Genome-Wide Comparative Chromosome Map between Human and the Forrest’s Pika
(Ochotona forresti) Established by Cross-Species Chromosome Painting: Further Support for the Glires Hypothesis

J. Ye, W. Nie, J. Wang, W. Su, M. Jing, A.S. Graphodatsky, F. Yang

State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, College of Life Sciences, Shaoxing University, Shaoxing, College of Life Sciences, Ludong University, Yantai, PR China; Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia; Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK

The order Lagomorpha consists of 2 families: Leporidae (hares and rabbits), and Ochotonidae (pikas), about 91 species [Hoffmann and Smith, 2007], of which the domestic rabbit (Oryctolagus cuniculus, 2n = 44) is the most well-studied species mainly due to its importance as a model for biomedical research [Korstanje et al., 1999].

The phylogenetic placement of Lagomorpha on the eutherian tree remains a topic of debate. Nevertheless, the grouping of Lagomorpha and Rodentia into a monophyletic clade called Glires represents the current consensus view that is supported overwhelmingly by both morphological and molecular evidence [Luckett and Hartenberger, 1993; Murphy et al., 2001a; Reyes et al., 2004; Asher et al., 2005; Kriegs et al., 2007; Arnason et al., 2008; Schneider and Cannarozi, 2009]. Furthermore, the Glires concept appears to be supported by molecular cytogenetic evidence. The existence of conserved association(s) of hu-
man (HSA) homologous chromosomal segments in do-

mestic rabbit [Korstanje et al., 1999] and squirrels [Rich-

ard et al., 2003; Stanyon et al., 2003; Li et al., 2004, 2006] has led to the suggestion that the HSA1/10p association and, to a lesser extent, the HSA9/11 association may re-

present the cytogenetic signature characterizing the clade Glires [Stanyon et al., 2003; Li et al., 2004]. Nevertheless, the presence of such signature rearrangements in Ochotonidae remains to be established.

Comparative cytogenetic studies have made a great contribution to our understanding of karyotype evolu-

tion of species within the families Leporidae and Ochotonidae, respectively [Hsu and Benirschke, 1971; Stock, 1976; Capanna et al., 1991; Ivanitskaya, 1991; Robi-

nson et al., 2002; Robinson, 2006 and literature cited therein]. However, earlier cross-family comparison based on G-banding has only revealed a few conserved chromo-

somal segments between representative species from Leporidae and Ochotonidae, leading to the suggestion that tandem fusion could be responsible for the family-

level karyotype divergence in Lagomorpha [Stock, 1976].

Cross-species chromosome painting is the most ro-

bust method for establishing genome-wide comparative chromosome maps in mammals. Such comparative chromo-

some maps, usually using human chromosomes as the reference, have enabled the reconstruction of ancestral eutherian genome organization and landscape chromo-

somal rearrangements accompanying the radiation of major phylogenetic lineages [see Ferguson-Smith and Trifonov, 2007 for review]. Up to now, the comparative chromosome maps between human and representative species of the extant 18 eutherian orders (including one Lagomorpha species, Oryctolagus cuniculus) [Korstanje et al., 1999] have been reported [see Ferguson-Smith and Trifonov, 2007 for review; Nie et al., 2008]. Nevertheless, genome-wide comparative chromosome maps between human and representative species of Ochotonidae, as well as between representative species of Leporidae and Ochotonidae have not been reported hitherto.

In this study, we have established the first genome-

wide chromosomal homology map between human and Forrest’s pika (Ochotona forresti, OFO, 2n = 54) by cross-

species chromosome painting with human chromosome-

specific painting probes. Such a map sheds further in-

sight into the ancestral lagomorph karyotype and chro-

mosomal rearrangements underlying the karyotype divergence between Leporidae and Ochotonidae and pro-

vides further support for the Glires hypothesis.

Materials and Methods

Metaphase Preparations and DAPI-Banding

The O. forresti fibroblast cell line was derived from skin biops-

ies of a male Forrest’s pika collected from Gaoligong Mountain, Yunnan, China. Metaphase preparations were made following conventional methods as previously described [Yang et al., 2003a]. The O. forresti chromosomes were karyotyped based on inverted DAPI-banding patterns that are similar to the G-banding pat-

terns.

