commune: apport de l'étude du polymorphisme des locus protéiques et microsatellites. *Génét. Sélect. Évol.* 26 (Suppl. 1):183.
SNPRCN (Serviço Nacional de Parques, Reservas e Conservação da Natureza) (1991). *Livro Vermelho dos Vertebrados de Portugal, Vol. II. Peixes Dulciaquícolas e Migradores*, Lisboa.
Raymond, M., and Rousset, F. (1995). Population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248.
Takezaki, N., and Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144:389.
Teisberg, P. (1970). High voltage agarose gel electrophoresis in the study of C_3 polymorphism. *Vox Sang.* 19:47.
Utter, F. M., and Hodgins, H. O. (1971). Biochemical polymorphisms in the Pacific hake (*Merluccius productus*). *Conseil Int. Explor. Mer. Rapports procès verb.* 161:87.
Weir, B. S., and Cockerham, C. C. (1984). Estimating *F*-statistic for the analysis of population structure. *Evolution* 38:1358.
Weiss, S., Antunes, A., Schlötterer, C., and Alexandrino, P. (2000). Mitochondrial haplotype diversity among Portuguese brown trout *Salmo trutta* L. populations: Relevance to the post-Pleistocene recolonization of northern Europe. *Mol. Ecol.* 9:691.

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Telomeres in Warm-Blooded Vertebrates Are Composed of GC-Rich Isochores

P. Perani,[1,3] S. Cacciò,[1] S. Saccone,[2] L. Andreozzi,[2] and G. Bernardi[1]

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We have hybridized the vertebrate telomeric sequence (TTAGGG)n on DNA compositional fractions from 13 mammalian species and 3 avian species, representing 9 and 3 orders, respectively. Our results indicate that the 50- to 100-kb fragments derived from telomeric regions are composed of GC-rich and GC-richest isochores. Previous works from our laboratory demonstrated that single-copy sequences from the human H3 isochore family (the GC-richest and gene-richest isochore in the human genome) share homology with compositionally correlated compartments of warm-blooded vertebrates. This correlation suggested that the GC-richest isochores are, as in the human genome, the gene-richest regions of warm-blooded vertebrates' genome. Moreover, this evidence suggests that telomeric regions are the most gene-dense region of all warm-blooded vertebrates. The implications of these findings are discussed.

KEY WORDS: isochores; GC composition; telomeres; warm-blooded vertebrates.

INTRODUCTION

Vertebrate genomes are mosaics of isochores, namely, of long, compositionally homogeneous DNA segments that can be subdivided into a small number of families characterized by different GC contents (Bernardi, 1989, 2000). In the human genome, which is by far the most extensively studied, the compositional range of isochores is 30–60% GC. Five families of isochores have been identified in this genome: two GC-poor families, L1 and L2, representing together

[1] Laboratoire de Génétique Moléculaire, Université de Paris VII, 2 Place Jussieu, 75005 Paris, France.
[2] Dipartimento di Biologia Animale, Università di Catania, via Androne 81, 95125 Catania, Italy.
[3] To whom correspondence should be addressed. Fax: +33 1 40 50 01 95.

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62% of the genome; and three GC-rich families, H1, H2, and H3, representing 22, 9, and 3–5% of the genome, respectively (the remaining DNA is formed by satellite and ribosomal DNAs).

A striking feature of the human genome is that the gene concentration is extremely nonuniform and parallels GC levels (Bernardi *et al.*, 1985; Mouchiroud *et al.*, 1991). The gene concentration is low and constant over GC-poor isochore families L1 and L2; it increases over GC-rich isochores and reaches the highest level, about 20 times that of the GC-poor isochores, in the GC-richest isochore family H3. This family has the highest CpG island concentration, as well as the highest transcriptional and recombinational activities (Aïssani and Bernardi, 1991; Rynditch *et al.*, 1991), and is also largely endowed with a distinct, open chromatin structure (Bernardi, 1993).

This genome organization appears to be conserved in warm-blooded vertebrates. In fact, the majority of the mammalian and the totality of the avian genomes that have been studied at the level of large DNA (Kadi *et al.*, 1994; Sabeur *et al.*, 1993) displayed an isochore pattern that is similar to that described for the human genome, that is, a pattern characterized by the presence of sizable amounts (6–10%) of very GC-rich isochores (operationally defined as main-band DNA with a buoyant density higher than 1.710 g/cm^3).

