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Cross-species chromosome painting in the golden mole and elephant-shrew: support for the mammalian clades Afrotheria and Afroinsectiphillia but not Afroinsectivora

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Cross-species painting (fluorescence *in situ* hybridization) with 23 human (*Homo sapiens* (HSA)) chromosome-specific painting probes (HSA 1–22 and the X) was used to delimit regions of homology on the chromosomes of the golden mole (*Chrysochloris asiaticus*) and elephant-shrew (*Elephantulus rupestris*). A cladistic interpretation of our data provides evidence of two unique associations, HSA 1/19p and 5/21/3, that support Afrotheria. The recognition of HSA 5/3/21 expands on the 3/21 synteny originally designated as an ancestral state for all eutherians. We have identified one adjacent segment combination (HSA2/8p/4) that is supportive of Afroinsectiphillia (aardvark, golden mole, elephant-shrew). Two segmental combinations (HSA 10q/17 and HSA 3/20) unite the aardvark and elephant-shrews as sister taxa. The finding that segmental syntenies in evolutionarily distant taxa can improve phylogenetic resolution suggests that they may be useful for testing sequence-based phylogenies of the early eutherian mammals. They may even suggest clades that sequence trees are not recovering with any consistency and thus encourage the search for additional rare genomic changes among afrotheres.

Keywords: cross-species chromosome painting; fluorescence *in situ* hybridization; Afroinsectiphillia; golden mole; elephant-shrew; phylogenetics

1. INTRODUCTION

Comprehensive molecular studies of higher-level mammalian phylogenetics (e.g. Madsen *et al.* 2001; Murphy *et al.* 2001*a*,*b*; Scally *et al.* 2001; Waddell *et al.* 2001) have generated relatively well-resolved phylogenies which suggest that extant placental (eutherian) mammals can be divided into four major supraordinal clades. One of these is the Afrotheria (Stanhope *et al.* 1998*a*), a clade of probable African origin that includes elephant-shrews (Macroscelidea), golden moles (Chrysochloridae) and tenrecs (Tenrecidae) that are grouped with aardvarks (Tubulidentata), hyraxes (Hyracoidea), elephants (Proboscidea) and the dugongs and manatees (Sirenia). Although there is little clear morphological evidence (Asher 1999; Carter 2001; Whidden 2002) to support the evolutionary affinities of this disparate morphological group, Werdelin & Nilsonne (1999) have argued that intraabdominal testes (testicondy) is a derived condition among therians. This would provide compelling morphological support for afrotherian monophyly. Among issues central to their recognition, and pertinent to our investigation, are the position of the afrotheres at the root of the eutherian tree, the relationships within the Afrotheria, and that their recognition contradicts the monophyly of the Insectivora (Lipotyphla; Stanhope *et al.* 1998*b*; Waddell *et al.* 1999*b*; Scally *et al.* 2001; Waddell *et al.* 2001; Delsuc *et al.* 2002; Malia *et al.* 2002; Waddell & Shelley 2003; Amrine-Madsen *et al.* 2003).

The molecular studies of Madsen *et al.* (2001), Murphy *et al.* (2001*a*,*b*), Scally *et al.* (2001), Waddell *et al.* (2001), Delsuc *et al.* (2002) and Waddell & Shelley (2003), among others, provide evidence for the placement of the root of the evolutionary tree between the Afrotheria and the remaining clades. However, they could not statistically exclude Xenarthra, or Xenarthra plus Afrotheria (Atlantogenata; Waddell *et al.* 1999*b*) as sister to all other placentals. Given the basal placement of the southern mammals in these phylogenies, the estimated times of divergence and the spatial coincidence with tectonic events, the break up of Gondwana is implicated in early placental evolution (Waddell *et al.* 1999*a*; Eizirik *et al.* 2001; Madsen *et al.* 2001; Murphy *et al.* 2001*a*,*b*). This has recently been challenged by Archibald (2003) who suggests that a Laurasian origin for eutherian mammals is still feasible even if Afrotheria is sister to all other mammals.

Within Afrotheria a number of clades have been suggested. There is anatomical and fossil evidence to suggest a close relationship of proboscideans, sirenians and hyracoids (the clade Paenungulata (Simpson 1945; Novacek 1992)), which has significant support in the molecular data already mentioned. Although an association between tenrecs and golden moles had been proposed on morphology, the lipotyphlan concept was fatally contradicted by the finding that sequences place these species and the