Fluorescence in situ Hybridization

Human chromosome-specific painting probes were made from flow-sorted chromosomes by degenerate oligonucleotide primed-PCR (DOP-PCR) [Telenius et al., 1992]. Cross-species chromosome painting between O. forresti and human was carried out as previously described [Yang et al., 1999]. After post-hybrid-

ization washes, biotin-labeled probes were visualized using Cy3-

avidin (final concentration 2 μg/ml, Amersham). After detection, slides were then mounted in Vectashield medium with DAPI (4,6-diamidino-2-phenylindole, Vector Laboratories) and cov-

ered with 22 × 32 mm2 coverslips.

Microscopy

Fluorescence in situ hybridization (FISH) images were cap-

tured using the Genus system (Applied Imaging Corp.) as previ-

ously described [Yang et al., 2004]. Hybridization signals were assigned to specific chromosomes or chromosomal regions as de-

fined by enhanced DAPI-banding patterns.

Results

Painting O. forresti Chromosomes with Human Paints

The male O. forresti has a 2n = 54 karyotype (fig. 1). The X and Y chromosomes are a large submetacentric chromosome and a small bi-armed chromosome, respec-

tively. To establish the chromosomal homology map be-

tween human and O. forresti, 22 human autosomal paints and the X chromosome paint were hybridized onto the metaphases of O. forresti. Representative FISH examples are shown in figure 2. The cross-species hybridization results are summarized on a DAPI-banded karyotype of O. forresti (fig. 1). In the genome of O. forresti, 9 human chromosome painting probes (HSA6, 9, 11, 13, 17, 18, 20, 21, and X) each hybridized onto one O. forresti chromo-

somal segment or chromosome; 9 human probes (HSA1, 2, 8, 10, 14–16, 19, and 22) each detected 2 chromosomal regions; 5 human probes (HSA3–5, 7, and 12) each deline-

ated 3 homologous segments. In total, 22 human auto-

somal painting probes defined 41 conserved segments in the O. forresti genome. All previously proposed ances-

tral human homologous segment associations such as HSA3/21, 4/8, 7/16, 12/22 (twice), 14/15, and 16/19 [Yang et al., 2003b and references cited therein] were also found

Cytogenet Genome Res 2011;132:41–46

Ye/Nie/Wang/Su/Jing/Graphodatsky/Yang
in the *O. forresti* genome. Besides these postulated ancestral eutherian syntenies, 6 adjacent segment associations (HSA1/10, 3/12, 9/15, 10/11, 12/14, and 18/20) were present in the *O. forresti* genome, with 5 of these associations (i.e. HSA3/12, 9/15, 10/11, 12/14, and 18/20) most likely resulting from Robertsonian translocations as suggested by the position of centromeres.

**Discussion**

Cross-species chromosome painting with human chromosome-specific painting probes has allowed us to construct the first genome-wide comparative chromosome map between human and *O. forresti*, a representative species of the pika family. An integrated analysis of our comparative chromosome map and the previously published maps [Korstanje et al., 1999; Robinson et al., 2002] provides new insight into the comparative genome organization of the Lagomorpha.

**Karyotype Relationship between *O. cuniculus* and *O. forresti***

Comparative maps between human and rabbit (*Oryctolagus cuniculus*) have been established by using various methods, including reciprocal chromosome painting with both human and *O. cuniculus* chromosome-specific probes, and comparative gene mapping [Korstanje et al., 1999; Hayes et al., 2002; Chantry-Darmon et al., 2003, 2005]. The comparison of our human – *O. forresti* comparative map with the published human – *O. cuniculus* comparative map has enabled us to deduce the subchro-
mosomal correspondence between *O. forresti* and *O. cuniculus* as well as human, based on the genome-wide homologies delimited by comparative chromosome painting and banding comparison (fig. 1). The comparative results demonstrate that these 2 lagomorph species, representing the 2 lagomorph families, share at least 29 conserved segments equivalent to human chromosomal segments or segment associations 1a, 1b/10p, 2pq, 2q, 3a/21, 3b, 4a/8p, 4b, 5a, 6, 7a, 7b/16p, 7c, 8q, 9, 10q, 11, 12p, 12q/22q13, 12q/22q14, 14a, 15/14b/15, 16q/19q, 17, 18, 19, 20 and X. Ignoring the unpainted heterochromatic regions, 8 *O. forresti* chromosomes (OFO4, 5, 15, 16, 18, 24, 25, and X) show complete chromosomal conservation with their *O. cuniculus* counterparts; thirteen *O. forresti* chromosomes (OFO6, 9–13, 17, 19–23, and 26) each correspond to one *O. cuniculus* chromosomal segment; 6 *O. forresti* chromosomes (OFO1–3, 7, 8, and 14) each correspond to 2 *O. cuniculus* chromosomal segments or chromosomes. Most homologous segments/chromosomes between *O. forresti* and *O. cuniculus* defined by human painting probes also display a high degree of conservation in banding patterns, with the exception of *O. forresti* chromosomes 1p, 3q, 4, 6 and 11 and their corresponding homologues in *O. cuniculus*. Intrachromosomal rearrangements such as inversion or centromere repositioning may have accounted for the banding variations within OFO1p–OCU16, OFO3q–OCU17, OFO4–OCU12, OFO6–OCU7q, and OFO11–OCU14q.