We have hybridized, under conditions in which repetitive sequences are competed out, the human H3 isochores on DNA compositional fractions of DNAs from 13 mammalian and 3 avian species. The probe hybridized with the GC-richest isochores of the genome of all the species studied (Cacciò *et al.*, 1994). In all likelihood highly conserved single-copy sequences (that is, coding sequences) are responsible for the hybridization, since the noncoding single-copy sequences are divergent enough not to contribute to the observed patterns. These experiments confirmed results obtained by comparison of isochore patterns (Sabeur *et al.*, 1993) and added further evidence to the fact that GC-richest isochores are also the gene-richest part of the genome of the warm-blooded vertebrates (De-Sario *et al.*, 1991; Ikemura *et al.*, 1990).

In situ hybridization experiments on human metaphase chromosomes showed that the H3 isochore family is located prevalently in telomeric bands, adding further evidence to the fact that the gene-richest regions are the telomeric regions (Saccone *et al.*, 1992).

Our present goal is to extend the correlation found at the genome level in warm-blooded vertebrates. For this reason we addressed the question of the compositional properties of the telomeric regions by means of hybridization of the vertebrate telomeric repeat on compositional DNA fractions of DNAs from warm-blooded vertebrates. The hybridization patterns observed are compatible with H3 isochore families in warm-blooded vertebrates having a prevalent telomeric localization.

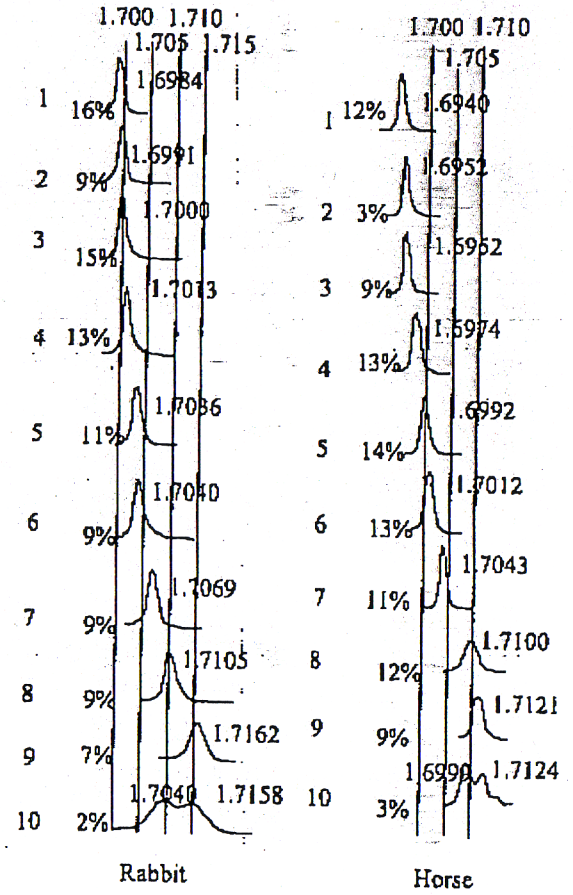

Fig. 1. Analytical CsCl profiles of fractions from a preparative Cs_2SO_4/BAMD density gradient from rabbit and horse. The relative amount of DNA and the modal buoyant density of the peaks are indicated.

MATERIALS AND METHODS

DNA Fractionation and Analysis

The compositional DNA fractions of mammalian and avian species used in this work were previously described by Cacciò *et al.* (1994). In Fig. 1 are presented two newly prepared gradients from horse and rabbit.

Southern and Slot-Blot Preparation and Hybridization

One microgram of each DNA fraction was digested overnight with *Eco*RI (Promega) at 37°C and at a final concentration of 10 units of enzyme per mg of DNA. The digested fractions were electrophoresed on an 0.7% agarose gel for 6–8 hr and alkaline transferred on a Bio-Rad membrane. Hybridizations were performed according to a standard procedure (Sambrook *et al.*, 1989). Filters were washed for 20 min at room temperature in 2× standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), and then for 30 min in 0.1 × SSC, 0.1% SDS at 68°C. Exposure times for autoradiography were 48–72 hr at −70°C.

The probe used was pHuR93, which contains 240 bp of the telomeric tandem repeat (Meyne *et al.*, 1990) and was purchased from the American Type Culture Collection; the probe was labeled by random-priming, using α-dCTP^{32}, to a specific activity of 10^8 cpm/mg.