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aardvark firmly within Afrotheria (Stanhope *et al.* 1998*a*). The suggestion that the African endemic insectivores were more closely related to the aardvark and the enigmatic elephant-shrews than to lipotyphlan shrews, moles and hedgehogs is not supported on morphological grounds (Asher 1999; Whidden 2002). The relationships within the Afrotheria (except for the basal split between Paenungulata and the other Afrotheria) remain hard to resolve with many of the internal groupings receiving low support (Stanhope *et al.* 1998*a*; Malia *et al.* 2002; Waddell & Shelley 2003). The majority of molecular studies with diverse genes support an elephant-shrew + golden mole/tenrecs + aardvark grouping (the Afroinsectiphillia (e.g. Madsen *et al.* 2001; Murphy *et al.* 2001*b*; Waddell *et al.* 2001; Delsuc *et al.* 2002) sister to Paenungulata, but there are occasional contradictions to this (Murata *et al.* 2003)). The Afroinsectivora hypothesis (Waddell *et al.* 2001) is also favoured in many analyses, but recent analyses of new genes failed to recover it unambiguously (Waddell & Shelley 2003).

Given the length of sequence included in recent studies (e.g. greater than 17 kb from 20 nuclear and three mtDNA genes for 42 placentals and two marsupials (Amrine-Madsen *et al.* 2003)), the short internodes, long branches and presence of base composition shifts, it seems unlikely that sequence models can conclusively resolve all relationships in the placental tree (Waddell *et al.* 1999*a*, 2001; Waddell & Shelley 2003). It is therefore likely that improved resolution within the Afrotheria will depend on the inclusion of new genetic markers that are unlikely to suffer from excessive homoplasy. Characters such as long indels, SINES (short interspersed nuclear elements), protein sequence signatures and chromosomal rearrangements (rare genomic changes (RGCs); reviewed in Rokas & Holland (2000)) have the advantage that they are similar to conventional morphological synapomorphies in that they are either present or absent, and they do not require complex models to explain their evolution (Amrine-Madsen *et al.* 2003). One class of RGCs that holds significant promise is the conserved adjacent homologous segments that result from translocations and intra-chromosomal rearrangements from an ancestral state. These adjacent segment combinations can be identified by fluorescence *in situ* hybridization (FISH) that most commonly involves the use of flow-sorted human chromosome painting probes. Because they occur infrequently (O'Brien *et al.* 1999), they can provide cladistic landmarks with low levels of homoplasy that link species phylogenetically (e.g. Müller *et al.* 2003).

In the present investigation we provide cross-species chromosome painting data for two additional afrotherians, the golden mole (*Chrysochloris asiatica*) and the elephant-shrew (*Elephantulus rupestris*). Our aims were twofold. First, we hoped to gain greater insight into the chromosomal evolution and genome organization of the afrotherians particularly given the basic dichotomy in diploid number within the group. The paenungulates all have high chromosome numbers (Sirenia $2n = 48$–56, Proboscidea $2n = 56$, Hyracoidea $2n = 54$), while the Afroinsectiphillia have low numbers (Tubulidentata $2n = 20$, Macroscelidea $2n = 26$–34, Afroinsectivora $2n = 28$–36 in golden moles). Because the size and number of the conserved adjacent homologous segments reflect the number of rearrangements that have occurred in evolution, and the difference in chromosome numbers of the species that are compared, we anticipated that the low-numbered afrotherians would provide additional information on the eutherian ancestral karyotype (Chowdhary *et al.* 1998; Haig 1999; Wienberg *et al.* 2000; Murphy *et al.* 2001*c*; Richard *et al.* 2003*b*; Yang *et al.* 2003). Second, given the uncertainty regarding many of the evolutionary relationships within the Afrotheria, we set out to determine whether we could identify shared derived chromosomal states that would provide evidence, independent of sequence data, of the phylogenetic associations among aardvark, golden moles and the elephant-shrews (Afroinsectiphillia). As this requires interpretation in a cladistic framework, and the Paenungulata and Afroinsectiphillia are reciprocally monophyletic (e.g. Murphy *et al.* 2001*b*; Delsuc *et al.* 2002; Murata *et al.* 2003), we used the elephant (*Loxodonta africana*) as outgroup together with comparable data from 14 out of the 18 generally recognized orders of eutherian mammal species (see Yang *et al.* (2003) and references therein, Richard *et al.* (2003*a*,*b*) and Stanyon *et al.* (2003)).

2. METHODS

(a) *Chromosome preparation and standard karyotype preparation*

Fibroblast cultures were established from skin or toe clips of the Cape golden mole (*C. asiaticus*) and Smith's rock elephant-shrew (*E. rupestris*), and metaphase preparations were obtained by conventional methods. The G-banded karyotypes of both the golden mole ($2n = 30$) and elephant-shrew ($2n = 26$) were arranged and numbered in decreasing size. Standard karyotypic features and the FISH data from these species (see below) were compared with those published for the aardvark (Yang *et al.* 2003) and elephant (Frönicke *et al.* 2003; Yang *et al.* 2003).

