Incorporating Mouse Genome
Mammalian Genome
Genes and Phenotypes

Comparative map between chicken Chromosome 15 and human chromosomal region 12q24 and 22q11-q12

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Abstract

The physical and comparative map of GGA15 was improved by the construction of 9 BAC contigs around loci previously mapped on GGA15 by linkage analysis. In total, 240 BAC clones were isolated, covering 30–35% of GGA15, and 120 STS were developed (104 STS derived from BAC end sequences and 18 STS derived within genes). Seventeen chicken orthologues of human genes located on human Chr 22q11-q12 were directly mapped within BAC contigs of GGA15. Furthermore, the partial sequences of the chicken BAC clones were compared with sequences present in the EMBL/GenBank databases and revealed matches to 26 genes, ESTs, and genomic clones located on HSA22q11-q12 and HSA12q24. These results provide a better alignment of GGA15 with the corresponding regions in human and mouse, and improve our knowledge of the evolution and dynamics of the vertebrate genome.

Introduction

Although birds and mammals diverged over 300 million years ago, several chromosomal segments of similar gene content are conserved between human and chicken (Burt et al. 1999). Comparative mapping studies have been shown to be very useful to identify such homologous chromosome segments in human and chicken. Recently, detailed comparative maps between human and chicken chromosomes have been published (Nanda et al. 2000; Crooijmans et al. 2001; Buitenhuis et al. 2002; Jennen et al. 2002).

GenBank Accession Numbers: The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers BZ592394-BZ592544.

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In our group, a bi-directional approach is used to improve the comparative map of chicken and human (Crooijmans et al. 2001; Buitenhuis et al. 2002; Jennen et al. 2002). First, a BAC contig is built, starting from loci whose positions on the chicken genome are known. Second, genes known to be located in the identified syntenic regions in human and mouse are used to map additional genes in these regions. The linkage map of chicken Chromosome (Chr) 15 (GGA15) is 71 cM in size and contains 19 markers (Groenen et al. 2000). Four chicken orthologues of human genes (*CRYBB1, CRYBA4, IGL@, MIF*), located in the human on Chr 22q11 (HSA22q11), have been mapped to this chicken microchromosome (Schmid et al. 2000).

The aim of this study is to improve the comparative map between GGA15 and HSA22q11 on the chicken genome.

Materials and methods

Chicken Chr 15 BAC clones. The Wageningen chicken BAC library was screened by PCR (Crooijmans et al. 2000) for all microsatellite markers and STS markers within genes located on GGA15. A detailed description of all loci used can be found at the ARKdb farm animal database (http://www.thearkdb.org/). This includes microsatellite marker *MCW0052* located within the gene *IGVPS*. Primers corresponding to all other genes mapped to GGA15 (*CRYBB1, CRYBA4, MIF*) were designed based on database sequences (Table 1). All identified BAC clones were tested for purity by PCR amplification of the marker directly on two single colonies (colony PCR).

Sequencing. BAC-end sequencing and sample sequencing were performed as described by Jennen et al. (2002). Sequences obtained were first analyzed with PREGAP4 of the STADEN software package

Table 1. Characteristics of markers developed in chicken genes

Gene	Accession number		Human cytogenetic map position	PCR size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
	Chicken	Human[a]				
ADRBK2	AJ397769	NM_005160	22q12.1	95	CTGACCATGAATGACTTCAG	CAGTGTCTGCTTTTCTGCAC
CDC45L	AJ393544	NM_003504	22q11.21	97	TATTGGAATTCTGCAGCGCC	TTCAAATGCTATTCGCATGC
CRYBA4	U18260	NM_001886	22q12.1	172	TGGTGTGTTTGCGCTTGGAG	TTGCAGCTCCTAATTCCTCC
CRYBB1	U09951	NM_001887	22q12.1	318	CCCTGTACGAGTCTGCTGAC	CGACGGATGGACTGGATCTG
CRYBB2	S52930	NM_000496	22q11.23	122	TCCAACTGAAGCCCTCGCAC	AGCTTTATTCGGTTGCGGTG
CRYBB3	U28146	NM_004076	22q11.23	198	GCCAGCACGTCTTTGAGAAG	AAGAACACCAAACCGATGGC
GSTT1	U13676	NM_000853	22q11.23	152	CTTTAACCACTGCTCTCCAC	GCAATTATGTGAAGGCTGTG
HIRA	X99375	NM_003325	22q11.21	138	AGGATTTACTGGGTCCAGTC	CTGTGAAAAGCCTCTGGAAG
MIF	M95776	NM_002415	22q11.23	97	AGTACATAGCCCGTGCACATC	TAGAACGGTTACGACATCTC
PITPNB	AI979795	NM_012399	22q12.1	115	GGTCAACTTTATTCGGTGGC	TGAGTATATTGTCCCTTCTC
PNUTL1	AJ393439	NM_002688	22q11.21	124	GGAAGCCCATCACTGACTAC	AAGGGCGAGATGAAGTAGAG
PPIL2	AW198371	NM_014337	22q11.21	97	AATACAGAGACCCGAGAGAC	CTTTCTTCTTTTCAGGAGCC
RANBP1	AF179468	NM_002882	22q11.21	138	GCCCTTTTGAAGATGTCTTC	AATCCTTCGGAGGCTGTTTC
SMARCB1	AJ398441	NM_003073	22q11.23	134	AATCGCGATTCGCAACACGG	CAGTGTTGGCCAAGCGTCTC
TFIP11	AJ396682	NM_012143	22q12.1	155	TCCTGCTTGGAACTGTGAAG	TATCATTTCACCTCCTGCCG
UFD1L	AF228284	NM_005659	22q11.21	127	GTTCTCCTTCAACATGTTCG	GCCTTTCTCCACATCTGACC
XBP1	AJ394086	NM_005080	22q12.1	120	CGCAGCACTCAGACTACGTG	GAATCTGAAGAGTCACTGCC
TBX3	AF033669	NM_016569	12q24.1	159	CATGTACTGTGCTGTTTAGAG	CTTCCTACTGCAGGAGTAGTC

[a] Accession number of human genes used in BLAST search to identify chicken orthologous genes.

