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Genome Organization and Species Formation in Vertebrates

Giorgio Bernardi

Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France

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Abstract. Some years ago Wilson and co-workers proposed that the higher rates of karyotypic change and species formation of mammals compared to cold-blooded vertebrates are due to the formation of small demes, as favored by the social structuring and brain development of the former. Here, evidence is reviewed which indicates that mammals are more prone to karyotypic change and species formation than cold-blooded vertebrates because of their different genome organization. Similar evidence has also recently become available for birds. While this different organization appears to be a necessary and, in all likelihood, a sufficient condition for the increased rates of karyotypic change and species formation found in mammals, it is still possible that social structuring and brain development may have played an additional accelerating role.

Key words: Species formation — Karyotypic change rate — Organismic evolution — Isochores — Genomes — Mammals — Birds

Introduction

Sixty years ago, Wright (1931; see also Wright 1940) proposed that adaptive evolution and species formation (an expression to be preferred to speciation; Goldschmidt 1940) take place fastest in those species with a marked propensity for population subdivision or deme formation. Some years later, Goldschmidt (1940) proposed that gene rearrangements may have played a major role in organismal evolution. The latter proposal was subsequently formulated in more detail.

According to White (1968) and Grant (1973), fixation of karyotypic mutations can facilitate species formation and adaptive evolution at the organismal level (see Bush et al. 1977) by acting (1) as a sterility barrier (stasipatric species formation; White 1968, 1978), the mutant karyotype functioning at the population level as a cytogenetic reproductive isolating mechanism; (2) as a regulatory mutation, producing an altered pattern of gene expression that results in an organism with a new and fitter phenotype (see, for example, Zieg et al. 1977); and (3) as a linker of loci that previously were far apart in the genome, thereby creating a particular combination of alleles (Dobzhansky 1970).

These points were reconsidered by Wilson et al. (1974, 1975; Levin and Wilson 1976; Bush et al. 1977) when they found that lower vertebrates exhibit a species formation rate which is, on the average, 20% that of mammals, and a karyotypic change rate which is close to 10% that of mammals. Bush et al. (1977) proposed that "The propensity to form small demes is attributable to several factors, one of which may be especially important for understanding how mammals have achieved remarkably high rates of adaptative evolution. This factor is social structuring. If it were not for this social factor, mammals, because of their high dispersal power, might have evolved at the same rate or more slowly than most lower vertebrates." In more re-

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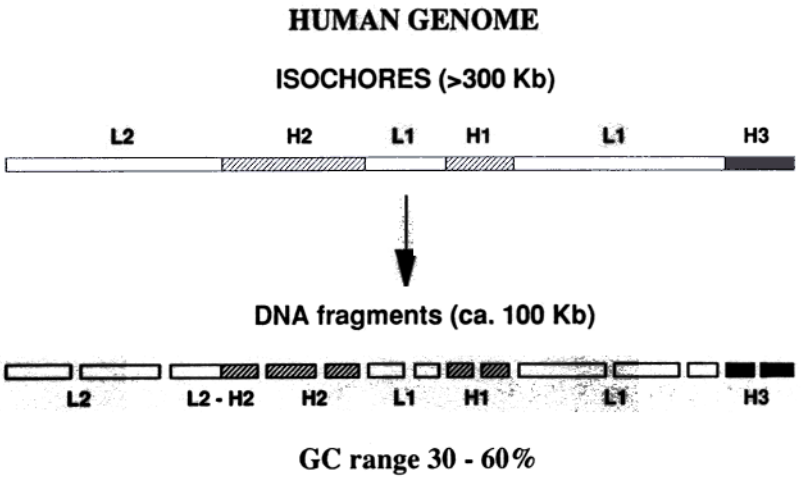

Fig. 1. Scheme of the compositional organization of the genomes from warm-blooded vertebrates. In the example shown, which concerns the human genome, the DNA consists of long (>300 kb, on the average) segments, the isochores, which are compositionally homogeneous (above a size of 3 kb) and belong to a small number of families, GC-poor (*L1* and *L2*), CG-rich (*H1* and *H2*), and very GC-rich (*H3*). Physical and enzymatic degradation occurring during DNA preparation generates large DNA fragments, routinely in a size range of 50–100 kb. (Modified from Bernardi et al. 1985.)

cent work, the importance of brain development in organismic evolution was stressed (Wyles et al. 1983; Wilson 1985).

