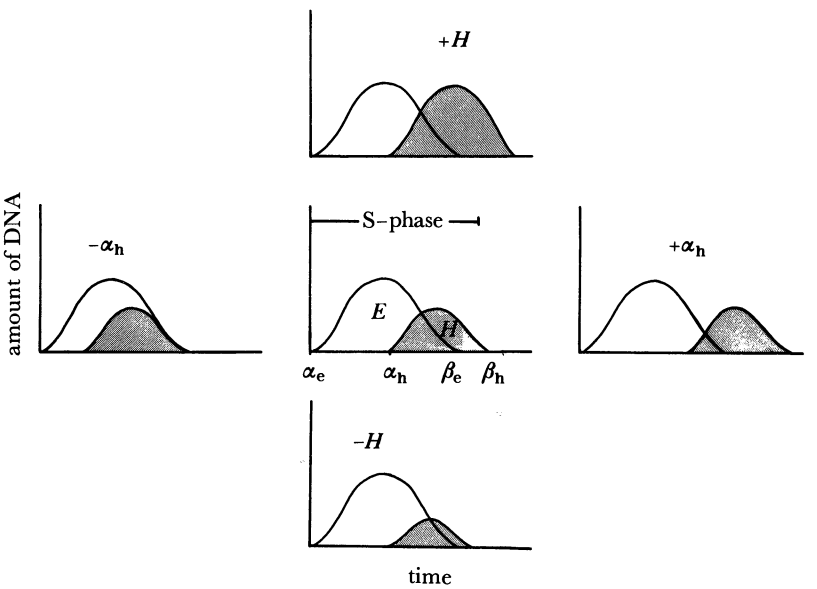

FIGURE 5. Hypothetical distribution curves of replicating euchromatin (E) and heterochromatin (H) during S-phase of the mitotic cell cycle. According to this model, S-phase is prolonged by an increase in the amount of heterochromatin ($+H$) or by a delay in the initiation of its replication ($+\alpha_h$). Similarly, S-phase is shortened by a decrease in the amount of heterochromatin ($-H$) or by an advance in the initiation of its replication ($-\alpha_h$). The curves of H and E are in reality each composed of many, perhaps thousands, of smaller curves representing diverse components with different replication schedules. The example illustrated here assumes a uniform average rate of synthesis for E and for H. This model may apply to individual chromosomes as well as to the genome as a whole.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The importance of these relationships to phenomena at the organismal level is the magnified effect that even a small change in cell cycle time, resulting from the addition of late-replicating heterochromatin, can have on overall growth rate and morphogenesis in a multicellular organism. For example, body size in a multicellular organism should be at least roughly proportional to the number of cells and the size of each cell. Figure 6 shows the kinds of effects, in terms of growth rate in relation to development, that could be produced by a 10% difference in cell cycle time or a twofold difference in cell size (assuming that growth is exponential over the ontogenetic period examined). From figure 6*a*, in which development is strictly age-dependent, at a given developmental stage, species B (cell cycle, 0.9) will be substantially larger than species C (cell cycle time, 1). In figure 6*b*, the same relationship is shown in a situation where developmental stage is size-dependent. That these kinds of argument may be applicable to newts and their satellite DNA and heterochromatin deserves further investigation. The large differences in body size observed among species of *Triturus* suggests extensive differentiation in rates of growth and development, despite similarity in genome sizes. Furthermore, two independent investigators have found that centromeric, and possibly also pericentric heterochromatin in *T. vulgaris*, a small newt, replicates late and outwith the span of replication of the remainder of the chromosomes (Callan & Taylor 1968; Ragghianti *et al.* 1973).