In addition to these intrachromosomal changes, karyotype differences between *O. forresti* and *O. cuniculus* appear to be mainly due to Robertsonian translocations. For example, *O. forresti* chromosomes 1 (homologous to HSA1/10/11) and 3 (homologous to HSA9/15/14/15) can be derived through a few Robertsonian translocations from *O. cuniculus* chromosomes 1 (homologous to HSA9/11), 16 (homologous to HSA1/10) and 17 (homologous to HSA15/14/15). The same situation is also observed in *O. forresti* chromosomes 2 and 13, 6 and 17, 10 and 14, 20 and 26, and 12 and 22 versus *O. cuniculus* chromosomes 3 and 15, 7, 8 and 20, 10, and 11, respectively (fig. 1). In contrast, previous G-banding comparison has suggested that tandem fusions have played a major role in the evolution of lagomorph karyotypes [Stock, 1976]. There is indeed one obvious example of chromosomal
evolution via tandem fusion: the evolution of *O. cuniculus* chromosome 2p apparently could be explained by a tandem fusion of *O. forresti* chromosomes 21 (= HSA4) and 23 (= HSA4/8p). However, it is most probably that *O. forresti* chromosomes 21 and 23 have evolved from *O. cuniculus* chromosome 2p via a fission event as the HSA4/8p association represents a conserved ancestral synteny. Therefore, together our results suggest that Robertsonian translocations, rather than tandem fusions, are the dominant type of interchromosomal rearrangements (fig. 1). However, further comparative chromosome painting data from more pika species are required to determine the direction of karyotype evolution of lagomorphs.

**Further Cytogenetic Evidence for the Superorder Glires**

Previous comparative analyses have indicated that HSA1/10p and HSA9/11 (to a lesser extent) associations are considered to be the cytogenetic signatures characterizing the clade Glires [Stanyon et al., 2003; Li et al., 2004]. Our results show that the HSA1/10p association appears to be present in the *O. forresti* chromosome 1. Thus, the existence of HSA1/10p association in the pika genome provides further cytogenetic evidence for the Glires hypothesis. However, the HSA1/10p association is absent in the genomes of *Pedetes capensis* (Pedetidae), *Sicista betulina* (Dipodidae), and *Castor fiber* (Castoridae) [Graphodatsky et al., 2008], it seems likely that this association has been disrupted in their common ancestor. Interestingly, HSA1/10 is also present in all Perissodactyla species for which the Zoo-FISH data are available [Yang et al., 2003a, 2004; Trifonov et al., 2008] and in the Malayan flying lemur (*Galeopterus variegatus*) [Nie et al., 2008]. However, reciprocal chromosome painting data show that the HSA1/10 association detected in both Perissodactyla species and the Malayan flying lemur came from the segments homologous to HSA1 and 10q [Yang et al., 2003a, 2004; Nie et al., 2008; Trifonov et al., 2008]. Therefore, the HSA1/10p association is indeed a cytogenetic signature that supports the grouping of orders Lagomorpha and Rodentia into the superorder Glires.

Although HSA9/11 association is found in domestic rabbit [Korstanje et al., 1999; Chantry-Darmon et al., 2005], it is absent in the genomes of other lagomorphs so far investigated. The karyotypic relationships within the leporids have been examined by chromosome painting using *O. cuniculus* chromosome-specific probes [Robinson et al., 2002]. The results showed that *O. cuniculus* chromosome 1 (homologous to HSA9/11) has broken up in the leporid lineage (i.e. homologous to HSA9 and 11, respectively) [Robinson et al., 2002]. Moreover, HSA9/11 association is also absent in the genome of *O. forresti* in our study. Nevertheless, comparative chromosome maps between human and representative species of the Rodentia [Richard et al., 2003; Stanyon et al., 2003; Li et al., 2004, 2006; Graphodatsky et al., 2008] have suggested that the HSA9/11 association could be regarded as a component of the ancestral karyotype of the Rodentia [Graphodatsky et al., 2008]. Considering that the family Ochotonidae consists of about 30 species, comparative chromosome painting data from more representative pika species, in particular those with lower diploid numbers, are needed to further validate if the HSA9/11 association could represent the shared derived cytogenetic character of the superorder Glires.