(Bonfield et al. 1995; http://www.mrc-lmb.cam.ac.uk/pubseq). The network BLAST client software (blastc13) of the NCBI was used to compare the sequences of good quality reads with sequences deposited in public databases. The BAC-end sequences were also used to develop new STS markers for chromosome walking. Sample sequences and BAC-end sequences, including STS markers have been submitted to GenBank and have been assigned the accession numbers BZ592394–BZ592544.

Mapping of genes. Genes of interest were either mapped to BACs that were already present within known BAC contigs or mapped by SNP typing as described by Buitenhuis et al. (2002). The SNP was first detected in the parents of the Wageningen mapping population (Groenen et al. 1998). A specific restriction enzyme for the SNP was used to map the gene as a PCR-RFLP on one selected family from the Wageningen mapping population.

Analysis of chromosomal rearrangements. Chromosomal rearrangements were analyzed by using GRIMM (Tesler 2002; http://www-cse.ucsd.edu/groups/bioinformatics/GRIMM). GRIMM enables the analysis of rearrangements in multichromosomal genomes and provides a new algorithm for analyzing comparative maps for which gene directions are unknown. Gene data sets based on the comparative map between human, mouse, and chicken were used for the calculation of the minimum possible number of rearrangements steps (the multichromosomal distance) between chicken and human, chicken and mouse, and human and mouse. The data sets were used with an unsigned gene order, because the gene orientation in chicken is unknown.

Results

Construction of GGA15 BAC contigs. BAC contigs of GGA15 were constructed around loci known to be located on this chromosome. The Wageningen chicken BAC library was initially screened with 17 markers. One BAC clone per marker was selected for end sequencing. The BAC-end sequences were used to design specific STS markers for chromosome walking. In total, 104 STS markers were designed and 240 BAC clones isolated, which resulted in the construction of nine BAC contigs.

Identification and mapping of genes. Since GGA15 showed conservation of synteny with HSA22q11 (Schmid et al. 2000), chicken orthologues of human genes from HSA22q11-q12 were identified to further increase the number of starting points for

chromosome walking. Chicken orthologues from 14 human genes were identified by using a BLAST search with the mRNA sequences of human genes known to be located on HSA22q11-q12. For these 14 genes, STS markers were developed to screen the BAC library (Table 1). Nine genes—*ADRBK2*, *CRYBB2*, *CRYBB3*, *GSTT1*, *PITPNB*, *RANBP1*, *SMARCB1*, *TFIP11*, and *XBP1*—were mapped to BACs that were already present within the BAC contigs of GGA15.

The other five genes—*CDC45L, HIRA, PNUTL1, PPIL2*, and *UFD1L*—were mapped to BACs that formed a single contig, which had not yet been assigned to a chromosome. With PCR-RFLP, this BAC contig could also be mapped genetically to GGA15. Restriction enzyme *Hha*I was used to map the BAC clone bW041F24 positive for locus *HIRA* on the chicken linkage map. *HIRA* and, therefore, the complete BAC contig, were mapped close to microsatellite marker *MCW0031* (recombination fraction = 0; LOD score = 12.64).

The chicken orthologue of *TBX3*, which in human is located on HSA12q24, was initially used within another project and, by using PCR-RFLP, was found to be located on GGA15. With restriction enzyme *Tsp* 509 I, BAC clone bW110C15 positive for locus *TBX3* was mapped on the chicken linkage map close to *ACW0169* (recombination fraction = 0.04; LOD score = 10.54).

To further increase the number of genes mapped to GGA15, 19 different BAC clones from GGA15 contigs were used for sample sequencing. The sequences obtained by sample sequencing and BAC-end sequencing were compared with sequences in Genbank and with the UMIST Chicken EST sequences (Boardman et al. 2002; http://www.chick.umist.ac.uk) by using the BLAST algorithm. In total, sequence identity was found to 66 genes, ESTs, and genomic clones from chicken, human, and other vertebrates. The BLAST hits showed homology to 10 sequences from HSA22q11-q12 and to 16 sequences from HSA12q24. Homology to two genes and two anonymous genome segments from HSA3 was also found (Fig. 1a; Table 2), clearly marking a conserved segment. Furthermore, a sample sequence of BAC clone bW086M10 showed homology to a genomic clone from HSA1 (Fig. 1a; Table 2), but did not show homology to any annotated gene. On average, sequence homology with chicken sequences was 96.9%, and with human sequences, 81.5%.

Analysis of chromosomal rearrangements. GRIMM was used for the calculation of the multichromosomal distance between chicken and human, chicken and mouse, and human and mouse.