(b) *Fluorescence* in situ *hybridization*

Human chromosome-specific painting probes were made by degenerate oligonucleotide-primed polymerase chain reaction amplification of flow-sorted chromosomes (Telenius *et al.* 1992; Ferguson-Smith *et al.* 1998). Cross-species chromosome painting followed Scherthan *et al.* (1994) and Yang *et al.* (1997).

In brief, 100–150 ng of biotin-labelled chromosome-specific paints were made up to 12 µl with hybridization buffer (50% deionized formamide, 10% dextran sulphate, $2 \times$ SSC, 0.5 mol l^{-1} phosphate buffer, pH 7.3, and $1 \times$ Denhardt's solution). The probes were denatured at 65 °C for 10 min and then preannealed by incubation at 37 °C for 15–60 min. Metaphase slides were denatured by incubation in 70% formamide/30% $2 \times$ SSC solution at 65–68 °C for 1.5–2 min, quenched in ice-cold 70% ethanol, and dehydrated through a 70%, 90% and 100% ethanol series. The pre-annealed paints were applied to slides, covered with 22×22 mm^2 cover-slips, sealed with rubber cement and incubated for 72 h at 37 °C. Post-hybridization washes involved two 5 min incubations in 50% formamide/50% $2 \times$ SSC (v/v) at 40 °C followed by two 5 min incubations in $2 \times$ SSC at 40 °C. Biotin-labelled probes were visualized using Cy3-avidin (1 : 500 dilution, Amersham). After detection, slides were mounted in Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI), Vector Laboratories). Images were captured using the Genus system (Applied Imaging). Hybridization signals were assigned to specific chromosomal regions defined by DAPI staining.

(c) *Two-colour fluorescence* in situ *hybridization*

To confirm problematic assignments of the human painting probes to the golden mole and elephant-shrew we performed two-colour FISH analyses. These were done specifically for the following syntenic associations: 1/19p, 3/21, 7/16, 12/22, 14/15 and 16/19. The FISH probes were labelled with biotin- and digoxigenin-dUTP and visualized with avidin-FITC and anti-digoxigenin-rhodamine.

3. RESULTS

The golden mole (*Chrysochloris asiaticus* (CAS)) has $2n = 30$, consistent with an earlier report based on conventionally stained chromosomes (Bronner 1995). The G-banded karyotype of the species is presented in figure 1*a*. The specimen used in our investigation shows heterochromatic difference in the short arms of CAS 8 and 10. Polymorphisms in the length of the short arms of several of the chromosomal pairs were also evident in the elephant-shrew (*Elephantulus rupestris* (ERU)) specimen and was most pronounced in ERU 5 and ERU 7. This species has $2n = 26$, which confirms an earlier report (Wenholdt & Robinson 1987); the G-banded karyotype is presented in figure 1*b*.

(a) *Cross-species chromosome painting of the golden mole and elephant-shrew*

The 22 human autosomal painting probes and the X paint produced hybridization signals that covered the entire euchromatic region of the target species. The cross-species hybridization results are mapped to the G-banded karyotypes of the two species (figure 1*a*,*b*). The heterochromatic regions were unhybridized.

The human painting probes delineated 32 homologous autosomal segments in the golden mole and 36 segments in the elephant-shrew (figures 1 and 2). Fourteen human (*Homo sapiens* (HSA)) chromosomes show conserved synteny (i.e. retained as single sites of hybridization) in the golden mole (HSA 1, 4–6, 9, 11, 13–15, 17, 18, 20, 21 and X) and the elephant-shrew, although often in combination with other chromosomes or chromosomal segments. The golden mole has retained one additional conserved synteny. HSA 3 is conserved *in toto* in this species but gives four sites of hybridization in the elephant-shrew. Additionally, one of these sites, the association between HSA 3/20 which is contiguous in the aardvark, has been disrupted by the amplification of heterochromatic sequences in the elephant-shrew (ERU 7; figure 1*b*). There were five conserved adjacent segment combinations comprising the compound products of three or more human chromosomal segments (reflecting multiple translocations with respect to the human karyotype) in the golden mole (2/8/4, 3/21/5, 8/1/19, 12/22/10/2/22) and five in the elephant-shrew (1/19/2/8/4, 13/3/21/5, 2/10/12/22, 3/15/14, 7/16/12/22). As is evident, all of the ancient human segmental associations HSA 3/21, 4/8,7b/16, 10/12/22, 12/22, 14/15 and 16q/19q that are conserved in the majority of mammals for which chromosome painting data exist (Yang *et al.* (2003) and references therein and Richard *et al.* (2003*b*)) are retained in the two afrotheres. The remaining adjacent segment combinations reflect simple fusions of two human chromosomal equivalents, none of which is shared between the golden mole and elephant-shrew.

