

Karyotype Evolution:
Evolutionary Chromosome Rearrangements in
Material Homologous to Human Chromosome
22q Studied in the Dog and Gibbon

by

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Thesis submitted for the degree of
Doctor of Philosophy

The Open University

31st December 2003

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*This thesis is dedicated to my husband, Gavin,
to my parents, Edward and Patricia,
and to my sister, Deborah*

Abstract

Following divergence from the common ancestor, mammalian karyotype evolution has been an ongoing process, by which chromosomes have been structurally and numerically reorganised by various intra- and inter-chromosomal rearrangements. Each mammalian species has a unique karyotype, but despite millions of years of divergent evolution, a balance has occurred between karyotype diversity and conservation. As a result, each mammalian species has a unique arrangement of homologous chromosome blocks known as evolutionarily conserved chromosome segments (ECCSs) within their karyotype.

The study of karyotype evolution requires the definition of ECCSs and the junctions between them, which can lead to an understanding of the underlying mechanisms, which drive evolutionary rearrangements. It is important for our understanding of phylogeny as well as normal and abnormal chromosome structure and sequence organisation in the mammals.

There are several different possible approaches to generating comparative maps of ECCSs, such as comparative sequence analysis. But, in the absence of genome sequence, alternative approaches are required, such as comparative chromosome painting (zoo-FISH), high-resolution cross-species FISH, and cloning and sequencing.

The aim of the work towards this thesis was to study evolutionary chromosome rearrangements involving material homologous to human chromosome 22 in the dog and gibbon. The dog is distantly related to the human and, due to its complex karyotype, had not previously been included in evolutionary studies. For the purposes of studying the dog, it was necessary to produce a standard karyotype, which was a useful outcome for the canine genetics research community. The gibbon is the primate most closely related to the human, which has material homologous to human chromosome 22 in two ECCSs within its karyotype.

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The two species were studied by reciprocal heterologous chromosome painting and cross-species FISH. The gibbon rearrangement was ultimately studied at the sequence level for a detailed analysis of the sequences surrounding the rearrangement breakpoint junctions involving material homologous to human chromosome 22q.

Acknowledgements

I would like to thank my supervisors Mark Ross and Nigel Carter for all their help, advice and guidance provided throughout the course of this thesis. I would also like to thank Margaret Leversha for her help with FISH during the first stages of the work. Thanks also to David Bentley, who acted as an academic sponsor for my registration.

A number of people at the Sanger Institute have made invaluable contributions, without which this thesis would not have been possible. Particular thanks go to Tamsin Wilmer for teaching me STS-PCR, Jackie Bye for help with library curation, Danita Pearson and Elizabeth Southeran for help with library protocols, Alison Coffey for her vectorette know-how, Elizabeth Huckle for sequencing the vectorette fragments, John Collins, Dave Beare and Ian Dunham for their knowledge of chromosome 22 and help with primer design, Graeme Bethel for help with EcoRI digest gels, Rebecca Pavitt, Matt Jones, Kirsten McLay, Claire Bagguley, James Gilbert, Stephen Keenan, Simon Mercer and Brian Wilson for putting my two cosmids through the Sanger sequencing and annotation pipeline, and Sarah Hunt and Carol Scott for primer design and creating Gibbonace,

I would also like to thank Joan and Andrew in the library for providing all the reprints, Richard Summers for all the scanning and binding and Matt Hurles for useful discussions about breakpoints.

Personal thanks go to all my friends, in particular Gareth and Simon (see you on June 5th!), Mark Ross, Dave Vetrie, Matthew Breen, Sheila, Paw, Gillian and Ian, Claire and Steve and Robin and Delphine, for their friendship, support and encouragement.

Final thanks go to my family: My husband, Gavin, my parents, Edward and Patricia and my sister, Deborah.

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Glossary of Abbreviations

1 ⁰	primary
2 ⁰	secondary
BAC	bacterial artificial chromosome
BLAST	Basic Local Alignment Search Tool
BrdU	5-bromodeoxyuridine
BSA	bovine serum albumen
CCD	charge-coupled device
CFA	<i>Canis familiaris</i>
CIAP	calf intestinal alkaline phosphatase
CISS	chromosomal <i>in situ</i> suppression (hybridisation)
C ₀ t	concentration × time (DNA reassociation kinetics)
cv	coefficient of variation
DAPI	4', 6-diamidino-2-phenylindole
dATP	2' deoxyadenosine 5'-triphosphate
dCTP	2' deoxycytidine 5'-triphosphate
dGTP	2' deoxyguanosine 5'-triphosphate
DMEM	Dulbecco's Minimal Essential Medium
DMSO	dimethyl sulphoxide
dNTP	any deoxynucleoside triphosphate, or a mixture of all four
dTTP	2' deoxythymidine 5'-triphosphate
dUTP	2' deoxyuridine 5'-triphosphate
DNA	deoxyribonucleic acid
DOP-PCR	degenerate oligonucleotide-primed polymerase chain reaction
DTT	dithiothreitol
EBV	Epstein-Barr virus
ECACC	European Collection of Animal Cell Cultures
EDTA	ethylenediamine tetraacetic acid (disodium salt)
EGTA	ethylene glycol- <i>bis</i> (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid

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FBS	foetal bovine serum
FISH	fluorescence <i>in situ</i> hybridisation
FITC	fluorescein isothiocyanate
HSA	<i>Homo sapiens</i>
HSRE	high salt restriction endonuclease buffer
HSY	<i>Hylobates syndactylus</i>
kb	kilobase (DNA)
LINE	long interspersed nuclear element
LSRE	low salt restriction endonuclease buffer
Mb	megabase (DNA)
NTA	nitrilotriacetic acid
OMIM	Online Mendelian Inheritance in Man
o/n	overnight
PAC	P1-derived artificial chromosome
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PHA	phytohaemagglutinin
PMSF	phenylmethylsulfonyl fluoride
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute (tissue culture medium)
SAM	S-adenosyl methionine
SDS	sodium dodecyl sulphate
SINE	short interspersed nuclear element
SSC	standard saline citrate
STS	sequence tagged site
TAPS	N-tris(hydroxymethyl)methyl-3-amino-propanesulphonic acid
Tris	tris(hydroxymethyl)aminomethane
U	units
UV	ultraviolet
v/v	volume/volume

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w/v weight/volume

Publications

Parts of the work presented in this thesis have appeared previously in the following publications:

Langford, C. F., Fischer, P. E., Binns, M.M., *et al.* (1996) Chromosome-specific paints from a high-resolution flow karyotype of the dog. *Chromosome Res* 4(2):115-23

Breen, M., Bullerdiek, J., Langford, C. F. (1999a) The DAPI banded karyotype of the domestic dog *Canis familiaris* generated using chromosome-specific paint probes, *Chromosome Res* 7:401-406

Breen, M., Thomas, R., Binns, M. M., Carter, N. P., Langford, C. F. (1999b) Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (*Canis familiaris*) and human. *Genomics*. 61(2):145-55

Langford, C.F. and Breen, M. (2003). Chromosome Structure and Function - Comparative Cytogenetics. In: Nature Encyclopedia of the Human Genome. Macmillan Publishers Ltd. Nature Publishing Group, Hampshire, U.K. www.ehgonline.net