The slot-blot was prepared as follows: each DNA fraction (0.5 mg) was transferred to a Bio-Rad membrane using a slot-blot apparatus (Bio-Rad) according to the manufacturer's instructions. The filters were hybridized and washed as described above.

RESULTS

Localization of the Telomeric Universal Repeat on Fractionated Mammalian DNAs

Figures 2 to 7 display the hybridization patterns obtained using the vertebrate telomeric sequence, $(TTAGGG)_n$, as a probe on compositional DNA fractions from 13 mammalian species and 3 avian species. In our exposition, we follow the same order as in Sabeur *et al.* (1993). Rabbit, mouse, horse, and cat DNAs were analyzed by Southern hybridization (Figs. 3, 4, 6, and 7, respectively).

In the case of the *shrew* the strongest hybridization signal was in fraction 7 (1.7107 g/cm^3); fractions 6 (1.7041 g/cm^3) and 8 (1.7108 g/cm^3) showed weaker signals (Fig. 2). Although the signals in fractions 6 and 8 have the same intensity, there is a great difference in the genomic percentages that these fractions represent. In fact while fraction 8 represents only 2%, fraction 6 represents 23.8% of the shrew genome (11 times more). As the DNA loads on the filter are identical for all the fractions, the signal of fraction 8 is, therefore, "enriched" almost 11-fold relative to fraction 6. Therefore the telomeric repeat is localized in compositional fractions of the shrew genome having a modal buoyant density between 1.7041 (density of fraction 6) and 1.7108 cm^3/ml (density of fraction 8) (Fig. 8). This result coincides with the H3 cross-hybridization experience, which shows a peak of hybridization centered at 1.708 g/cm^3 (Cacciò *et al.*, 1994).

The *fruit bat* genome showed hybridization signals in fractions 6 (1.7044 g/cm^3) and, much more so, in fraction 7 (1.7117 g/cm^3) (Fig. 2). As in the case of the shrew, fraction 8 is not considered (being only 3.9% of the fruit bat genome). Fraction 7 contained satellite DNAs together with main-band DNA (for discussion see (Cacciò *et al.*, 1994; Sabeur *et al.*, 1993). The hybridization density range of the telomeric probe is from 1.7044 to 1.708 g/cm^3 (Fig. 8). The presence of this main-band DNA component in fraction 7 is confirmed by both present experience and previous experience with H3 cross-hybridization (Cacciò *et al.*, 1994).

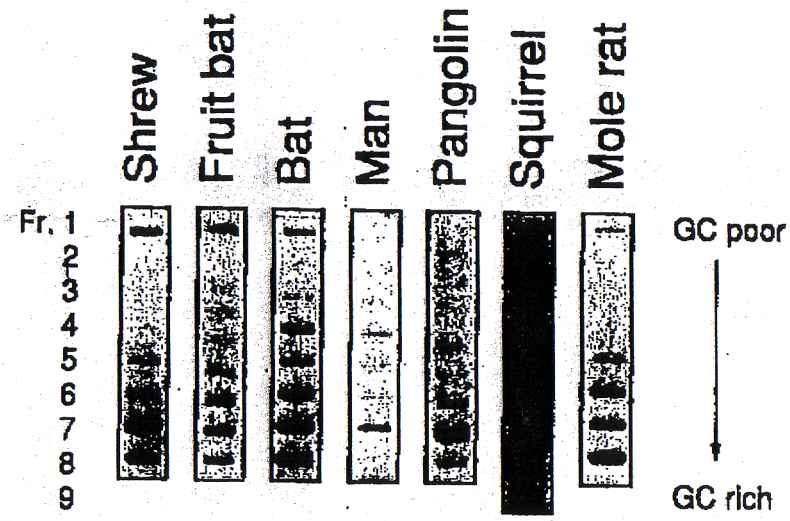

Fig. 2. Slot-blot hybridization patterns obtained using the vertebrate telomeric repeat $(TTAGGG)_n$ as a probe. Species are indicated at the top of each line. The modal buoyant density of the fractions increases from the top to the bottom of the line, as indicated at the right. The fraction numbers are indicated at the left; fraction 1 always corresponds to the pellet.