4. DISCUSSION

To place our data in a phylogenetic context we broadened our comparisons to include the aardvark and elephant. Because this requires interpretation in a cladistic framework and the Paenungulata and Afroinsectiphillia are reciprocally monophyletic (e.g. Murphy *et al.* 2001*b*; Murata *et al.* 2003), we use the elephant as outgroup for the Afroinsectiphillia. To facilitate our discussion of the interspecific comparisons, we present the half-karyotype of the aardvark for which regions of homology with human have been established by reciprocal painting (Yang *et al.* 2003), and the corresponding chromosomal regions of the elephant-shrew and the golden mole as established in the present study (figure 3). In addition, the recent human : xenarthran data from Richard *et al.* (2003*b*) allow us to further clarify the presumed synapomorphies uniting the Afrotheria. However, confirmation that these cytogenetic characters constitute Afrotherian synapomorphies as opposed to ancestral placental states would be strengthened by outgroup comparison to a marsupial representative which is lacking (Frönicke *et al.* 2003; Yang *et al.* 2003).

(a) *Homologies with other Afrotheria and phylogenetic inferences*

(i) *Support for the Afrotherian clade*

Frönicke *et al.* (2003) identified HSA 5/21 in the elephant, suggesting that this composite, together with HSA 1/19p, may be a synapomorphy that unites Afrotheria. Our data (figure 1*a*,*b*; figure 3) confirm and extend the observations, particularly with respect to the former synteny. We suggest that the proposed ancestral eutherian association of HSA 3/21 (Yang *et al.* 2003) be expanded to include segments homologous to human chromosome 5 forming an HSA 3/21/5 segmental combination defining Afrotheria. The most parsimonious explanation for the observed patterns (HSA 3 paints a single chromosome in the golden mole, the HSA3/21/5 configuration is present in aardvark and elephant-shrew) is to argue that this chromosome appears to have undergone a fission in the elephant lineage (*Loxodonta africana* (LAF)) giving rise to the HSA 5/21 (LAF 3) and the HSA 1/3/21 association on LAF 21 (see Frönicke *et al.* 2003). Other non-sequence-based synapomorphies consistent with afrotherian monophyly are detection of a unique 9 bp deletion in exon 11 of the BRCA 1 gene (Madsen *et al.* 2001), the protein sequence signatures ('protein-morphological synapomorphies') of Van Dijk *et al.* (2001), the 5′ and 3′ deletions present in exon 26 of APOB (Amrine-Madsen *et al.* 2003), and the so-called AfroSINES reported by Nikaido *et al.* (2003).

(ii) *Support for the Afroinsectiphillia*

The HSA 2/8p/4 configuration is present in the golden mole, elephant-shrew and aardvark (figure 3) but is absent from the elephant (our outgroup species). Although it could be argued that this configuration has been disrupted in the elephant, these particular associations have not been observed in any of the remaining orders for which painting data are available except the rabbit, *Oryctolagus cuniculus* (family Leporidae, order Lagomorpha (Korstanje *et al.* 1999)). This association is, however, absent from the pika, *Ochotona hyperborea* (A. S. Graphodatsky and F. Yang, unpublished data), suggesting that its presence in leporids