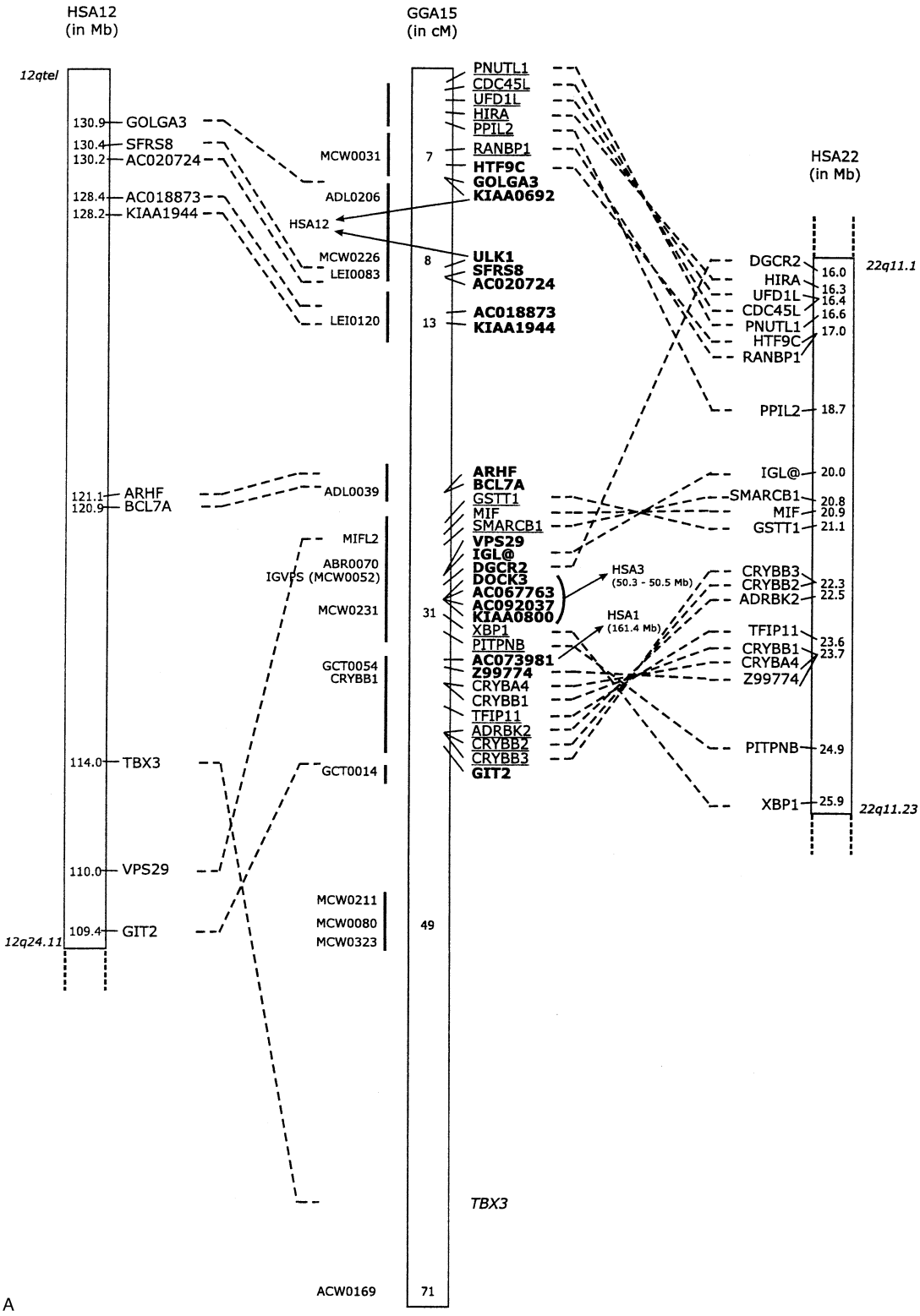
Based on the comparative map between human, mouse, and chicken as shown in Fig. 2, the following data sets were generated (format as needed for GRIMM):

```
>chicken
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 2728 29 30 $
>human
8 9 10 11 12 30 16 29 $
18 4 3 2 1 7 6 5 17 15 14 13 28 27 26 25 24 23 21 $
19 20 $
22 $
>mouse
5 18 6 7 1 2 3 4 17 $
13 14 15 $
19 20 $
8 9 21 23 24 25 26 27 28 29 30 16 12 11 10 $
22 $
```

These data sets were used with an unsigned gene order, because the gene orientation in chicken is unknown. The gene order for human and mouse is given per chromosome. Calculations resulted in a multichromosomal distance between chicken and human of 11, between chicken and mouse of 12, and between human and mouse of 6.