A convincing demonstration that the differences in species formation rates between mammals and lower vertebrates are due to differences in effective population sizes is, however, still lacking, simply because estimates of N_e (the effective population size) are not available. As far as the effect of brain development on organismic evolution is concerned, it can only be considered as a working hypothesis.

I will propose here a different explanation for the different karyotypic change rate of lower vertebrates and mammals. The basic idea, first mentioned 4 years ago (Bernardi 1989), is that the genomes of mammals are more prone to karyotypic changes than those of cold-blooded vertebrates because of their different organization. The evidence in favor of this explanation will be reviewed. (In fact, similar evidence has also recently become available for birds.) While the genome organization of mammals appears to be a necessary and, in all likelihood, a sufficient condition for their increased rates of karyotypic change and species formation, the factors stressed by Wilson and co-workers may have played an additional accelerating role.

Needless to say, we adhere here to the classical view (see above) that karyotypic change and species formation are closely linked. While this does not apply to all cases of species formation, it appears to do so in the case of vertebrates, as supported by the parallel changes in karyotypic change and species formation.

Genome Organization is Different in Mammals and Cold-blooded Vertebrates

Twenty years ago, we discovered that the bovine genome could be fractionated in a small number of discrete major DNA components characterized by different GC (molar percentage of G + C) levels (Filipski et al. 1973; satellite and minor DNA components, like ribosomal DNA, will be neglected here). Subsequent investigations (Thiery et al. 1976; Macaya et al. 1976) showed (1) that the compositional pattern of the bovine genome (namely, the profile of a plot of relative DNA amount vs GC) was present in other mammals as well; (2) that mammalian genomes consisted of long (>300 kb), heterogeneous DNA segments (which were later called "isochores" for similar regions; Cuny et al. 1981); and (3) that cold-blooded vertebrates exhibited a less heterogeneous compositional pattern compared to mammals.

In mammals, isochores (or, more precisely, the large DNA fragments ca. 100 kb in size derived from them; see Fig. 1) cover a very wide GC range and attain very high GC values. In cold-blooded vertebrates, isochores are much more uniform in composition and never attain the highest GC levels of the genomes of mammals, as shown by the investigations of Hudson et al. (1980) and Bernardi and Bernardi (1990a,b), which confirmed and greatly expanded the initial observations. On the other hand, histograms of GC levels of third-codon positions of genes from mammals are very highly biased toward very high GC levels, the highest mode being located at over 72% GC. In cold-blooded vertebrates, in contrast, the distribution covers a lower GC range and is more symmetrical (Bernardi et al. 1985; 1988; Bernardi and Bernardi 1991). In conclusion, the compositional patterns of mammals and cold-blooded vertebrates are very different, at both the DNA level and the coding sequence level.

Chromosome Organization is Different in Mammals and Cold-blooded Vertebrates

Differences related to those just described exist at the chromosomal level. Indeed, in reviewing pub-