The *bat* genome showed strong hybridization signals in fractions 7 and 8 (1.7123 and 1.7146 g/cm^3, respectively) (Fig. 2). Fractions 4, 5, and 6 (densities of 1.7014, 1.7034, and 1.7075 g/cm^3, respectively) displayed less intense signals. The hybridization density range is therefore from 1.7014 to 1.7146 g/cm^3 (Fig. 8).

Telomeric probes hybridize in *human* DNA from fraction 4 to fraction 7 (Fig. 2) (with a buoyant density range between 1.7017 and 1.7136 g/cm^3) (Fig. 8). This range is largely comparable with that (1.7021–1.7145 g/cm^3) already reported by De Sario *et al.* (1991).

Pangolin DNA showed a strong signal in fraction 7 (1.7090 g/cm^3) and weak signals in fraction 6 (1.7065 g/cm^3) and fraction 8, whose modal buoyant

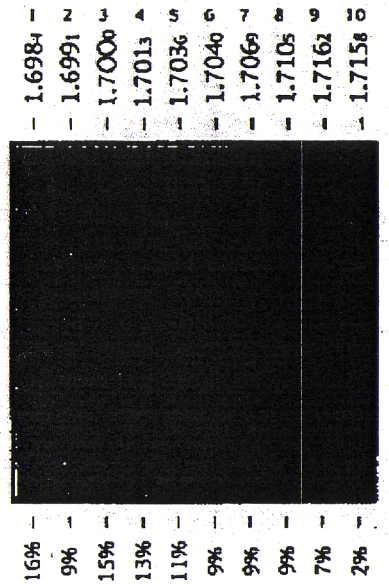

Fig. 3. Southern blot hybridization pattern of rabbit genomic fractionations using the telomeric repeat $(TTAGGG)_n$ as a probe. The modal buoyant density and the number of each fraction are indicated at the top. The relative genomic amount of each fraction is indicated at the bottom.

density of the major band is 1.7099 g/cm^3 as estimated by Sabeur *et al.* (1993) (Fig. 2). Therefore the hybridization density range is from 1.7065 (fraction 6) to 1.7099 g/cm^3 (Fig. 8). Sabeur *et al.* estimated fraction 7 to be composed of satellite DNA but H3 cross-hybridization experiments revealed the presence of main-band DNA. This is further confirmed by the present experiment.

Analysis of the *rabbit* Southern blot (Fig. 3) shows weak hybridization in fractions 7 (1.7069 g/cm^3) and 8 (1.7105 g/cm^3) and very strong hybridization in fraction 9 (1.7162 g/cm^3). For the last fraction the estimation of main band DNA density is difficult. Probably a component of 1.713 g/cm^3 is hidden by the huge satellites present in this fraction. Accordingly the estimated density range of the hybridization is 1.7069 to 1.713 g/cm^3 (this uncertainty about the upper limit of the density range is shown as a dashed line in Fig. 8).

Squirrel DNA showed hybridization in fractions 6 and 7 (densities of 1.7043 and 1.7094 g/cm^3, respectively) and fractions 8 and 9, with the highest intensity in fraction 8 (Fig. 2). The density of the main band DNA in fractions 8 and 9 is difficult to evaluate because of the presence of a huge satellite. Sabeur *et al.* (1993) considered a value of 1.737 g/cm^3 for the main-band component of fraction 8. The same authors considered fraction 9 to be composed of three components: two satellite DNAs (densities of 1.7004 and 1.7187 g/cm^3) and the main-band component, with a density of 1.7150 g/cm^3. We should thus consider