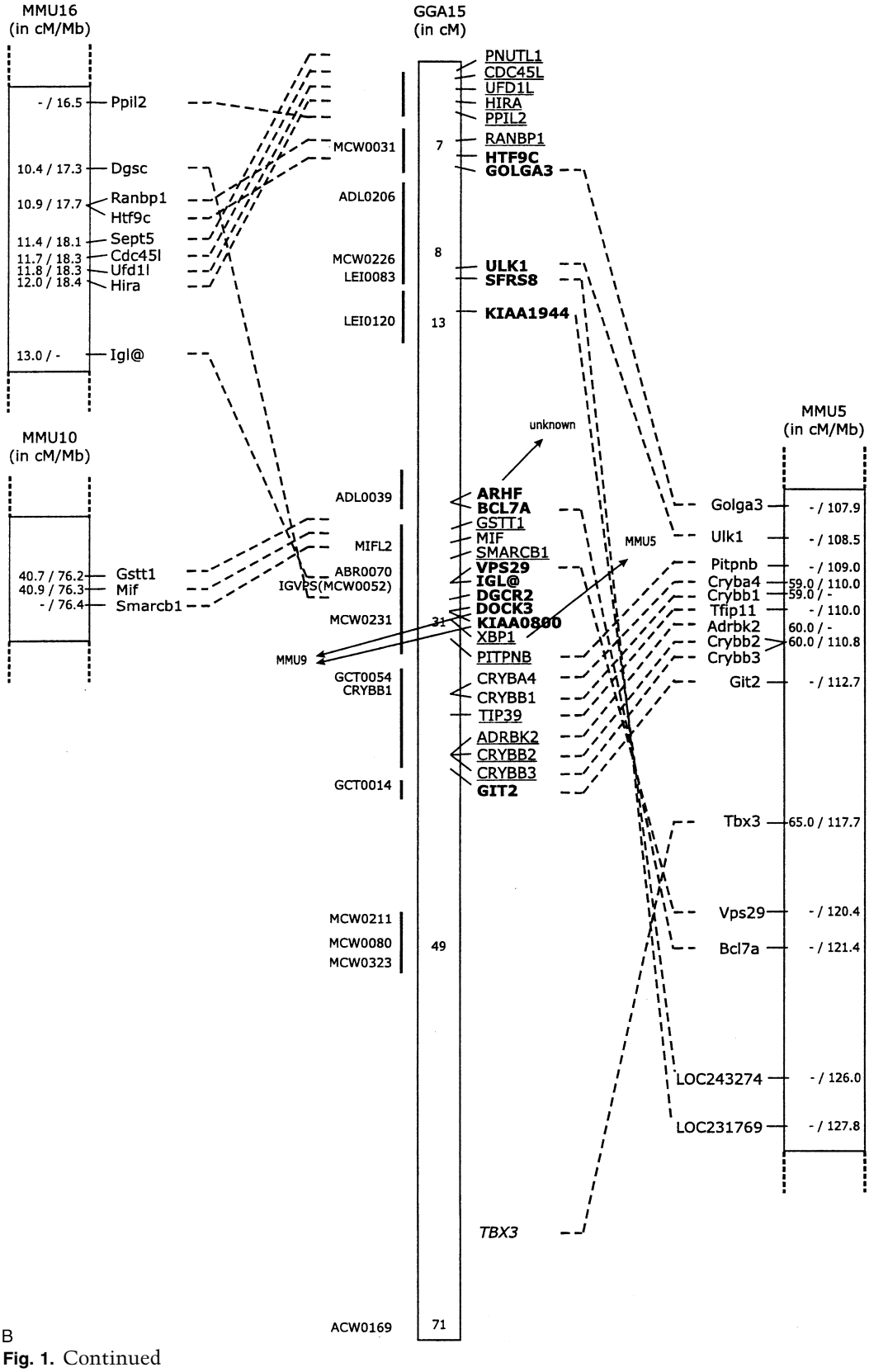
Discussion

GGA15 BAC contigs. The linkage map of GGA15 is 71 cM, which is about 1.8% of the total chicken genome (~4000 cM) (Groenen et al. 2000; Groenen and Crooijmans 2003). On the basis of the physical size of the chicken genome of 1.2×10^9 bp, 1 cM on average corresponds to 300 kb. Although there are some indications that this ratio is somewhat different for macrochromosomes versus microchromosomes, the estimated physical size for GGA15 would be around 21 Mb. From the average insert size of the BACs of 134 kb (Crooijmans et al., 2000) and correcting for the overlap between the different BACs, we calculated that the BACs would cover around 30–35% of GGA15. However, because no markers were identified between microsatellite marker *MCW0323* and AFLP marker *ACW0169* (distance 22 cM) to screen the Wageningen BAC library, no BAC clones could be found in this region, except for the BAC clone positive for *TBX3*. Therefore, only the first 49 cM of GGA15 (between *ADL0206* and *MCW0323)* are covered, with BAC clones in nine contigs. For this region the coverage is estimated to be almost 50%. On average, 4.8 BAC clones were obtained per marker, which is comparable to the previously reported number (Crooijmans et al. 2001; Buitenhuis et al. 2002; Jennen et al. 2002).



A

Fig. 1. Comparative map of (**a**) GGA15 and part of HSA12 and 22, and (**b**) GGA15 and part of MMU5, 10, and 16. Estimated positions for mouse and human are given in cM and/or Mb, according to the Map Viewer from Entrez Genomes (http://www.ncbi.nlm.nih.gov/). The loci located on the linkage map of the chicken genome (Groenen et al. 2000) are shown on the left of the vertical bar. The numbers inside the vertical bar of GGA15 represent the relative positions in cM of the chicken loci. On the left of the vertical bar from GGA15 the 9 BAC contigs are shown in solid bars (not to scale). The genes (human name) and genomic clones (human accession number) located on GGA15 are shown on the right site. Genes mapped in this study by sequence comparison using the BLAST algorithm are in bold; genes mapped by chromosome walking are underlined; and genes used in PCR-RFLP studies are in italics.