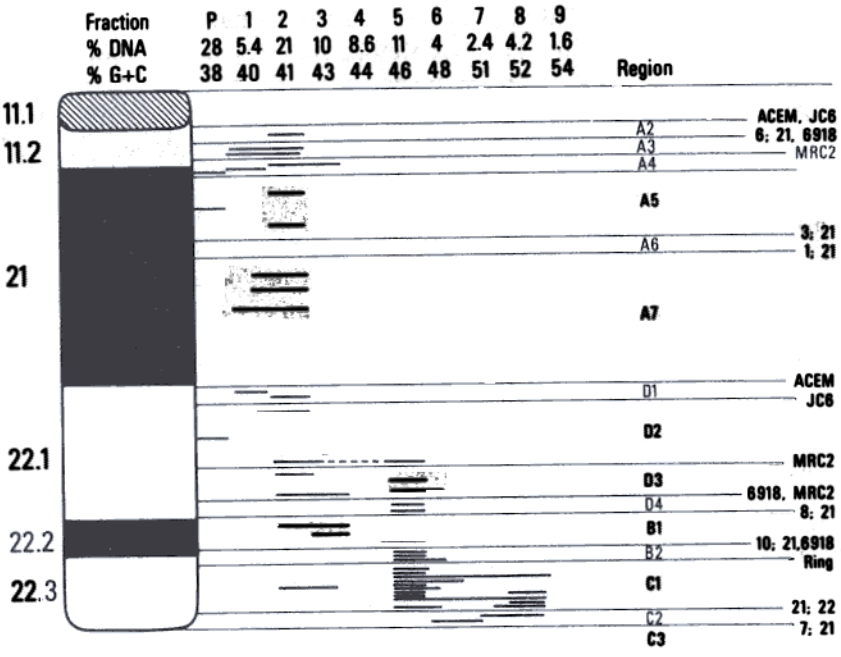

Fig. 2. Compositional map of the long arm of human chromosome 21. *Long horizontal lines* indicate positions of the breakpoints associated with the rearranged chromosomes listed at the right of the figure. *Short horizontal lines* indicate the compositional DNA fractions hybridizing single-copy probes localized in different Giemsa-positive (*dark*) and Giemsa-negative (*light*) or Reverse bands. From Gardiner et al. (1990).

lished and unpublished work by other authors, we noticed that banding patterns of chromosomes from cold-blooded vertebrates were poor or absent, whereas those from warm-blooded vertebrates were strong and distinct (Cuny et al. 1981). We attributed these differences to the fact that GC-poor sequences and GC-rich sequences, as present in the genomes of warm-blooded vertebrates, were responsible for Giemsa positive- and Giemsa-negative (or Reverse) bands, respectively, of metaphase chromosomes, as had been previously suggested on other grounds. This general conclusion has been confirmed since (Medrano et al. 1988; Schmid and Guttenbach 1988; Gardiner et al. 1990; Saccone et al. 1992; Bernardi 1993a).

The correlations between the isochore organization of the genome and the banding patterns of chromosomes have been investigated in further detail by compositional mapping (Bernardi 1989; Gardiner et al. 1990; see Fig. 2). Wherever long-range physical maps are available, compositional maps can be constructed by assessing GC levels around landmarks (localized on the physical maps) that can be probed. This simply requires the hybridization of the probes on DNA fractionated according to base composition. If DNA preparations about 100 kb in size are used, compositional mapping defines the base composition of DNA stretches of about 200 kb around the landmark which was probed (Bernardi 1989).

When this approach was tried for the long arm of chromosome 21, it provided a direct demonstration that G-bands generally are homogeneous in composition and GC-poor, whereas R-bands are compositionally heterogeneous, the highest GC levels (corresponding to the H3 isochore family) being in the telomere-proximal region (Gardiner et al. 1990; see Fig. 2). The latter observation agreed with cytogenetic evidence that telomeres almost always correspond to R-bands and that the terminal regions of 20 of them (including that of the long arm of chromosome 21) are the most denaturation-resistant regions of human chromosomes (Dutrillaux 1973) and the richest in GC, as shown by their chromomycin A3 positivity and DAPI negativity (Ambros and Sumner 1987). This suggested that isochore family H3 might correspond to these denaturation-resistant telomeric regions and to some similar intercalary regions mainly located on chromosomes 1, 3, 11, 15, 19, and 22.

This suggestion (Bernardi 1989; Gardiner et al. 1990) recently received an experimental confirmation by in situ compositional mapping. Chromosome in situ suppression hybridization was carried out to localize on human metaphase chromosomes the GC-richest human isochore family H3 (representing about 3% of the genome), as isolated by preparative equilibrium centrifugation in Cs_2SO_4/BAMD density gradient. [BAMD, 3,6-*bis* (acetato mercuri methyl dioxane) is a sequence-specific DNA ligand; see Bernardi 1989.] This isochore family has the highest gene concentration (Mouchiroud et al. 1991), the highest concentration in CpG islands (Aïssani and Bernardi 1991a,b), the highest transcriptional and recombinational activity (Rynditch et al. 1991), as well as an abundance of open chromatin structures, in which histone H1 is absent, histones H3 and H4 are acetylated, and nucleosomes are more widely spaced (Tazi and Bird 1990; see also Aïssani and Bernardi 1991a,b). The in situ hybridization results obtained showed that sin-

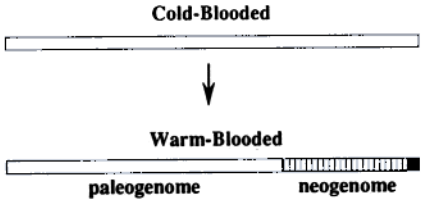

Fig. 3. Scheme of the compositional genome transition accompanying the emergence of warm-blooded from cold-blooded vertebrates. The compositionally homogeneous genomes of cold-blooded vertebrates are changed into the compositionally heterogeneous genomes of warm-blooded vertebrates. The latter comprise a paleogenome (corresponding to about two-thirds of the genome) which did not undergo any large compositional change and a neogenome (corresponding to the remaining third of the genome, with the GC-richest part only representing 3% of the genome). In the scheme, GC-poor isochores (*open bar*), GC-rich isochores (*hatched bar*), and GC-richest isochores (*black bar*) are represented as three contiguous regions, neglecting the mosaic structure of the isochores in the warm-blooded vertebrate genome (see Fig. 1).

gle-copy sequences from the H3 isochore family are localized in the two coincident sets of T-bands and chromomycin bands of human metaphase chromosomes (Saccone et al. 1992) which mainly correspond to about 20 telomeric regions.