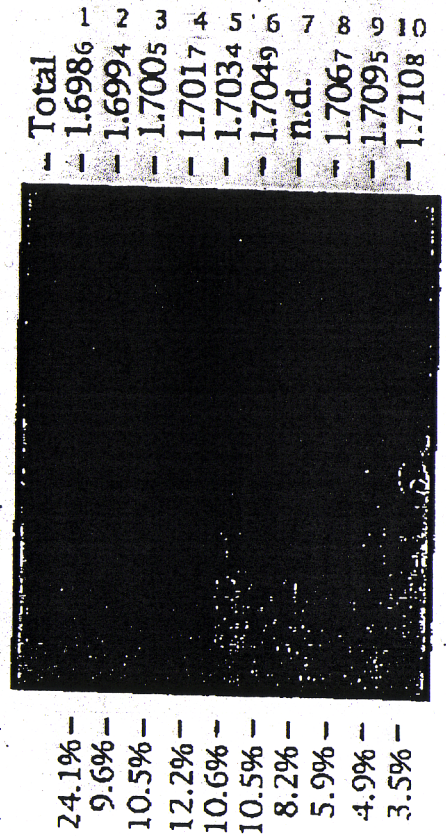

Fig. 4. Southern blot hybridization pattern of mouse genomic fractionations using the telomeric repeat $(TTAGGG)_n$ as a probe. Fraction 7 of the mouse gradient was not determined. The modal buoyant density and the number of each fraction are indicated at the top. Total: unfractionated genomic DNA. The relative genomic amount of each fraction is indicated at the bottom.

that the hybridization density range of the telomeric probe is from 1.7043 to 1.7150 g/cm^3 (Fig. 8).

Mole rat DNA showed hybridization in fractions 6, 7, and 8 (densities of 1.7064, 1.7097, and 1.7104 g/cm^3) (Fig. 2); therefore the hybridization range is from 1.7064 to 1.7104 g/cm^3 (Fig. 8).

Mouse DNA showed, as analyzed by Southern hybridization, a smear in fraction 10 (Fig. 4); there is also an intense signal in the pellet, probably due to contamination of the pellet with some GC-rich isochores, which occurred during the collection of the fraction. Faint bands are seen in fractions 7, 8, and 9 at high molecular weights; this is probably due to the presence in these fractions of telemetric repeats physically linked to mouse minor satellite repeats that do not contain *Eco*RI sites (and therefore produce discrete bands) (Kipling *et al.*, 1991).

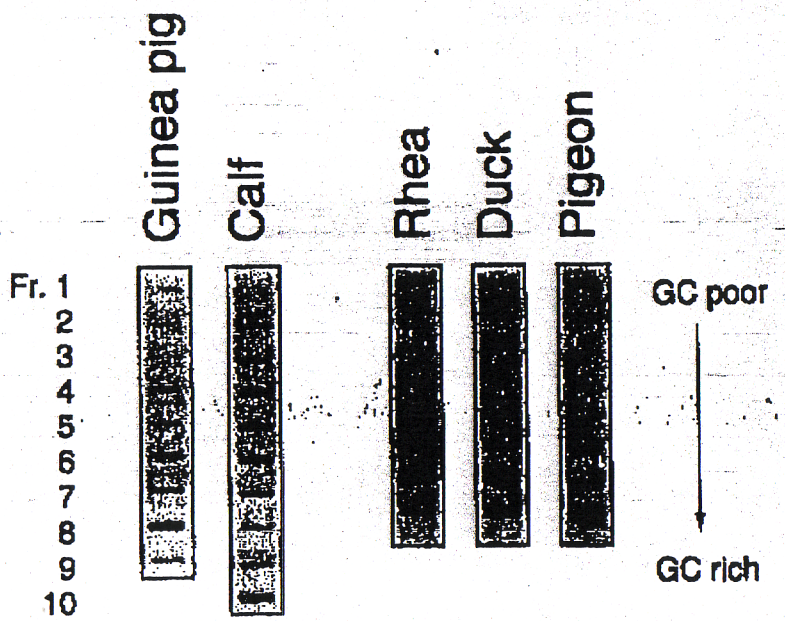

Fig. 5. Slot blot hybridization patterns obtained using the vertebrate telomeric repeat (TTAGGG)n as a probe. Species are indicated at the top of each line. The modal buoyant density of the fractions increases from the top to the bottom, as indicated at the right. The fraction numbers are indicated at the left; fraction 1 always corresponds to the pellet.

The main-band density hybridizing with the telomeric probe is 1.7108 g/cm^3 (fraction 10) (Fig. 8).

Guinea pig DNA showed hybridization in fractions 7 and 8 (Fig. 5) that contains satellite DNAs. Sabeur *et al's.* (1993) estimations of the main-bands DNAs densities are 1.7112 and 1.7130 g/cm^3, respectively. The hybridization range is therefore from 1.7112 to 1.7130 g/cm^3 (Fig. 8).