B

Fig. 1. Continued

Table 2. BAC sample sequencing hits and accession numbers

Locus[a]	*BAC clone*	*Accession number*[b]	*BLAST hit*[c]	*Accession number*[d]	*Human orthologue*[e]	*bp Homology*	*% Identity*	*HSA map position*		*MMU map position*
								Cytogenetic map	*Sequence map*[f] (Mb)	
ST15BE059	bW128N01	BZ592498	**chicken EST**	AL584815		590	96			
		BZ592499	**chicken EST**	*334464.5*	RANBP1	659	97	22q11.21	17.0	16 (10.9 cM)
		BZ592500	**chicken EST**	*334464.5*	RANBP1	702	95	22q11.21	17.0	16 (10.9 cM)
		BZ592501	**chicken EST**	AL584937		590	95			
		BZ592502	**chicken EST**	AF179468	RANBP1	119	100	22q11.21	17.0	16 (10.9 cM)
		BZ592503	**chicken EST**	AF179468	RANBP1	58	100	22q11.21	17.0	16 (10.9 cM)
		BZ592504	**chicken EST**	*043373.1*		392	98			
MCW0031	bW109B14	BZ592505	**chicken EST**	*043373.1*		461	99			
		BZ592506	**chicken EST**	AL584937		555	93			
		BZ592507	**chicken EST**	*336214.1*	HTF9C	183	100	22q11.21	17.0	16 (10.9 cM)
			chicken EST	AJ445064	HTF9C	175	100	22q11.21	17.0	16 (10.9 cM)
		BZ592405	**chicken EST**	AJ397158		248	98			
			chicken EST	*333056.2*		635	96			
ST15BE009	bW044P19	BZ592432	**chicken EST**	*022848.1*		149	98			
ST15BE074	bW050B14	BZ592508	KIAA0692	AK024061		152	86	12	cyt	
		BZ592509	**chicken EST**	*002176.1*		134	98			
		BZ592510	**chicken EST**	*016090.1*		423	96			
		BZ592511	**chicken EST**	*331940.5*	GOLGA3	330	96	12q24.33	103.9	5 (62.0 cM)
		BZ592512	**chicken EST**	*331940.5*	GOLGA3	470	96	12q24.33	103.9	5 (62.0 cM)
		BZ592452	**chicken EST**	*333056.2*		439	95			
			chicken EST	*050929.1*		302	96			
ST15BE131	bW058E06	BZ592482	**chicken EST**	BM440415		239	92			
			chicken EST	*355017.8*		588	96			
ADL0206	bW091F08	BZ592513	**chicken EST**	*344548.1*		275	96			
ST15BE055	bW037I08	BZ592514	rat BAC clone	AC115194		146	84			
		BZ592515	**chicken EST**	*038082.1*		124	96			
		BZ592516	**chicken EST**	*056038.1*	ULK1	149	99	12q24.3	cyt	5
		BZ592517	**chicken EST**	*050266.1*		579	95			
LEI0083	bW017J22	BZ592518	**chicken EST**	*319066.1*		328	98			
		BZ592519	SFRS8	AA374595		68	88	12q24.13	130.4	5
		BZ592520	**chicken EST**	AJ442844	SFRS8	178	94	12q24.13	130.4	5
		BZ592521	human BAC clone	AC020724		260	95	12	130.2	
			chicken EST	*355445.1*		594	99			
		BZ592404	**chicken EST**	*018980.1*		265	97			
ST15BE002	bW034D23	BZ592448	human BAC clone	AC020724		227	89	12	130.2	
			SFRS8	NM_004592		126	89	12q24.13	130.4	5
ST15BE118	bW054N17	BZ592481	**chicken EST**	*019152.1*		545	97			
ST15BE089	bW034E12	BZ592522	KIAA1944	AB075824		80	88	12q24.33	128.2	
LEI0120	bW049K11	BZ592523	**chicken EST**	*021433.1*		246	99			
ST15BE005	bW020E20	BZ592427	human BAC clone	AC018873		85	92	12	128.4	
ADL0039	BW113E05	BZ592524	**chicken EST**	AJ447520	BCL7A	104	94	12q24.13	120.9	5
		BZ592525	**chicken EST**	*044129.1*	BCL7A	256	99	12q24.13	120.9	5
		BZ592526	**chicken EST**	AJ453931	ARHF	229	96	12q24.31	121.1	
		BZ592527	**chicken EST**	BQ037409		271	97			

Table 2. continued

		BZ592408	**chicken EST**	*044925.2*		587	98			
ST15BE093	bW017B07	BZ592461	**chicken EST**	AJ444684		193	95			
			chicken EST	AJ396350		60	98			
			chicken EST	*334980.3*		504	98			
		BZ592528	**chicken EST**	BI394105		63	95			
		BZ592529	**chicken EST**	*340810.4*		199	95			
ST15BE017	bW014A05	BZ592425	**chicken genomic clone**	AF079888[a]	VPS29	860	95	12q24	110.0	5
			chicken EST	*051914.1*	VPS29	103	99	12q24	110.0	5
MCW0052	bW122C13	BZ592530	**chicken genomic clone**	AF079888[a]	VPS29	520	93	12q24	110.0	5
		BZ592531	**chicken genomic clone**	AF079888[a]	VPS29	596	97	12q24	110.0	5
		BZ592532	**chicken genomic clone**	AF079888[a]	VPS29	462	96	12q24	110.0	5
		BZ592533	**chicken EST**	*051914.1*	VPS29	406	97	12q24	110.0	5
		BZ592534	**IGL@**	M97945	IGL@	254	91	22q	20.0	16 (13.0 cM)
		BZ592535	**IGL@**	M15141	IGL@	212	97	22q	20.0	16 (13.0 cM)
		BZ592536	**IGL@**	M24403	IGL@	544	95	22q	20.0	16 (13.0 cM)
		BZ592537	**Cidd**	X95885	DGCR2	42	97	22q11.21	16.1	16 (10.37 cM)
		BZ592409	**chicken EST**	BI392070	DGCR2	118	98	22q11.21	16.1	16 (10.37 cM)
			chicken EST	*356683.3*		117	98			
ST15BE091	bW033N17	BZ592538	human BAC clone	AC092037		97	87	3	50.5	
		BZ592539	human BAC clone	AC067763		65	87	3	50.3	
		BZ592540	**chicken EST**	*056914.3*	DOCK3	389	96	3p21.3	50.3	9
		BZ592541	**chicken EST**	AJ443413	KIAA0800	90	94	3p21.31	50.5	9
		BZ592542	**chicken EST**	T20324		172	93			
			chicken EST	*056914.3*	DOCK3	442	99	3p21.3	50.3	9
		BZ592543	**chicken EST**	*056914.3*	DOCK3	368	98	3p21.3	50.3	9
MCW0231	bW107C20	BZ592402	**chicken EST**	*053822.1*		263	100			
ST15BE125	bW086M10	BZ592483	human BAC clone	AC073981		152	84	1	161.4	
GCT0054	bW043G23	BZ592544	**chicken EST**	*356750.1*		674	97			
			chicken EST	AI982143		546	94			
		BZ592439	human BAC clone	Z99774		57	92	22q11.22-12.2	23.8	
		BZ592440	**chicken EST**	BQ038575		551	92			
ST15BE122	bW017H24	BZ592489	**p95PKL**	AF112366	GIT2	367	98	12q24.1	109.4	5
			chicken EST	*3326211*	GIT2	367	97	12q24.1	109.4	5
GCT0014	bW047O08	BZ592477	**chicken EST**	BM491459		498	98			
			chicken EST	*043045.1*		413	98			

[a] Order of loci according to the chicken linkage map (Groenen et al. 2000) and Fig. 1.
[b] Genbank accession number of BAC sample sequence.
[c] BLAST hits to chicken genes and ESTs are in bold; the other hits are directly to human and other vertebrates.
[d] Genbank accession number or UMIST cluster number (in italics) of BLAST hit.
[e] Human orthologues of chicken sequences; anonymous ESTs and genome segments are not shown.
[f] Sequence map position is according to NCBI Map Viewer build30 (http://www.ncbi.nlm.nih.gov/).
[g] AF079888 is known as Gallus gallus immunoglobulin light chain gene, 5′ DNaseI hypersensitive site sequence.