Interestingly, these results extend previous observations showing (1) that genes localized in T-bands have much higher GC levels than genes localized in G- or R-bands, the latter being higher than the former (Ikemura and Wada 1991; De Sario et al. 1991); and (2) that telomeric probes corresponding to T-bands hybridize on the GC-richest isochore family H3, whereas telomeric probes corresponding to R-bands hybridize on GC-rich families H1 and H2 (De Sario et al. 1991); in agreement with these findings, the telomeric repeat common to all chromosomes hybridized on isochore families H1, H2 and H3, but not on isochores families L1 and L2.

Rearrangements are Most Frequent at Compositional Discontinuities

The results discussed above show that the genomes of mammals differ compositionally from those of cold-blooded vertebrates in that about one-third of the mammalian genome underwent GC increases, whereas the remaining two-thirds did not. These two compartments were called the neogenome and the paleogenome, respectively (Bernardi 1989; see Fig. 3). Such changes took place through the fixation of directional point mutations, as shown by sequence comparisons of homologous genes from warm- and cold-blooded vertebrates (Perrin and Bernardi 1987; Bernardi et al. 1988; Bernardi and Bernardi 1991).

The crucial point now is that recombinational events are much more frequent in the neogenome than in the paleogenome of mammals or, in all likelihood, than in the genomes of cold-blooded vertebrates which correspond to the paleogenome of warm-blooded vertebrates and are similar to it both at the DNA and at the chromosomal level.

Detailed information, mainly concerning the human genome, shows that translocation breakpoints are not randomly located on chromosomes (Sutherland and Hecht 1985; Sutherland 1991; see also Fig. 2). Indeed, R-bands and G/R borders are the predominant sites of exchange processes, including spontaneous translocations, spontaneous and induced sister-chromatid exchanges, and the chromosomal abnormalities seen after X-ray and chemical damage. They also include the *hot spots* for the occurrence of mitotic chiasmata (Hecht 1988; Kuhn and Therman 1986; Morgan and Crossen 1977; Sutherland and Hecht 1985). Likewise, fragile sties tend to be more frequent in R-bands or near the border of R- and G-bands (Aurias et al. 1978; Yunis and Soreng 1984; Hecht 1988). It has been suggested that fragile sites could be the points at which chromosomes break to form rearrangements (Hecht and Hecht 1984a,b). These observations indicate that R-bands and G/R borders are particularly prone to recombination and suggest that these phenomena are associated with the compositional discontinuities at G/R borders and within R bands, as well as with the genomic distribution of repeated sequences. Cancer-associated chromosomal aberrations are also nonrandom, with a limited number of genomic sites consistently involved and frequently associated with cellular oncogenes and fragile sites (Mitelman and Heim 1988). Incidentally, chromosomal rearrangements may have as an important consequence the activation of oncogenes by strong promotors that have been put upstream of them by the rearrangement (Klein 1983).

At this point, the question should be raised as to why recombination phenomena are so frequent in the neogenome of warm-blooded vertebrates. The high GC level *per se* is unlikely to be responsible because, although rare, genomes which are relatively high in GC (but in a uniform way) are also found among cold-blooded vertebrates (Bernardi and Bernardi 1990a,b). What seems to matter is, therefore, the presence of compositional discontinuities which exist not only at the borders of G- and R- (or T-) bands, but also within R-bands (Gardiner et al. 1990). The latter are due to the presence of thin G-bands (which can be detected at high resolution; Yunis 1981) and also to the compositional heterogeneity of the isochores (from H1 and H2 families) present in R-bands.