**Conservation of Ancestral Eutherian Syntenies in *O. forresti***

The comparative chromosome maps between human and representative species of the extant 18 eutherian orders have been used to infer the composition of the ancestral eutherian karyotype (AEK) [Chowdhary et al., 1998; Murphy et al., 2001b; Richard et al., 2003; Yang et al., 2003b; Svartman et al., 2006; Ferguson-Smith and Trifonov, 2007]. Our results show that the ancestral syntenies equivalent to human chromosomes 2pq, 2q, 6, 8q, 9, 10q, 11, 13, 17, 18, 19p, 20, and X have been conserved in the genome of *O. forresti*, while the ancestral syntenies of HSA1, 5, and 7a each have been fragmented by additional fission events in the *O. forresti* genome. In addition, our results demonstrate that the postulated ancestral eutherian syntenic associations (i.e. HSA3/21, 4/8p, 7/16p, 14/15, 12pq/22qt, 12qt/22q, 16q/19q) are all present in the *O. forresti* genome. Therefore, most of the proposed AEK have been retained in the *O. forresti* genome. However, *O. forresti* clearly has a derived karyotype. By comparing our result with the putative AEK as suggested by Ferguson-Smith and Trifonov [2007], we can speculate that at least 11 chromosome fissions, 7 chromosome fusions, 1 inversion, and 3 centromere repositionings were needed to convert the karyotype of 2n = 46 AEK to that of 2n = 54 *O. forresti*. Therefore, the *O. forresti*, like the *O. cuniculus*, has a relatively conserved genome organization.

**Acknowledgements**

This study is supported partly by a grant from the National Natural Science Foundation of China (No. 30770293) and MCB, SB RAS, RFBR grants (A.S.G.). F.Y. is supported by the Wellcome Trust.
References

Arnason U, Adegoke JA, Gullberg A, Harley EH, Janke A, et al: Mitogenomic relationships of placental mammals and molecular estimates of their divergences. Gene 421:37–51 (2008).

Asher RJ, Meng J, Wible JR, Mckenna MC, Rougier GW, et al: Stem Lagomorpha and the antiquity of Gliridae. Science 307:1091–1094 (2005).

Capanna E, Bonomo M, Civitelli MV, Simonetta A, Capanna E: The chromosomes of Royle’s pika, Ochotona roylei (Mammalia, Lagomorpha). Rendiconti Lincei 2:59–67 (1991).

Chantry-Darmon C, Rogel-Gaillard C, Bertaud M, Urien C, Perrocheau M, et al: 133 new gene localizations on the rabbit cytogenetic map. Cytogenet Genome Res 103:192–201 (2003).

Chantry-Darmon C, Bertaud M, Urien C, Chadi-Taouirit S, Perrocheau M, et al: Expanded comparative mapping between man and rabbit and detection of a new conserved segment between HSA22 and OCU4. Cytogenet Genome Res 111:134–139 (2005).

Chowdhary BP, Raudsepp T, Frönicke L, Scherthan H: Emerging patterns of comparative genome organization in some mammalian species as revealed by Zoo-FISH. Genome Res 8:577–589 (1998).

Ferguson-Smith MA, Trifonov V: Mammalian karyotype evolution. Nat Rev Genet 8:950–962 (2007).

Graphodatsky AS, Yang F, Dobigny G, Romanenko SA, Bilueva LS, et al: Tracking genome organization in rodents by Zoo-FISH. Chromosome Res 16:261–274 (2008).

Hayes H, Rogel-Gaillard C, Zijlstra C, De Haan NA, Urien C, et al: Establishment of an R-banded rabbit karyotype nomenclature by FISH localization of 23 chromosome-specific genes on both G- and R-banded chromosomes. Cytogenet Genome Res 98:199–205 (2002).

Hoffmann RS, Smith AT: Lagomorphs, in Wilson DE, Reeder DM (eds): Mammal species of the World (Smithsonian Institution Press, Washington, 2007).

Hsu TC, Benirschke K: An Atlas of Mammalian Chromosomes. Vol 6, Folio 265 (Springer, Berlin 1971).

Ivanitskaya EV: The comparative analysis of G-banding chromosomes of pikas and superspecies system of the genus Ochotona (Ochotonidae, Lagomorpha), in Zaitsev EV (ed): Questions of Systematics, Faunistics and Palaeontology of Small Mammals (Proceeding of the Zoological Institute Press, Petersburg 1991).