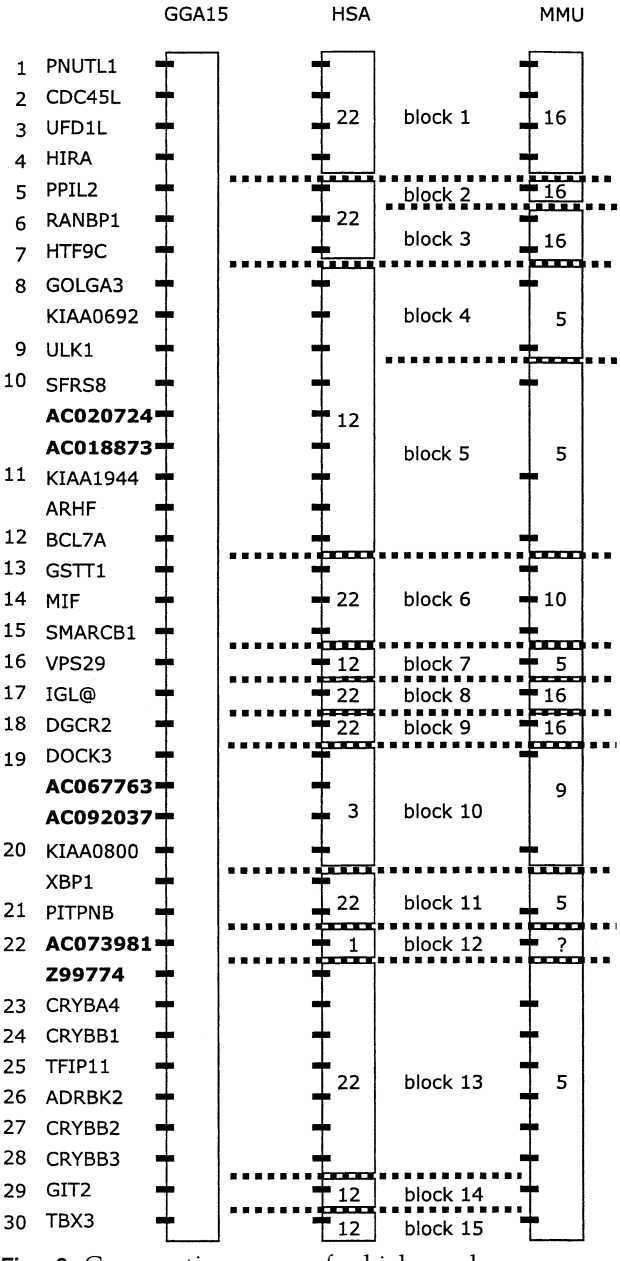


Fig. 2. Comparative map of chicken chromosome 15 (GGA15) to human (HSA) and mouse (MMU). A number is assigned to each gene and genomic clone (1–30), which were mapped in all three species. Chromosome segments in which the gene order in all three species is the same, are indicated by block 1–15. Positions of chromosomal rearrangements are indicated by dotted lines, with the chicken gene order as a start. The numbers of the human and mouse chromosomes are shown inside the vertical bars of HSA and MMU respectively.

The beta crystallin gene cluster. In human, *CRYBB1* and *CRYBA4* form a gene cluster with *CRYBB2*, *CRYBB2P1*, and *CRYBB3*, the beta-crystalline gene cluster. Orthologues of all human members of this gene cluster, except for the pseudogene *CRYBB2P1*, are present in mouse as a gene cluster on chromosome 5 (MMU5) (Hulsebos et al. 1995a, 1995b). From the comparative map, we expected the chicken orthologues of human *CRYBB2* and *CRYBB3* to form a gene cluster with chicken *CRYBB1* and *CRYBA4*. The results above confirm our expectation of the four chicken beta crystallin genes to form a gene cluster. Figure 3 shows that all four beta crystallin genes are located on overlapping BAC clones in the same BAC contig; *CRYBB1* and *CRYBA4* are located on BAC clones bW043G23 and bW093I01, whereas *CRYBB2* and *CRYBB3* are located on bW031O19, bW058F23 and bW070F04. In chicken, *CRYBB1* and *CRYBA4* are linked head to head, with 2147 nt of intervening sequence (Duncan et al. 1995). Also in human both genes are tightly linked head to head, with 3890 bp in between (NCBI Map Viewer build30; http://www.ncbi.nlm.nih.gov/). It can be expected that chicken *CRYBB2* and *CRYBB3* are also tightly linked, because the distance between the human genes is 2235 bp.

Furthermore, in our study the distance between *CRYBB1* and *CRYBB2* is equal to one BAC clone (~134 kb), which is about 10 times smaller than in human. This in not according to the whole-chicken genome size, which is on average three times smaller than the human. Nevertheless, it could be that genome sizes differ more on specific spots.

Chicken versus human and mouse. Although, GGA15 initially showed conservation of synteny only with HSA22, our results also showed conservation of synteny with HSA12. However, from an ancestral point of view, our findings are not at all surprising. Several reports on the evolution of the mammalian genome (Murphy et al. 2001; Haig 1999; O'Brien and Stanyon 1999; Chowdhary et al. 1998) describe two ancestral chromosomes, which are both a combination of human Chrs 12 and 22. These ancestral chromosomes were reconstructed by using chromosome paints and comparative maps of several primates, rodents, and other mammalian species. The first ancestral chromosome is a combination of HSA12p-q and HSA22qtel (12pq-22qtel) and shows conservation of synteny with segments of GGA1 (Murphy et al. 2001; Schmid et al. 2000). The second ancestral chromosome comprises HSA12qtel and HSA22q (22-12qtel) and is syntenic to GGA15 (this paper).