Concerning the molecular basis for the associa-

tion between chromosome breakpoints and compositional discontinuities, one possibility is that chromatin structure is more "open" at compositional discontinuities (as was shown to be the case for CpG islands and the very GC-rich genes which are associated with them; Tazi and Bird 1990; see also Aïssani and Bernardi 1991a,b), so allowing recombination to take place, possibly using the many repeated sequences concentrated in those regions. Indeed, the regions which are most susceptible to recombination correspond to high concentrations of Alu sequences (Zerial et al. 1986), CpG islands (Aïssani and Bernardi 1991a,b), and minisatellites (Jeffreys et al. 1985).

Conclusion

In conclusion, what is proposed here is that the two major compositional shifts which occurred in the genomes of vertebrates and which led to the formation of the compositionally compartmentalized genomes of birds and mammals also led to a remarkable degree of instability and to a propensity for karyotypic change and species formation.

This conclusion deserves several comments. The first one is that there is no contradiction between (1) the proposal (Bernardi and Bernardi 1986; Bernardi et al. 1988; Bernardi 1993b) that an increased thermodynamic stability of DNA, RNA, and proteins in warm-blooded vertebrates was the reason (i.e., the selective advantage) for the directional fixation of mutations which led to the formation of GC-rich isochores and (2) the genetic instability which was a consequence of it. Indeed, the latter can be visualized as the cost to be paid for satisfying the inescapable requirements of the former. In fact, genetic instability also provided a positive contribution to the evolution of warm-blooded vertebrates (see below). Likewise, the expected increased incidence of cancers associated with chromosomal translocations in mammals and birds compared to cold-blooded vertebrates might also be considered as the price to be paid to evolve faster.

The second comment concerns the possible correlation between the formation of compositionally compartmentalized genomes prone to karyotypic changes and mammalian radiation. It has been proposed (Crompton et al. 1978; see also Carroll 1987) that the body temperature of early mammals may have been lower than even that of the most primitive of living mammals, the monotremes, which have a temperature of only 30–32°C, and that mammalian homeothermy was acquired in two steps. The first step enabled mammals to invade a nocturnal niche without an increase in resting metabolic rate. The second step enabled them to invade a diurnal niche and involved the acquisition of higher body temperature and metabolic rates. Modern mammals require approximately 10 times more food and oxygen than do reptiles of comparable size. The higher metabolic rate allows mammals to be active more continuously and to maintain a high, constant body temperature that is independent of the environment. Such a radical difference in metabolic rate affects nearly all the systems of the body and is responsible, directly or indirectly, for nearly all the differences observed between reptiles (be they modern or Paleozoic) and mammals (Carroll 1987). Incidentally, these considerations suggest that the compositional genome transition under consideration may have taken a very long time, from the appearance of early mammals to that of present-day mammals.

If one takes into consideration the proposed link between body temperature and genome compartmentalization (Bernardi and Bernardi 1986; Bernardi et al. 1988; Bernardi 1993b), the stepwise increase in body temperature may suggest that the genome compartmentalization developed slowly in early mammals (which appeared over 220 Myr ago and remained in a nocturnal insectivorous niche for a very long time) and in the ancestors of present-day mammals. If such was the case, the strong compositional compartmentalization was simultaneous with the mammalian radiation of about 65 Myr ago and possibly played a causative role.

The third comment is that the main proposal made here is further supported by the following recent observations. Avian genomes have a higher compositional heterogeneity than mammalian genomes, and third-codon positions of avian genes reach 100% GC (F. Kadi, D. Mouchiroud, and G. Bernardi, in preparation). This very large compositional heterogeneity is accompanied by an extremely high speciation rate. Indeed, while some 9,000 species of birds exist today (a number roughly double of that of mammalian species), it has been estimated that about 150,000 species of birds have existed (P. Brodkorb, quoted by Sibley and Ahlquist 1986). It should be mentioned here that, while chromosomal repatterning (mainly chromosomal inversions and microchromosome fusions) are well known in birds (see Christidis 1990), the above proposal is in disagreement with the view that "allopatric speciation is clearly the dominant mode in birds" (Sibley and Ahlquist 1990), a view also contradicted by the widespread hybridization of bird species (Grant and Grant 1992).

Finally, while we do not believe that "if it were not for social structuring, mammals, because of their high dispersal power, might have evolved at the same rate or more slowly than most cold-blooded vertebrate" (Bush et al. 1977), we do not rule out that social structuring and brain develop-

ment may have played an additional accelerating role. Moreover, we agree that species formation in the virtual absence of chromosomal evolution probably mainly occurred according to the classical allopatric theory, as when a species becomes divided by a geographic barrier into two large populations (Bush et al. 1977).

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