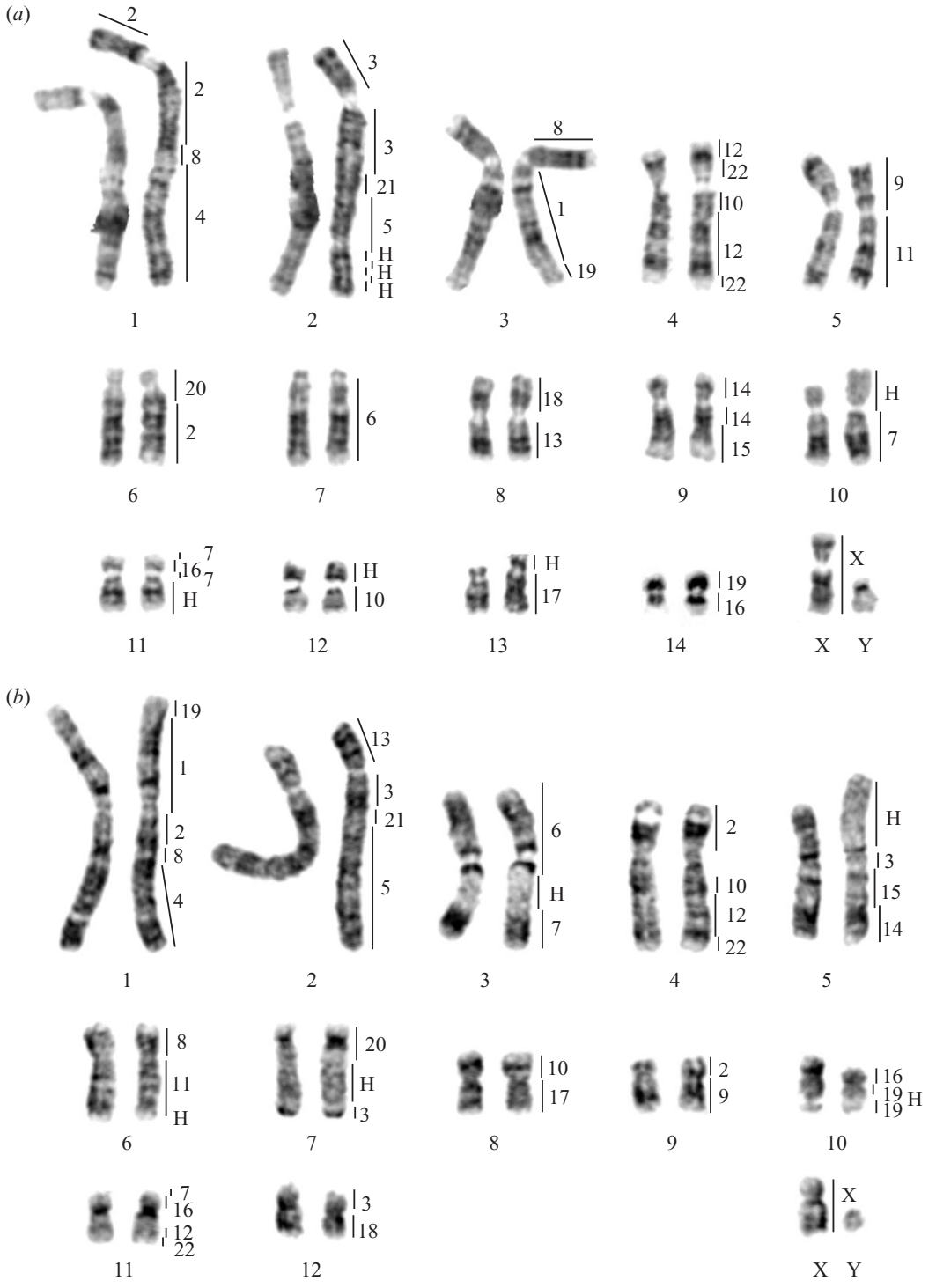

Figure 1. G-banded karyotypes of the (*a*) golden mole, *Chrysochloris asiaticus*, and (*b*) elephant-shrew, *Elephantulus rupestris*. Vertical lines to the right of each pair of chromosomes delimit the hybridization of the respective human chromosome painting probes. H, amplified heterochromatic regions.

is a result of convergence. The broadened outgroup comparison therefore strengthens claims for its recognition as a likely synapomorphy uniting the Afroinsectiphillia. This finding is at odds with morphology, which suggests that the elephant-shrews have cranial, post-cranial and dental features that are suggestive of an alignment with the paenungulates (Seiffert 2003). Our data, however, place it within the Afroinsectiphillia, as do the concatenated sequences of Madsen *et al.* (2001), Murphy *et al.* (2001*b*) and Waddell *et al.* (2001). Interestingly, a monophyletic Afroinsectiphillia could provide a morphological explanation for afrotherian monophyly by implying that the apomorphic morphological features shared by elephant-shrews and paenungulates are actually afrotherian synapomorphies, and that the 'insectivore'-like features of tenrecs and golden moles represent evolutionary reversals (Seiffert 2003).

The conserved syntenic grouping HSA 10/12/22, which is shared by the elephant-shrew, the aardvark and the elephant, has been observed in other mammalian orders and forms part of the hypothesized eutherian ancestral