Korstanje R, O’Brien PCM, Yang F, Rens W, Bosma AA, et al: Complete homology maps of the rabbit (Oryctolagus cuniculus) and human by reciprocal chromosome painting. Cytogenet Cell Genet 86:317–322 (1999).

Kriegs JO, Churakov G, Jurka J, Brosius J, Schmitz J: Evolutionary history of 7SL RNA-derived SINEs in Supraprimates. Trends Genet 23:158–161 (2007).

Li T, O’Brien PC, Bilueva L, Fu B, Wang J, et al: Evolution of genome organizations of squirrels (Sciuridae) revealed by cross-species chromosome painting. Chromosome Res 12:317–335 (2004).

Li T, Wang J, Su W, Nie W, Yang F: Karyotypic evolution of the family Sciuridae: inferences from the genome organizations of ground squirrels. Cytogenet Genome Res 112:270–276 (2006).

Lukett WP, Haptenberger JL: Monosomy or polyphly of the order Rodentia: possible conflict between morphological and molecular interpretations. J Mamm Evol 1:127–147 (1993).

Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, et al: Molecular phylogenetics and the origins of placental mammals. Nature 409:614–618 (2001a).

Murphy WJ, Stanyon R, O’Brien SJ: Evolution of mammalian genome organization inferred from comparative gene mapping. Genome Biol 2:1–8 (2001b).

Nie W, Fu B, O’Brien PC, Wang J, Su W, et al: Flying lemmurs – the flying tree shrews? Molecular cytogenetic evidence for a Scandentia-Dermoptera sister clade. BMC Biol 6:18 (2008).

Reyes A, Gissi C, Catzeflis F, Nevo E, Pesole G, et al: Congruent mammalian trees from mitochondrial and nuclear genes using Bayesian methods. Mol Biol Evol 21:397–403 (2004).

Richard F, Messaoudi C, Bonnet-Garnier A, Lombard M, Dutrillaux B: Highly conserved chromosomes in an Asian squirrel (Menetes bermudoi, Rodentia Sciuridae) as demonstrated by Zoo-FISH with human probes. Chromosome Res 11:597–603 (2003).

Robinson TJ, O’Brien PC, Robinson TJ, Ryder OA, et al: Karyotypic relationships of horses and zebras: results of cross-species chromosome painting. Cytogenet Genome Res 102:235–243 (2003a).

Robinson TJ, Yang F, Harrison WR: Chromosome painting refines the history of genome evolution in hares and rabbits (order Lagomorpha). Cytogenet Genome Res 96:223–227 (2002).

Schneider A, Cannarozzi GM: Support patterns from different outgroups provide a strong phylogenetic signal. Mol Biol Evol 26:1259–1272 (2009).

Sparrow R, Stouffer G, Garcia M, Froenicke L: Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization. Genomcs 82:245–249 (2003).

Stock AD: Chromosome banding pattern relationships of hares, rabbits and pikas (order Lagomorpha). A phyletic interpretation. Cytogenet Cell Genet 17:78–88 (1976).

Svrtafe M, Stone G, Stanyon R: The ancestral eutherian karyotype is present in Xenarthra. PLoS Genet 2:e109 (2006).

Telenius H, Pielme AH, Tunnaliui C, Behmel A, et al: Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. Genes Chromosomes Cancer 4:257–263 (1992).

Trifonov VA, Stanyon R, Nesterenok AI, Fu B, Perelman PL, et al: Multidirectional cross-species painting illuminates the history of karyotypic evolution in Perissodactyla. Chromosome Res 16:89–107 (2008).

Yang F, O’Brien PCM, Milne BS, Graphodatsky AS, Solancy N, et al: A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. Genomics 62:199–202 (1999).

Yang F, Fu B, O’Brien PC, Robinson TJ, Ryder OA, et al: Karyotypic relationships of horses and zebras: results of cross-species chromosome painting. Cytogenet Genome Res 102:235–243 (2003a).

Yang F, Alkalave EZ, Perelman PL, Pardini AT, Harrison WR, et al: Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype. Proc Natl Acad Sci USA 100:1062–1066 (2003b).

Yang F, Fu B, O’Brien PC, Nie W, Ryder OA, et al: Refined genome-wide comparative map of the domestic horse, donkey and human based on cross-species chromosome painting: insight into the occasional fertility of mules. Chromosome Res 12:65–76 (2004).