In order to reconstruct the common ancestor of mouse, human, and chicken, we compared the gene order in chicken with the human and mouse maps. This comparison clearly shows a large number of intra- and interchromosomal rearrangements. For example, the BAC clone bW122C13 within the

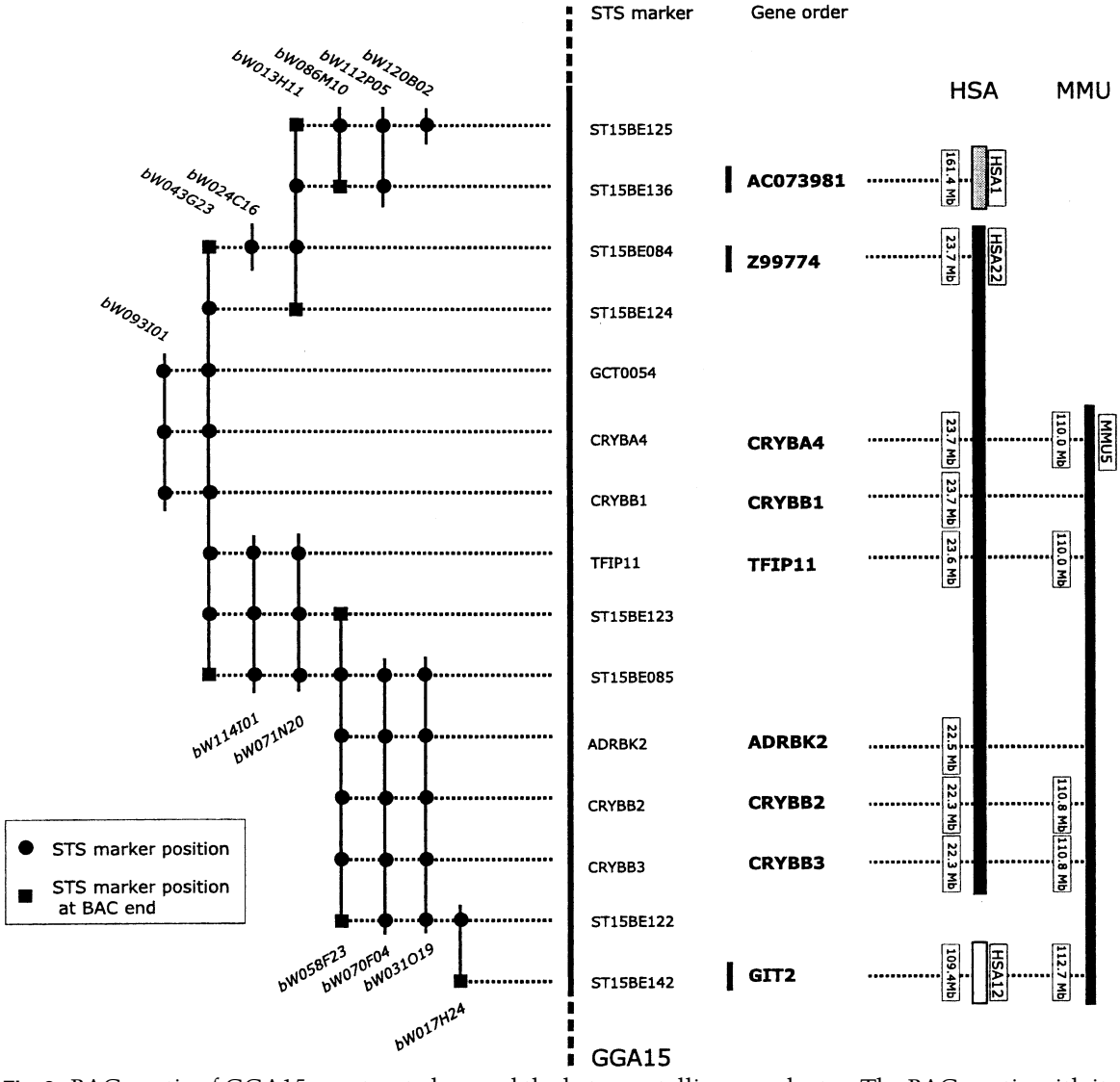


Fig. 3. BAC contig of GGA15 constructed around the beta crystallin gene cluster. The BAC contig with its BAC clones and STS markers is shown on the left and the comparative map with human and mouse on the right. Coloured bars give the positions of the genes and genomic clones in human and mouse. Human and mouse map positions are given in Mb.

contig of markers *ABR0070*, *MCW0052*, and *MCW0231* contains the gene *DGCR2* and gene cluster *IGL@* (including IGVPS), both located in human on HSA22q, and the gene *VPS29*, which is located in human on HSA12q24 (Fig. 1a). The presence of *DGCR2* and *IGL@* from HSA22 as well as *VPS29* from HSA12 on the same BAC clone suggests that a chromosomal breakpoint is located between *IGL@* and *VPS29*. This chromosomal breakpoint can also be found in mouse, where *IGL@* is located on MMU16 and *VPS29* is located on MMU5 (Fig. 1b).

Another example of a chromosomal breakpoint can be seen in the BAC contig containing the beta crystallin gene cluster (Fig. 2). Conserved blocks of genes are shown in all three species within this region. In human, the chromosomal breakpoint can be found between the genes *CRYBB3* (HSA22q11) and *GIT2* (HSA12q24). This breakpoint is absent in mouse, which is in good agreement with the mammalian ancestor 22-12qtel.

Both examples indicate that during evolution the breakpoints occurred after the separation of the mammalian and bird lineages. Moreover, the second example clearly shows that the breakpoint occurred in the human lineage after the human and mouse lineages separated, suggesting that the origin of the chromosome breaks was in human.