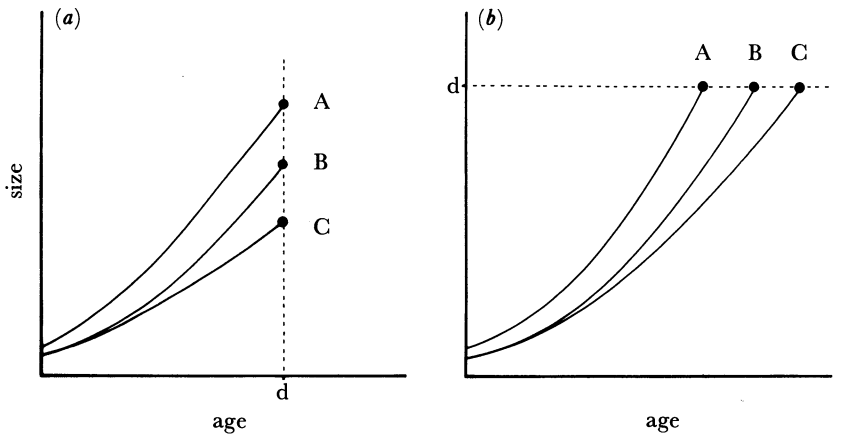

FIGURE 6. Theoretical effect of differences in cell cycle time or cell size, or both, on overall body size of multicellular organisms, assuming a constant rate of cell division over the growth period considered. If t is cell cycle time, s is cell size, a is age of organism, n is number of cell cycles which is a/t, 2^n is the number of cells after n cell cycles, then size of organism is proportional to $s(2^n)$. For species A, $t = 1$, $s = 2$; for species B, $t = 0.9$, $s = 1$; for species C, $t = 1$, $s = 1$. In figure 6*a* showing age-dependent development, species A, B and C reach the same stage of development at the same age: species B will have more cells and a larger overall size than species C; species A will have the same number of cells as species C and fewer cells than species B, but will have a larger overall body size than either C or B. In figure 6*b*, showing size-dependent development, the three species reach the same developmental stage at a given body size: species A will reach this stage earlier (that is at a younger age) than species B, and species B will reach it earlier than C.

Unequal sister chromatid exchange before mitosis or meiosis is probably the main mechanism for producing quantitative changes in satellite DNA in a genome. Each event of unequal exchange produces, by definition, an increase in satellite repeats on one participating chromatid and a decrease on the other. Therefore in the absence of selection, or some other 'driving' force, random exchanges over time may lead to increase, decrease, or no change in the amount of satellite DNA in chromosomes among individuals of a population. The postulated effect of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

satellite DNA on cell cycle time, however, provides a substrate on which natural selection can act. Individuals that carry, by chance, reduced products of unequal satellite DNA exchange will have correspondingly reduced cell cycle times, and they will be favoured by natural selection in circumstances in which rapid rates of growth are advantageous at some stage of development. In such circumstances, quantitative variation in satellite DNA amount and genome size among individuals of a given population is constrained by natural selection, and stasis or decrease should result. Under conditions of relaxed selection for growth rate, however, satellite DNA probably tends to increase in amount, at least in salamanders (Sessions 1985).

Heterochromatin variation in *Triturus*

Newts of the genus *Triturus* show substantial variation in Giemsa C-band heterochromatin (figures 2 and 3). Altogether eight species are considered here (from Sims 1984), and for each of these the sample size is small with respect to both numbers of individuals and populations. Nevertheless, we consider that our information is broadly representative, and we know enough about the other species to be confident that they will fit the general pattern that we present here. For example, both *T. vittatus* and *T. italicus* have chromosomal patterns of heterochromatin characteristic of 'small newts' (Schmid *et al.* 1979; Sims 1984). Four parameters of variation can be identified: (i) total amount of heterochromatin in the genome; (ii) amount of heterochromatin per chromosome; (iii) total number of heterochromatic bands in the chromosome set, and (iv) the chromosomal positions of the bands, that is, centromeric, pericentric, interstitial and telomeric. *Triturus* species fall into two groups on the basis of the pattern of variation in these parameters. These groups partly correspond to the major taxonomic groups identified by osteology and electrophoresis (Bolkay 1928; Frelow *et al.* 1985; Rafinski & Arntzen 1985). The most striking difference concerns the largest chromosome in the karyotypes of the large-bodied, relatively paedomorphic newts of the subgenus *Neotriton* (*T. cristatus* and *T. marmoratus*). In these newts, most of the long arm of chromosome 1 is heterochromatic and rich in satellite DNA (Macgregor 1979; Sims *et al.* 1984). This is obviously a derived situation, unique to *Neotriton*, and it is of undeniable phenotypic (developmental) significance (Horner & Macgregor 1983). Aside from chromosome 1, the banding patterns in these large newts are simple, and most of the heterochromatin is centromeric or pericentric. In this group, most of the phylogenetic variation in heterochromatin concerns simply the amount of centromeric heterochromatin (figures 2, 3, 7 and 8).