Calf DNA showed hybridization in fractions 4 (1.7043 g/cm^3), 5 (1.7076 g/cm^3), 6 (1.7107 g/cm^3), 7 (1.7110 g/cm^3), and 8 (whose main-band DNA density is difficult to estimate because of the very large amount of satellite DNA), with the highest intensity in fraction 6. Our estimation of the main-band DNA density of fraction 8 is 1.712 g/cm^3. This is because the increase in the main-band DNA density from fraction 6 to 7 is ~1 mg. The increase is theoretically to be kept constant passing from fraction 7 to fraction 8, thus giving a value of 1.711 ± 0.001 g/cm^3. The hybridization range is therefore from 1.7043 to 1.712 g/cm^3 (this uncertainty about the upper limit is given as a dashed line in Fig. 8).

Horse fractionation analyzed by Southern hybridization shows strong signals with fraction 9 (Fig. 6) and, to a lesser extent, fractions 6, 7, and 8. Fractions 8 and 9 contain a small amount of satellite DNA (Fig. 1). The hybridization range of the telomeric probe is therefore from 1.7043 (fraction 6 is not considered being too weak) to 1.7121 g/cm^3 (Fig. 8).

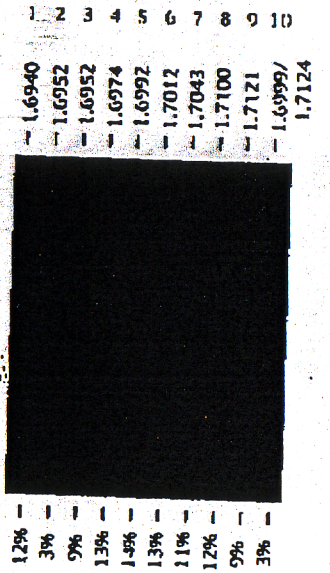

Fig. 6. Southern blot hybridization pattern of horse genomic fractionations using the telomeric repeat $(TTAGGG)_n$ as a probe. The modal buoyant density and the number of each fraction are indicated at the top. The relative genomic amount of each fraction is indicated at the bottom.

The *cat* shows, in the Southern blot, a hybridization signal localized in fraction 8 (Fig. 7). This fraction is composed mainly of satellite DNA but a main-band component should be present. The estimation of the main band DNA component of fraction 8 is 1.710 g/cm^3. The hybridization range of the telomeric probe is therefore located around this value (Fig. 8).

Localization of the Telomeric Universal Repeat on Fractionated Avian DNAs

Rhea DNA showed hybridization in fractions 5 (1.7058 g/cm^3), 6 (1.7120 g/cm^3), and 7 (1.7172 g/cm^3) (Fig. 5). Fraction 7 is composed mainly of satellite DNA. Cacciò *et al.* estimated the main band component of this fraction to be 1.712. Therefore the hybridization signals should range from 1.7058 to 1.712 g/cm^3 (Fig. 8).

Duck DNA showed the strongest hybridization signal in fractions 6 (1.7043 g/cm^3), 7 (1.7094 g/cm^3), and 8, which shows a broad profile. Kadi *et al.* (1994)

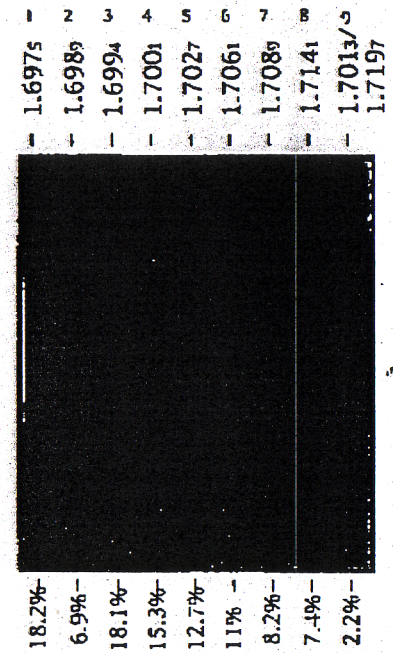

Fig. 7. Southern blot hybridization pattern of cat genomic fractionations using the telomeric repeat $(TTAGGG)_n$ as a probe. The modal buoyant density and the number of each fraction are indicated at the top. The relative genomic amount of each fraction is indicated at the bottom.

estimated the main-band density of fraction 8 to be 1.7109 g/cm^3. The range of the hybridization signal is therefore from 1.7043 to 1.7109 g/cm^3.