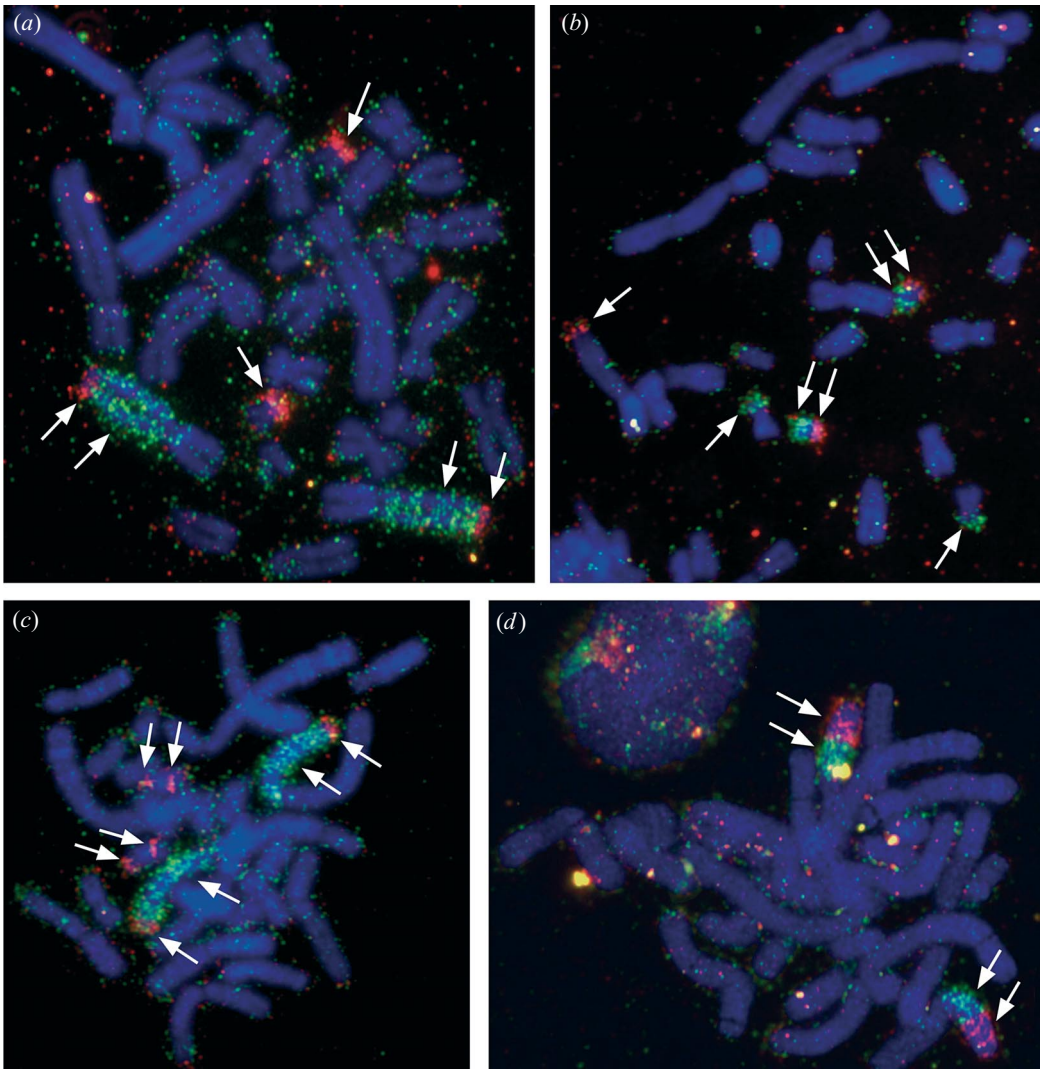

Figure 2. Examples of cross-species FISH using human-specific painting probes showing the conservation of the adjacent segment associations HSA 1/19p, HSA 19/16 and HSA 14/15 (arrows). (*a*) HSA 1 (green) and HSA 19 (red) on the golden mole; (*b*) HSA 16 (green) and HSA 19 (red) on the golden mole (partial spread); (*c*) HSA 1 (green) and HSA 19 (red) on the elephant-shrew; and (*d*) HSA 14 (red) and HSA 15 (green) on the elephant-shrew.

karyotype. The HSA 12/22/10/12/22 association in the golden mole is the result of the fusion of two ancestral syntenies (HSA 12qter/22 and 10/12/22) and is an autapomorphy in this lineage. HSA 19q/16q is present in the golden mole and elephant-shrew. It is also present in both aardvark (*Orycteropus afer* (OAF)) and elephant but fused other human chromosomal segments (in LAF 19q/16q/4; in OAF 19q/16q/13/2pq-prox/8p/4). The fact that the HSA 16q/19q combination exists in the majority of eutherian species studied, and is thought to be an ancestral state for all placentals (Murphy *et al.* 2001*c*; Frönicke *et al.* 2003; Richard *et al.* 2003*b*; Yang *et al.* 2003), suggests that it is plesiomorphic in these afrotherians. Because shared primitive conditions are not the best guide to phylogenetic relationships we do not regard HSA 16q/19q as being of consequence for these two species.

(iii) *Support for the recognition of elephant-shrew and aardvark as sister taxa*

HSA 10q/17 and HSA 3/20 are present in both the elephant-shrew and aardvark but absent from the golden mole and also from the elephant. This phylogenetic association has been detected (although with relatively weak bootstrap support) by Stanhope *et al.* (1998*b*), in some trees based on protein residues by Van Dijk *et al.* (2001) and in Waddell *et al.* (2001). Importantly, we find no support for a sister relationship between the Afroinsectivora represented by the golden mole and the Macroscelidea. While this is in conflict with Madsen *et al.* (2001), Murphy *et al.* (2001*b*), Delsuc *et al.* (2002) and Murata *et al.* (2003), among others, it is more in keeping with Waddell & Shelley's (2003) more recent study.