Relative to the large *Neotriton* species, the smaller species of the subgenera *Mesotriton* (*T. alpestris*) and *Palaeotriton* (*T. boscai*, *T. vulgaris*, and *T. helveticus*) have complex banding patterns, and most of their heterochromatin is non-centromeric (figures 2, 3, 7 and 8). *T. helveticus* stands out in this group as having the least amount of heterochromatin and the simplest banding patterns, resembling *Neotriton* species in this respect. *T. vulgaris* and *T. alpestris* represent the opposite extreme, with abundant heterochromatin distributed in numerous interstitial, telomeric and pericentric bands (figures 3*c*, *d* and 8). Not only do these small newts have more complex banding patterns than the larger species, but they also show more differentiation in chromosome morphology, as if their karyotypes have experienced more extensive rearrangements and repatterning than those of the large *Neotriton* species (Sims 1984) (figures 2 and 3).

In assessing the biological significance of heterochromatin in *Triturus* it is first necessary to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

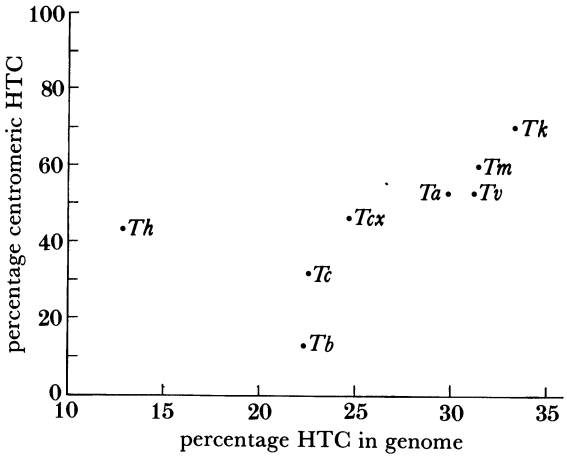

FIGURE 7. Amount of centromeric heterochromatin (HTC) plotted against the total amount of heterochromatin in the genomes of each of eight species of *Triturus*. In this figure, the heterochromatic long arm of chromosome 1 of the *cristatus* group is included. Note that *Th* is similar to *Tc* and *Tcx* in the amount of centromeric heterochromatin but it lacks the heterochromatic chromosome 1. Compare this figure with figure 8. Abbreviations as in figure 2.

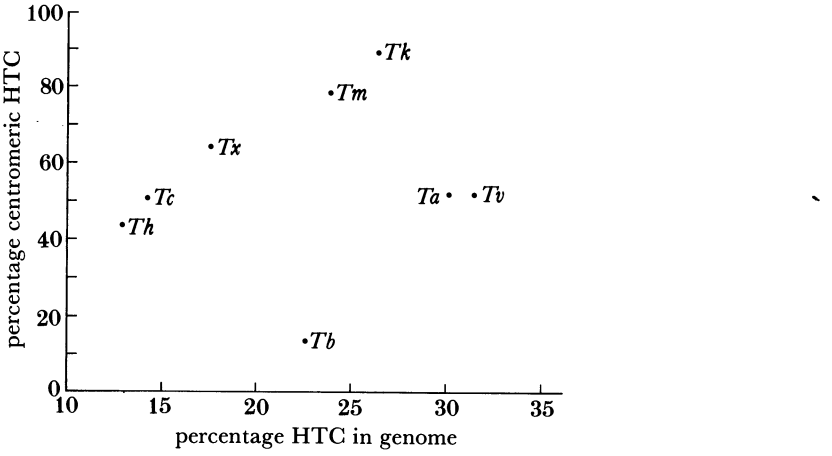

FIGURE 8. Same as figure 7 but excluding chromosome 1 of the *cristatus* group (*Tm*, *Tc*, *Tcx*, *Tk*). In this graph, the species form two groups. The *cristatus* group (*Neotriton*) plus *T. helveticus* (*Palaeotriton*) show a positive correlation between the amount of centromeric heterochromatin and total heterochromatin. The three 'small' newt species, *T. alpestris* (*Mesotriton*), *T. boscai*, and *T. vulgaris* (*Palaeotriton*), show larger amounts of non-centromeric heterochromatin relative to total heterochromatin.

determine how much of the observed patterns of variation simply reflect phylogenetic history. For example, all species of *Neotriton* have a highly modified chromosome 1 probably because they inherited it from a common ancestor subsequent to divergence from other lineages in the genus (see figure 1). It is more difficult to explain, on phylogenetic grounds (figure 1), the resemblance in heterochromatin amounts and banding pattern between *T. helveticus* and T. cristatus, or between *T. alpestris* and *T. vulgaris* (figure 8). If a simple, primarily centromeric banding pattern with small amounts of heterochromatin is a primitive feature, then the resemblance between *T. helveticus* and *T. cristatus* is due to retention of a common primitive pattern, and *T. vulgaris* and *T. alpestris* have converged on each other in a derived banding pattern (figures 3*c*, *d* and 8). In either case, *T. boscai* (another small newt belonging to the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Palaeotriton group) has a uniquely derived pattern of heterochromatin with a large amount of heterochromatin concentrated in pericentric regions (figures 3*b* and 8).