The case of the *pigeon* is similar to that of the duck because it shows a signal in fractions 6 (1.7051 g/cm^3), 7 (1.7097 g/cm^3), and 8. Fraction 8 contains a very small amount of main-band DNA (whose density is difficult to estimate). The peak of the hybridization signal ranges from 1.7051 to a value higher than 1.710 g/cm^3. This lack of confidence is indicated by dashed lines in Fig. 8.

Figure 8 presents a scheme of the estimated buoyant density of main-band DNA components from the genomes of the warm-blooded vertebrates investigated here that hybridize with the telomeric repeat.

DISCUSSION

We reported the use of hybridization techniques in the comparative analysis of the isochore organization in the genomes of warm-blooded vertebrates (Cacciò *et al.*, 1994). Our results further confirmed that the single-copy sequences present in the H3 isochore families are compositionally correlated among different

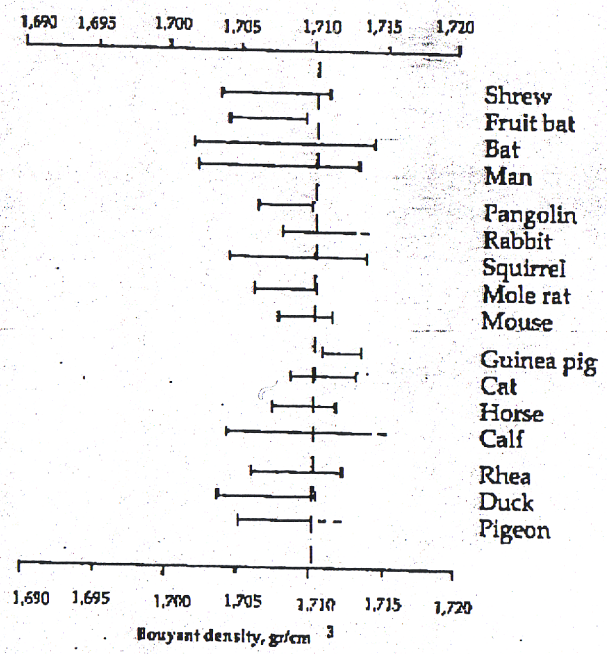

Fig. 8. Schematic representation of the hybridization patterns observed. Horizontal dashed lines represent the incertitude in the evaluation of the buoyant density (see text).

mammalian and avian species, in agreement with data obtained at the level of large DNA fragments (Kadi *et al.*, 1994; Sabeur *et al.*, 1993).

In the human genome, the H3 isochore family is located mainly in telomeric bands, as revealed by *in situ* hybridization of the H3 isochore on metaphase chromosomes (Saccone *et al.*, 1992, 1993). A complementary approach, hybridization of the telomeric sequence to human compositional DNA fractions, demonstrated that telomeric regions are formed by GC-rich and very GC-rich isochores (our data and De Sario *et al.*, 1991). Moreover, telomeric regions in man are the gene-richest regions, as revealed by the analysis of gene distribution [see Bernardi (2000) for a recent review] and by data from *in situ* hybridization at a high resolution (Craig and Bickmore, 1993; De Sario *et al.*, 1991; Ikemura *et al.*, 1990). All this evidence prompted our analysis of the correlation between telomeric regions and isochores in warm-blooded vertebrates, which is the subject of this report.

We have investigated the isochore structure at telomeric regions by hybridizing the vertebrate telomeric sequence, $(TTAGGG)_n$, on compositional DNA fractions from several mammalian and avian genomes; Figs. 2 through 7 display the hybrid-

ization patterns obtained using the telomeric probe. All the signals are located in the GC-rich and GC-richest isochores of the investigated species (see Fig. 8).

We interpreted those results as evidence in favor of a common chromosomal organization: in warm-blooded vertebrates, not only are the GC-richest isochore families correlated in terms of the sequences that they harbor, but also they occupy telomeric regions. Direct evidence in favor of this proposal has been obtained by *in situ* hybridization on mouse chromosomes using the GC-richest murine isochore as probe (Saccone *et al.*, 1997). This experiment shows that this isochore is located at both telomeric and intercalated R-bands, with a distribution pattern close to that described for the human genome (Saccone *et al.*, 1992). Furthermore, other authors have noted that human intercalate T-bands often correspond to terminal bands of other mammals, and vice versa (papers cited by Ikemura *et al.*, 1990).