A more detailed comparison of the conserved chromosome segments between GGA15 and the human and mouse chromosomes is shown in Fig. 2. The order of the conserved segments is based on a

combination of genetic mapping, chromosome walking results, and sequencing. We can identify at least 15 blocks, which is the minimum number of conserved segments between GGA15 and the human and mouse chromosomes. The number of genes/genomic clones per block varies from 1 (blocks 2, 7, 8, 9, 12, 14, and 15) to 7 (block 13). The dotted lines indicate the points where chromosomal rearrangements took place. For analyzing these rearrangements, we used GRIMM. Between human and mouse the lowest multichromosomal distance (6) is calculated. The distances between chicken and human (11) and between chicken and mouse (12) are comparable. These results are in agreement with the fact that human and mouse evolved from a common ancestor and, therefore, are more closely related than either one is to chicken. This is in contrast to the findings of Burt et al. (1999), who looked at the whole chicken genome and concluded that the genomes of chicken and human are more alike than those of mouse and human. However, the conclusion of Burt et al. (1999) was based on a limited number of mapped genes and conserved segments.

Using the same approach as Burt et al. (1999) and assuming that the GGA15 data are representative for the whole chicken genome, we predict the total number of conserved segments in the chicken–human–mouse comparison to be at least 800 and the total number of chromosomal rearrangements to be in the same size order. Crooijmans et al. (2001) estimated, in the comparative mapping study between GGA10 and HSA15, the total number of conserved segments to be at least 600. From these numbers, we estimate the rate of chromosomal change in the chicken lineage to range from 1.6 to 2 rearrangements per Myr since the divergence 300 Myr ago, which is slightly higher than the estimate of 1.5 in the human and mouse lineages (Pevzner and Tesler 2003).

The comparison between the results of Burt et al. (1999) and our results indicates that a higher gene density as well as the exact gene order and orientation are needed to better understand the evolutionary events that took place in the lineage leading to human, mouse, and chicken.

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References

1. Boardman PE, Sanz-Ezquerro J, Overton IM, Burt DW, Bosch E et al. (2002) A Comprehensive collection of chicken cDNAs. Curr Biol 12, 1965–1969
2. Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Res 25, 4992–4999
3. Buitenhuis AJ, Crooijmans RPMA, Bruijnesteijn van Coppenraet ES, Veenendaal A, Groenen MAM et al. (2002) Improvement of the comparative map of chicken chromosome 13. Anim Genet 33, 249–254
4. Burt DW, Bruley C, Dunn IC, Jones CT, Ramage A et al. (1999) The dynamics of chromosome evolution in birds and mammals. Nature 402, 411–413
5. Chowdhary BP, Raudsepp T, Frönicke L, Scherthan H (1998) Emerging patterns of comparative genome organization in some mammalian species as revealed by Zoo-FISH. Genome Res 8, 577–589
6. Crooijmans RPMA, Vrebalov J, Dijkhof RJM, Van der Poel JJ, Groenen MAM (2000) Two-dimensional screening of the Wageningen chicken BAC library. Mamm Genome 11, 360–363
7. Crooijmans RPMA, Dijkhof RJM, Veenendaal T, van der Poel JJ, Nicholls RD et al. (2001) The gene orders on human chromosome 15 and chicken chromosome 10 reveal multiple inter- and intra-chromosomal rearrangements. Mol Biol Evol 18, 2102–2109
8. Duncan MK, Haynes II JI, Piatigorsky J (1995) The chicken betaA4- and betaB1-crystallin-encoding genes are tightly linked. Gene 162, 189–196
9. Groenen MAM, Crooijmans RPMA (2003) Chapter 26. Structural genomics: integrating linkage, physical and sequence maps. In: Muir WM, Aggzey SE (eds) Poultry genetics, breeding and technology. CAB International, Wallingford, UK, pp 497–536
10. Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M et al. (1998) A comprehensive microsatellite linkage map of the chicken genome. Genomics 49, 265–274
11. Groenen MAM, Cheng HH, Bumstead N, Benkel BF, Briles WE et al. (2000) A consensus linkage map of the chicken genome. Genome Res 10, 137–147
12. Haig D (1999) A brief history of human autosomes. Philos Trans R Soc Lond B Biol Sci 354, 1447–1470
13. Hulsebos TJM, Jenkins NA, Gilbert DJ, Copeland NG (1995a) The beta crystallin genes on human chromosome 22 define a new region of homology with mouse chromosome 5. Genomics 25, 574–576
14. Hulsebos TJM, Gilbert DJ, Delattre O, Smink LJ, Dunham I et al. (1995b) Assignment of the beta B1 crystallin gene (CRYBB1) to human chromosome 22 and mouse chromosome 5. Genomics 29, 712–718
15. Jennen DGJ, Crooijmans RPMA, Kamps B, Açar R, Veenendaal A et al. (2002) A comparative map of chicken chromosome 24 and human chromosome 11. Anim Genet 33, 205–210

16. Murphy WJ, Stanyon R, O'Brien SJ (2001) Evolution of mammalian genome organization inferred from comparative gene mapping. Genome Biol 2, reviews 0005.1–0005.8
17. Nanda I, Zend-Ajusch E, Shan Z, Grützner F, Schartl M et al. (2000) Conserved synteny between the chicken Z sex chromosome and human chromosome 9 includes the male regulatory gene *DMRT1*: a comparative (re)-view on avian sex determination. Cytogenet Cell Genet 89, 67–78
18. O'Brien SJ, Stanyon R (1999) Ancestral primate reviewed. Nature 402, 365–366
19. Pevzner P, Tesler G (2003) Genome rearrangements in mammalian evolution: lessons from human and mouse genomes. Genome Res. 13, 37–45
20. Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn H et al. (2000) First report on chicken genes and chromosomes 2000. Cytogenet Cell Genet 90, 169–218
21. Tesler G (2002) GRIMM: genome rearrangements web server. Bioinformatics 18, 492–493