The significance of newt heterochromatin and its possible effects on cell proliferation and rates of organismal growth and development depends on newt ecology. The interaction of these factors is undoubtedly complex. At a very simple level we can imagine that in stable ponds under mild conditions of seasonal temperature shifts and with no (or little) interspecific competition, newt growing seasons will be extended and natural selection on growth rate will be relaxed. Satellite DNA would be expected to increase under such conditions, if our hypothesis is correct. *T. karelinii* may be a good example. It is the largest of all the *cristatus* group, it lives in southeastern Europe, and it has more heterochromatin overall than any other species (figures 2*c*, 7 and 8). On the other hand, if growing seasons are short, temperatures are low, and interspecific competition for resources is high, then rapid somatic and reproductive growth will be an advantage, and selection will constrain or reduce satellite DNA. *T. cristatus* provides a suitable example here because its range extends far into the northernmost parts of Europe, where growing seasons are relatively short, and it is almost always found together with at least one other species of *Triturus* in the same ponds.

Such factors may play an important role in the interaction between sympatric populations of newt species. For example, *T. vulgaris* and *T. cristatus* are broadly sympatric in many parts of Europe, including the British Isles. *T. cristatus* is a faster growing newt than *T. vulgaris*, and may be a major predator on smaller newt species, including *T. vulgaris* (Frazer 1983). Does *T. vulgaris* grow more slowly because overall it has more late-replicating satellite DNA in its genome than *T. cristatus*? One consequence of slow growth in *T. vulgaris* is small larval and adult body size, which makes it the more vulnerable to predation by *T. cristatus*. The latter feed freely on all stages of *vulgaris* larvae and adults. Is *T. vulgaris* trapped, irreversibly burdened by past excesses of 'selfish' DNA? If not, then why does it not grow faster and larger? What prevents *T. cristatus* from exterminating *T. vulgaris*? Is it perhaps that *T. cristatus* is constrained in its population growth by the balanced lethals in its own chromosome 1, which kill off half of all the offspring (Macgregor & Horner 1980)? Or does fast growth and large size give *T. cristatus* an advantage over smaller, slower growing newts that counteracts the reproductive devastation produced by its chromosome 1?

Whatever the answers to these questions, we present them to highlight the need to take careful account of a whole range of factors when attempting to evaluate the possible adaptive significance of satellite DNA and heterochromatin. Is it late replicating? Does its replication *add* to the cell cycle? If so, then what are the consequences likely to be in terms of growth and development, and how might these relate to the ecology of the species? In this field, as in so many others in biology, information gained exclusively at the molecular level is not enough. A strong and carefully planned comparative approach, supported by a 'feeling for the (whole) organism' (McLintock 1983 in Keller 1983) is absolutely essential for reasonable progress.

A mechanism for evolutionary changes in satellite DNA and heterochromatin

We conclude this paper by proposing a model for the mechanism and direction of evolutionary change in satellite DNA and heterochromatin that is based on patterns of variation observed in *Triturus*. In our model, karyotypes experience cycles of growth, dispersal and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

gradual degradation of satellite DNA (figure 9). We propose that satellite DNA tends to accumulate locally through some mechanism that generates tandem duplication. This growth is concentrated in the regions of the centromeres, possibly because late replication is most easily tolerated there. The first step is a burst of growth of satellite which eventually produces a cytologically visible lump of centromeric heterochromatin. Subsequently, small chromosomal