In eukaryotic organisms, telomeres are responsible for essential functions, such as preservation of chromosome integrity and complete DNA replication at chromosome ends (Biessmann and Mason, 1992). Those are probably common telomeric functions in eukaryotes. Other evidence suggested that telomeres are also involved in the shaping of nuclear architecture, for example, by tight association with the nuclear matrix (de Lange, 1992). Our results show that, in warm-blooded vertebrates, telomeres could also be the gene-richest regions. The clustering of genes at specific positions along the ends of vertebrate chromosomes could have important functional reasons.

REFERENCES

Aïssani, B., and Bernardi, G. (1991). CpG islands: Features and distribution in the genome of vertebrates. *Gene* 106:173.

Bernardi, G. (1989). The isochore organization of the human genome. *Annu. Rev. Genet.* 23:637.

Bernardi, G. (1993). The vertebrate genome: Isochores and evolution. *Mol. Biol. Evol.* 10:186.

Bernardi, G. (2000). Isochores and the evolutionary genomics of vertebrates. *Gene* 241:3.

Bernardi, G., Olofsson, B., Filipski, J., Zerial, M., Salinas, J., Cuny, G., Meunier-Rotival, M., and Rodier, F. (1985). The mosaic genome of warm-blooded vertebrates. *Science* 228:953.

Biessmann, H., and Mason, J. M. (1992). Genetics and molecular biology of telomeres. *Adv. Genet.* 30:185.

Cacciò, S., Perani, P., Saccone, S., Kadi, F., and Bernardi, G. (1994). Single-copy sequence homology among the GC-richest isochores of the genomes from warm-blooded vertebrates. *J. Mol. Evol.* 39:331.

Craig, J. M., and Bickmore, W. A. (1993). Chromosome bands—flavours to savour. *Bioessays* 15:349.

de Lange, T. (1992). Human telomeres are attached to the nuclear matrix. *EMBO J.* 11:717.

De Sario, A., Aïssani, B., and Bernardi, G. (1991). Compositional properties of telomeric regions from human chromosomes. *FEBS Lett.* 295:22.

Ikemura, T., Wada, K., and Aota, S. (1990). Giant G + C% mosaic structures of the human genome found by arrangement of GenBank human DNA sequences according to genetic positions. *Genomics* 8:207.

Kadi, F., Mouchiroud, D., Sabeur, G., and Bernardi, G. (1994). The compositional patterns of the avian genomes and their evolutionary implications. *J. Mol. Evol.* ●●●.

Kipling, D., Ackford, H. E., Taylor, B. A., and Cooke, H. J. (1991). Mouse minor satellite DNA genetically maps to the centromere and is physically linked to the proximal telomere. *Genomics* 11:235.

Meyne, J., Baker, R. J., Hobart, H. H., Hsu, T. C., Ryder, O. A., Ward, O. G., Wiley, J. E., Wurster-Hill, D. H., Yates, T. L., and Moyzis, R. K. (1990). Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes. *Chromosoma* 99:3.

Mouchiroud, D., D'Onofrio, G., Aïssani, B., Macaya, G., Gautier, C., and Bernardi, G. (1991). The distribution of genes in the human genome. *Gene* 100:181.

Rynditch, A., Kadi, F., Geryk, J., Zoubak, S., Svoboda, J., and Bernardi, G. (1991). The isopycnic, compartmentalized integration of Rous sarcoma virus sequences. *Gene* 106:165.

Sabeur, G., Macaya, G., Kadi, F., and Bernardi, G. (1993). The isochore patterns of mammalian genomes and their phylogenetic implications. *J. Mol. Evol.* 37:93.

Saccone, S., De Sario, A., Della Valle, G., and Bernardi, G. (1992). The highest gene concentrations in the human genome are in telomeric bands of metaphase chromosomes. *Proc. Natl. Acad. Sci. USA* 89:4913.

Saccone, S., De Sario, A., Wiegant, J., Raap, A. K., Della Valle, G., and Bernardi, G. (1993). Correlations between isochores and chromosomal bands in the human genome. *Proc. Natl. Acad. Sci. USA* 90:11929.

Saccone, S., Caccio, S., Perani, P., Andreozzi, L., Rapisarda, A., Motta, S., and Bernardi, G. (1997). Compositional mapping of mouse chromosomes and identification of the gene-rich regions. *Chromosome Res.* 5:293.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.