(**b**) *Genome conservation*

If we focus more fully on the high chromosome number (elephant, hyrax sirenians) versus low chromosome number (Afroinsectiphillia) dichotomy in the Afrotheria, several salient features emerge from our investigation of the chromosome conservation shown by the low-numbered afrotheres (figure 3). We have previously identified 30 syntenic autosomal segments in the aardvark genome (Yang *et al.* 2003). This is the lowest number identified in non-primate species which, together with the presence of all the putative primitive syntenies, suggests that the

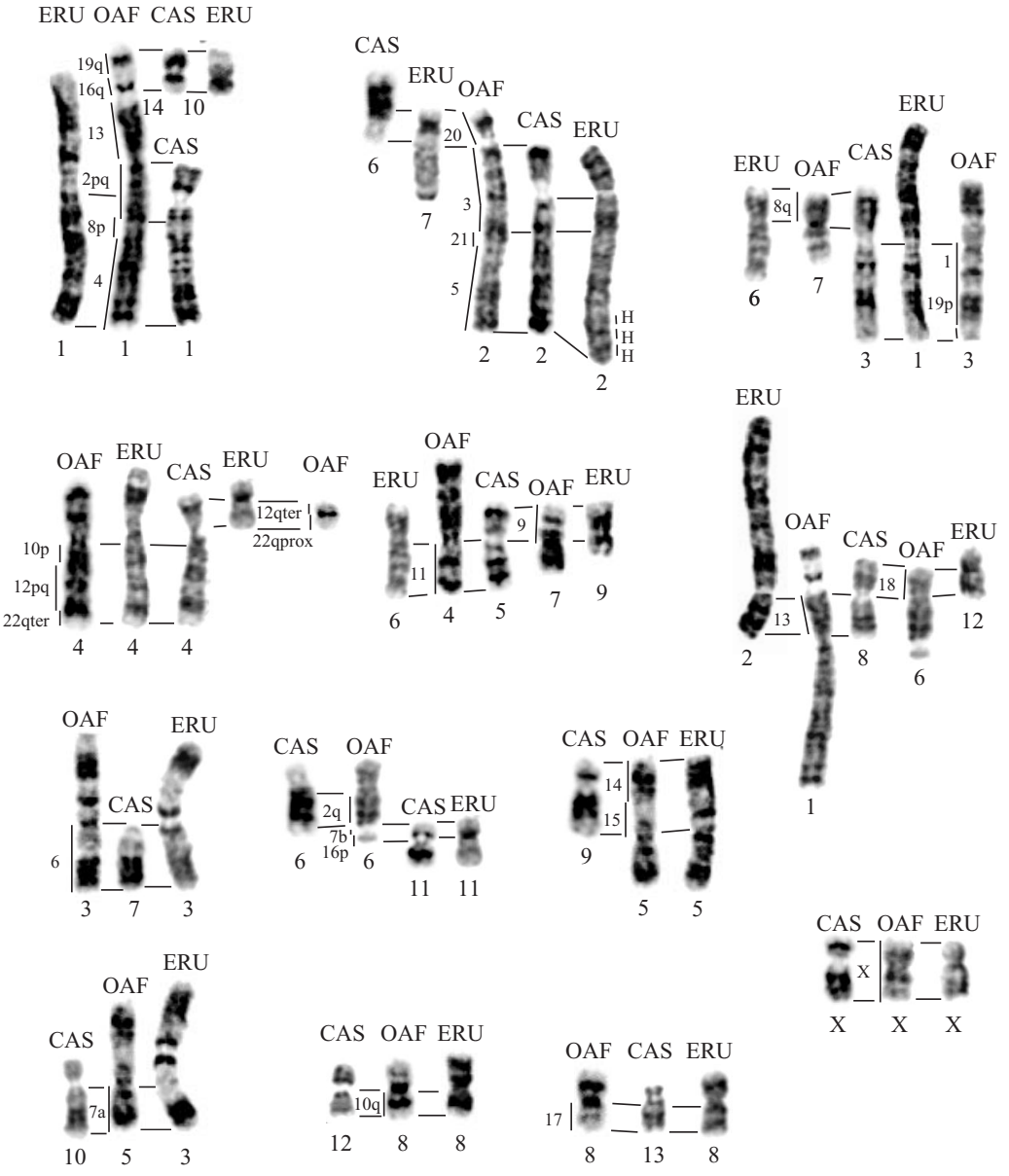

Figure 3. Chromosome comparisons between the elephant-shrew (ERU), aardvark (OAF) and golden mole (CAS) showing the approximate extent of correspondence among homologous regions detected by FISH and corresponding G-band patterns. We use the aardvark as a reference because homology to human chromosomes (left) was established by reciprocal painting. Regions that show conserved banding patterns are: HSA 4/8p (between OAF and CAS); HSA 5/21 (between OAF and CAS); HSA 1/19p between OAF and ERU (part), while this region appears fully conserved between CAS and ERU; HSA 6 in all three species; HSA 7a which is fully conserved in all three species; HSA 10q shows good correspondence between OAF and ERU, and HSA 11 which is conserved between OAF and CAS. Chromosome numbers correspond to the species karyotypes presented in figure 1*a*,*b* and, in the case of the aardvark, fig. 3 in Yang *et al.* (2003). H shows the presence of heterochromatic bands that are unhybridized in ERU 2.