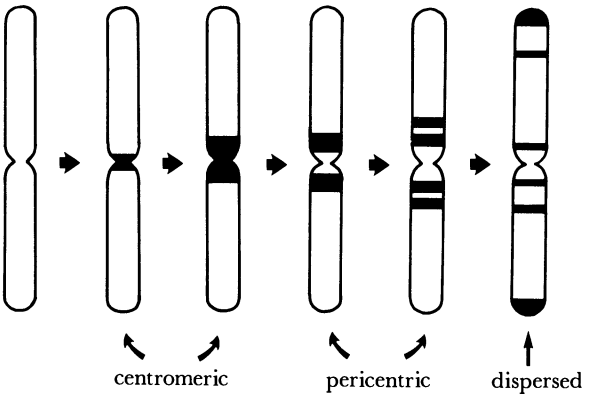

FIGURE 9. Hypothetical model for the localized growth and subsequent dispersal of heterochromatin and satellite DNA in a single chromosome. See text for details.

rearrangements, including inversions and unequal exchanges, occur at random in the vicinity of the centromeres and cause break-up and dispersal of satellite and heterochromatic blocks. This results in the formation of pericentric C-bands at the expense of centromeric C-bands. Further chromosome rearrangements lead to the progressive dispersal of satellite DNA and heterochromatin away from the pericentric regions and out to places in the chromosome arms and to the telomeres. Eventually, particular satellite DNAs will cease to be detectable as heterochromatin because of the break-up and dispersal of their tandem arrays or changes in their sequences. The whole process may happen again and again in rapid succession, one wave overlapping another, or it may happen once over a long period of evolutionary time, involving completely independent satellite families. Dispersed satellite positions may be more stable than centromeric locations; once established, a dispersed bit of satellite can persist, whereas there is faster turnover at the centromeres. In conjunction with these changes in major groups of satellites there may also be numerous, very small satellites (for example, 'minisatellites', Jeffreys *et al.* 1985) that undergo rapid fluctuation at interstitial locations. These satellites represent a kind of background 'noise', and never give rise to the major satellites that grow to enormous proportions and become heterochromatic in centromeric regions.

The model can be tested in newts by examining the patterns of distribution of specific satellites and heterochromatin in living species in a phylogenetic context. Three main patterns are clearly evident. In *Triturus*, heterochromatin is either mainly centromeric, mainly pericentric, or mainly dispersed (interstitial or telomeric) (figures 2 and 3). According to our model, dispersed distributions are 'older' than localized pericentric or centromeric concentrations of heterochromatin. A dispersed pattern is shared by members of two subgenera, *Palaeotriton* and *Mesotriton*, indicating that it is old. A primarily centromeric–pericentric pattern characterizes the relatively young subgenus, *Neotriton*.

Each of these patterns can be demonstrated with reference to a particular known satellite DNA (see table 1).

(i) TkS1 is confined to newts of the *T. cristatus* group and is much more abundant in *T. karelinii* than in any other species. It is therefore a relatively 'young' satellite. It is strictly centromerically localized. It represents around 10% of the genome of *T. karelinii*.

(ii) Tvm1 is represented in varied amounts in all the European *Triturus* species, although it is most abundant in *T. vulgaris*. It is therefore an 'older' satellite than TkS1, and it is located in pericentric C-bands. It represents about 1% of the genome of *T. vulgaris meridionalis* (Andronico *et al.* 1981).

(iii) TcS2 is represented at roughly equivalent levels in all the European newts and in the North American genera *Notophthalmus* and *Taricha*. It follows that it is a very 'old' satellite. Although it is concentrated in the extraordinary heterochromatin of the long arm of chromosome 1, it is dispersed in small amounts in many places throughout the karyotype, none of these identifiable as a C-band. It represents only about 0.1% of the genome (Varley *et al.* 1980; J. G. Gall, personal communication).

(iv) The same principles apply to both Nv1, a young satellite strictly confined to centromeric regions and, oddly enough, to a region immediately adjacent to the histone gene cluster, and to TcS1 which is an old satellite with a widely dispersed distribution, apart from some concentration on the heterochromatic long arm of chromosome 1. Both these satellites represent between 0.5% and 1% of the genome (Varley *et al.* 1980; J. G. Gall, personal communication).

We believe that this model represents the most parsimonious hypothesis of the primary mechanism of evolutionary change in satellite DNA and heterochromatin based on the available data in newts. The simple mechanism proposed could be the basis not only of the observed patterns of variation in heterochromatin and satellite DNA among living species, but also of large evolutionary changes in genome size that involve proportional growth of chromosomes without change in chromosome number or chromosome shape. Such proportional changes in chromosomes with genome size are characteristic of most salamander groups (Mizuno & Macgregor 1974).

We thank Alma Swan and Lesley Barnett for their helpful comments on the text of this paper and for production of the illustrations. We also thank Pim Arntzen, Lise Baldwin, Terry Burke, David Wake, and Graham Wallis for helpful discussion during the preparation of this manuscript. S.K.S. and much of the work reported here were supported by S.E.R.C. grants GR/C 05250 and B/SF/151.

References

Andronico F., Vitelli, L., DeLucchini, S., Serra, V. & Barsacchi, G. 1981 Caratterizzazione e localizzazione cromosomica di un DNA satellite in *Triturus vulgaris meridionalis* (Anfibi, Urodeli). *Atti Ass. Genet. Ital.* **28**, 25–27.

Ayala, F. 1975 Genetic differentiation during the speciation process. *Evol. Biol.* **8**, 1–78.

Baldwin, L. & Macgregor, H. C. 1985 Centromeric satellite DNA in the newt *Triturus cristatus karelinii* and related species: its distribution and transcription on lampbrush chromosomes. *Chromosoma* **92**, 100–107.

Bennett, M. D. 1983 The spatial distribution of chromosomes. In *Proc. Kew Chromosome Conference*, vol. II (ed. G. A. Dover & M. D. Bennett), pp. 71–79. London: G. Allen and Unwin.

Bolkay, S. J. 1928 Die Schadel der Salamandrinen, mit besonderer Rucksicht auf ihre systematische Bedeutung. *Z. Anat. Entwgesch.* **86**, 259–319.