aardvark has retained a karyotype that largely resembles that of the last common ancestor of all eutherians. Fifteen human chromosomes were retained intact in the aardvark genome as single homologous segments (HSA 1, 3, 4–6, 9, 11, 13–15, 17, 18, 20, 21 and X). Our cross-species painting data on the golden mole identify the conservation of the same 15 chromosomes, while the elephant-shrew has one additional disruption (HSA 3 occurs as four discrete regions of hybridization on ERU 2, 5, 7 and 12, respectively). In addition to giving credence to the identity of many of the chromosomes thought to have been present in the last common ancestor to all eutherian mammals, the conservation of the karyotypes in the low-numbered afrotheres extends, in many instances, quite convincingly to their G-banding patterns (figure 3). This is particularly evident in the interspecific comparisons comprising the regions of synteny delineated by HSA 4/8p, 5/21 and HSA 1/19p. Additionally, the regions defined by HSA 6 and HSA 7a appear to be fully conserved in all three species, as is HSA 11 (details in figure 3). Although there are some similarities with other regions, these are either too small or the banding patterns have been disrupted by internal rearrangements. Further refinements will depend on high-resolution mapping data and cross-species painting schemes that involve highly rearranged and fragmented genomes such as the dog (Yang *et al.* 2000). The

conserved homologies are all the more striking given that molecular dating places the interordinal splits among the Afroinsectiphillia between 77 and 66 Myr ago (Springer *et al.* 2003). When taken together with these data, our results suggest that relatively little interchromosomal repatterning has occurred subsequent to divergence from their last common ancestor, further emphasizing the slow rate of chromosomal evolution in the low-chromosome-numbered Afrotheria.

Interestingly, the conservative nature of chromosomal evolution detected in the low-numbered afrotherians extends also to man. In addition to the X chromosome, we and others (Haig 1999; Murphy *et al.* 2003; Richard *et al.* 2003*b*; Yang *et al.* 2003) have shown that 14 out of the 22 human autosomes (HSA 1, 3, 4–6, 9, 11, 13–15, 17–18, 20–21) have been retained *in toto* in representatives of the four major eutherian clades. This reflects their probable occurrence in the last common ancestor of all Placentalia and emphasizes the long conserved evolutionary history of these chromosomes, and by extension that of the human genome. This clearly underscores its appropriateness as the reference genome in comparative mammalian genomics. Additionally, although the genomic syntenies of HSA 2, 7, 8, 10, 12, 16, 19 and 22 are not fully maintained across Eutheria, reciprocal painting (Müller *et al.* 1999; Yang *et al.* 1999, 2000, 2003) suggests that these human configurations are mostly (but not exclusively) the fusion products of two conserved segments that occurred during primate evolution. Obviously, the regions of synteny detected by cross-species chromosome painting give no insights to the preservation, or otherwise, of gene order and the rate of subtle intrachromosomal disruption in these chromosomes.

In conclusion, we have used cross-species painting involving FISH of all 23 human chromosome painting probes (i.e. HSA 1–22 and the X) to delimit regions of homology on the chromosomes of two additional afrotherians. This brings to four the number of orders analysed in this uniquely African clade of mammals. A cladistic interpretation of our data provides evidence of one adjacent segment combination (HSA 2/8p/4) that supports the recognition of Afroinsectiphillia (golden mole, elephant-shrew, aardvark), two segmental combinations (HSA 10q/17, 3/20) that unite the aardvark and elephant-shrews as sister taxa, and two unique associations, HSA 1/19p and HSA 3/5/21, that define Afrotheria. The position of both the elephant-shrew and the aardvark has been largely unresolved and is pivotal to our understanding of the origin of living and extinct afrotherian orders. We think that these findings will lead to the development of a more refined set of hypotheses that encourage the search for additional independent characters such as rare indels, gene arrangements, protein signatures and SINES. These, together with chromosomal exchanges that define segmental syntenies in phylogenetically distant taxa, may provide improved resolution among early eutherian mammals.

Note added in proof. Recently Svartman *et al.* (2004) published cross-species chromosome painting results of human to another species of elephant shrew (*Macroscelides proboscideus*) confirming the regions of synteny reported by us for *Elephantulus rupestris*.

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