Bucci-Innocenti, S., Ragghianti, M. & Mancino, G. 1983 Investigations of karyology and hybrids in *Triturus boscai* and *T. vittatus*, with a reinterpretation of the species groups within *Triturus* (Caudata; Salamandridae). *Copeia* 1983 (3), 662–672.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Callan, H. G. 1942 Heterochromatin in Triton. *Proc. R. Soc. Lond.* B **130**, 324–335.
Callan, H. G. 1972 Replication of DNA in the chromosomes of eukaryotes. *Proc. R. Soc. Lond.* B **181**, 19–41.
Callan, H. G. & Taylor, J. H. 1968 A radioautographic study of the time course of male meiosis in the newt *Triturus vulgaris*. *J. Cell Sci.* **3**, 615–626.
Collins, J. M. 1978 Rates of DNA synthesis during the S-phase of HeLa cells. *J. biol. Chem.* **253**, 8570–8577.
Diaz, M. O., Barsacchi-Pilone, G., Mahon, K. & Gall, J. G. 1981 Transcripts from both strands of a satellite DNA occur on lampbrush chromosome loops of the newt *Notophthalmus*. *Cell* **24**, 649–659.
Diaz, M. O. & Gall, J. G. 1985 Giant readthrough transcription units at the histone loci on lampbrush chromosomes of the newt *Notophthalmus*. *Chromosoma* (In the press.)
Doolittle, W. F. & Sapienza, C. 1980 Selfish genes, the phenotype paradigm and genome evolution. *Nature, Lond.* **284**, 601–603.
Frazer, D. 1983 *Reptiles and amphibians in Britain*. London: Collins.
Frelow, M., Macgregor, H. C. & Wake, D. B. 1985 Genetic differentiation among newts of the genus *Triturus* in western Europe. (In preparation.)
Heslop-Harrison, J. S. & Bennett, M. D. 1984 Chromosome order – possible implications for development. *J. Embryol. exp. Morph.* **83**, suppl., 51–73.
Highton, R. & Larson, A. 1979 The genetic relationships of the salamanders of the genus *Plethodon*. *Syst. Zool.* **28**, 579–599.
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.
Jeffreys, A., Wilson, V. & Thein, S. L. 1985 Hypervariable 'minisatellite' regions in human DNA. *Nature, Lond.* **314**, 67–73.
John, B. & Miklos, G. L. G. 1979 Functional aspects of satellite DNA and heterochromatin. *Int. Rev. Cytol.* **58**, 1–114.
Kalezic, M. L. & Hedgecock, D. 1980 Genetic variation and differentiation of three common European newts (*Triturus*) in Yugoslavia. *Br. J. Herpetol.* **6**, 49–57.
Keller, E. F. 1983 A feeling for the organism. New York and San Francisco: W. H. Freeman.
Kit, S. 1961 Equilibrium centrifugation in density gradients of DNA preparations from animal tissues. *J. molec. Biol.* **3**, 711–716.
Larson, A. 1984 Neontological inference of evolutionary pattern and process in the salamander family Plethodontidae. *Evol. Biol.* **17**, 119–212.
Lewin, B. 1980 *Gene expression 2*, 2nd edn. Chichester: Wiley and Sons.
Lewin, R. 1982 Repeated DNA still in search of a function. *Science, Wash.* **217**, 621–623.
Macgregor, H. C. 1979 *In situ* hybridization of highly repetitive DNA to chromosomes of *Triturus cristatus*. *Chromosoma* **71**, 57–64.
Macgregor, H. C. & Horner, H. A. 1980 Heteromorphism for chromosome 1, a requirement for normal development in crested newts. *Chromosoma* **76**, 111–122.
Miklos, G. L. G. & Gill, A. C. 1982 Nucleotide sequences of highly repeated DNAs; compilation and comments. *Genet. Res., Camb.* **39**, 1–30.
Mizuno, S. & Macgregor, H. C. 1974 Chromosomes, DNA sequences and evolution in salamanders of the genus *Plethodon*. *Chromosoma* **48**, 239–296.
Oeldorf, E. M., Nishioka, M. & Bachman, K. 1978 Nuclear DNA amounts and development rate in holarctic Anura. *Sondersdruck Z. Zool. System. Evol.* **16**, 216–224.
Orgel, L. E. & Crick, F. H. C. 1980 Selfish DNA: the ultimate parasite. *Nature, Lond.* **284**, 604–607.
Rafinski, J. & Arntzen, J. W. 1985 Biochemical systematics of the Old World newts, genus *Triturus*: isozyme data. (In preparation.)
Ragghianti, N. M., Innocenti, S. B. & Mancino, G. 1973 Bandeggiatura indotta da 'C- e Q-staining methods' e pattern di replicazione dei cromosomi di *Triturus*. *Lincei–Rend. Sci. fis. Mat. Nat.* **55**, 764–770.
Schmid, M. 1980 Cytogenetical aspects of DNA synthesis in the late replicating regions of the human genome. *Genet. Polonica* **21**, 211–220.
Schmid, M., Olert, J. & Klett, C. H. 1979 Chromosome banding in amphibia III sex chromosomes in *Triturus*. *Chromosoma* **71**, 29–55.
Sessions, S. K. 1985 Cytogenetics and evolution in salamanders. Ph.D. dissertation, University of California, Berkeley, U.S.A.
Sims, S. H. 1984 Some aspects of the cytology of European newts (genus *Triturus*). Ph.D. dissertation, University of Leicester, England.
Sims, S., Macgregor, H. C., Pellatt, P. S. & Horner, H. A. 1984 Chromosome 1 in crested and marbled newts (*Triturus*). An extraordinary case of heteromorphism and independent chromosome evolution. *Chromosoma* **89**, 169–185.
Singh, L., Purdom, I. F. & Jones, K. W. 1981 Conserved sex-chromosome-associated nucleotide sequences in eukaryotes. *Cold Spring Harbor Symp. quant. Biol.* **45**, 805–814.
Skinner, D. M. 1977 Satellite DNAs. *BioSci.* **27**, 790–796.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Tone, M., Nakano, N., Takao, E., Narisawa, S. & Mizuno, S. 1982 Demonstration of W chromosome-specific repetitive DNA sequences in the domestic fowl, *Gallus gallus domesticus*. *Chromosoma* **86**, 551–569.

Tone, M., Sakaki, Y., Hashiguchi, T. & Mizuno, S. 1984 Genus specificity and extensive methylation of the W chromosome-specific DNA sequences from the domestic fowl, *Gallus gallus domesticus*. *Chromosoma* **89**, 228–237.

Varley, J. M., Macgregor, H. C., Nardi, I., Andrews, C. & Erba, H. P. 1980 Cytological evidence of transcription of highly repeated DNA sequences during the lampbrush stage in *Triturus cristatus carnifex*. *Chromosoma* **80**, 289–307.

Vig. B. K. 1982 Sequence of centromere separation: role of centromeric heterochromatin. *Genetics* **102**, 795–806.

Wake, D. B., Maxson, L. R. & Wurst, G. Z. 1978 Genetic differentiation, albumin evolution, and their biogeographic implications in plethodontid salamanders of California and southern Europe. *Evolution* **32**, 529–539.

Wallace, H. & Maden, M. 1976 The cell cycle during amphibian limb regeneration. *J. Cell Sci.* **20**, 539–547.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES