Second report on chicken genes and chromosomes 2005

Cytogenet Genome Res 109:415–479 (2005)
DOI: 10.1159/000084205

Cytogenetic and Genome Research

Second Report on Chicken Genes and Chromosomes 2005

Organized by
Michael Schmid
Indrajit Nanda
David W. Burt

Painting on a dinner plate. French, possibly Lunéville, early 1800's. Royal Ontario Museum, Toronto.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1424–8581/05/1094–0415$22.00/0

Accessible online at:
www.karger.com/cgr

Second report on chicken genes and chromosomes 2005

Prepared by

M. Schmid,[a] I. Nanda,[a] H. Hoehn,[a] M. Schartl,[b] T. Haaf,[c] J.-M. Buerstedde,[d] H. Arakawa,[d] R.B. Caldwell,[d] S. Weigend,[e] D.W. Burt,[f] J. Smith,[f] D.K. Griffin,[g] J.S. Masabanda,[g] M.A.M. Groenen,[h] R.P.M.A. Crooijmans,[h] A. Vignal,[i] V. Fillon,[i] M. Morisson,[i] F. Pitel,[i] M. Vignoles,[i] A. Garrigues,[i] J. Gellin,[i] A.V. Rodionov,[j] S.A. Galkina,[k] N.A. Lukina,[k] G. Ben-Ari,[l] S. Blum,[l] J. Hillel,[l] T. Twito,[m] U. Lavi,[m] L. David,[n] M.W. Feldman,[n] M.E. Delany,[o] C.A. Conley,[p] V.M. Fowler,[q] S.B. Hedges,[r] R. Godbout,[s] S. Katyal,[s] C. Smith,[t] Q. Hudson,[t] A. Sinclair[t] and S. Mizuno[u]

[a] Department of Human Genetics, University of Würzburg, Würzburg (Germany);
[b] Department of Physiological Chemistry I, University of Würzburg, Würzburg (Germany);
[c] Department of Human Genetics, University of Mainz, Mainz (Germany);
[d] Department of Molecular Radiobiology, GSF, Neuherberg (Germany);
[e] Department of Animal Science and Animal Behavior, Mariensee, Federal Agricultural Research Center (Germany);
[f] Department of Genetics and Genomics, Roslin Institute, Roslin (Edinburgh), Midlothian (UK);
[g] Department of Biological Sciences, University of Brunel, Uxbridge (UK);
[h] Animal Breeding and Genetics Group, Wageningen University, Wageningen (The Netherlands);
[i] Institut National de la Recherche Agronomique, Centre INRA de Toulouse (France);
[j] Komarov Botanical Institute, St. Petersburg (Russia);
[k] Biological Institute, St. Petersburg State University, St. Petersburg (Russia);
[l] The Hebrew University, Rehovot (Israel); [m] ARO, Volcani Center (Israel); [n] Stanford University, Stanford (USA);
[o] Department of Animal Science, University of California, Davis (USA);
[p] NASA Ames Research Center, Space Life Sciences Research Branch, Moffet Field (USA);
[q] Department of Cell Biology, The Scripps Research Institute, La Jolla (USA);
[r] Department of Biology, Pennsylvania State University, University Park (USA);
[s] Department of Oncology, University of Alberta, Cross Cancer Institute, Edmonton (Canada);
[t] Department of Pediatrics and Murdoch Childrens Research Institute, University of Melbourne, Melbourne (Australia);
[u] Department of Agricultural and Biological Chemistry, College of Bioresource Sciences, Nihon University, Fujisawa (Japan)

Contributions received from 7 March 2003 to 17 November 2004; accepted for publication by M. Schmid 25 November 2004.

Supported by the UK Biotechnology and Biological Research Council, by grant RFBR 03-04-49477 and grant MK-4046.2004.04 from the President of the Russian Federation, by the Deutsche Forschungsgemeinschaft, grant SCHM484/21-1 and SCHM 484/21-2, by Grants-in-Aid for Scientific Research on Priority Areas (2) 14014248 and 15011252 from the Ministry of Education, Culture, Sports, Science and Technology and Grant-in-Aid for Scientific Research (B) 12460036 from Japan Society for the Promotion of Science, by the United States Department of Agriculture 02-35205-11568, by a research grant from the National Cancer Institute of Canada with funds from the Canadian Cancer Society, by the EU Framework VI program "Geninteg", and by grants from the National Science Foundation and NASA.

This report is dedicated to the memory of Shigeki Mizuno (Nihon University, Fujisawa).

Request reprints from:
Dr. Michael Schmid
Department of Human Genetics, University of Würzburg
Biozentrum, Am Hubland, D–97074 Würzburg (Germany)
telephone: 0049-931-888-4077; fax: 0049-931-888-4058
e-mail: m.schmid@biozentrum.uni-wuerzburg.de

Dr. Indrajit Nanda, Department of Human Genetics, University of Würzburg
Biozentrum, Am Hubland, DE–97074 Würzburg (Germany)
telephone: 0049-931-888-4078; fax: 0049-931-888-4058
e-mail: ijn@biozentrum.uni-wuerzburg.de

Dr. David W. Burt, Department of Genomics and Bioinformatics
Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS (UK)
telephone: 0044-131-527-4200; fax: 0044-131-440-0434
e-mail: Dave.Burt@BBSRC.AC.UK

KARGER
Fax + 41 61 306 12 34
E-mail karger@karger.ch
www.karger.com

1424–8581/05/1094–0415$22.00/0

Accessible online at:
www.karger.com/cgr

The chicken: a powerful model organism comes of age

(Prepared by D.W. Burt)

The First Report on Chicken Genes and Chromosomes (Schmid et al., 2000) marked a significant point in the history of chicken genomics. It was a time when practical, high-resolution genetic and physical maps were first made available. Even at that time it was possible to reveal significant similarities in the genome organization of species as diverse as chicken and man. This, the Second Report on Chicken Genes and Chromosomes marks another landmark with the release of the chicken genome sequence (Burt, 2004a, b; International Chicken Genome Sequencing Consortium, 2004). The assembly of the chicken genome sequence was based on a combination of shotgun sequencing and integration with available genetic and physical mapping resources. In this report, we have summaries on these cytogenetic, genetic, BAC and radiation hybrid physical mapping resources. The future of genetic studies will be based on dense maps of single nucleotide polymorphisms. The potential of these SNP maps is described (see Chicken Variation Database web site http://chicken.genomics.org.cn/index.jsp for a summary of the 2.8 million SNPs now available in the chicken). Besides being critical for the assembly of the chicken genome sequence itself these resources will now serve as the basis for comparative genome mapping studies in other birds, as illustrated in this issue. Furthermore, this report contains a variety of topics relevant for avian genetics and genome evolution. The chicken has now an impressive array of tools (see ARK-genomics web site http://www.ark-genomics.org/) and genome resources (see the AvianNET web site http://www.chicken-genome.org/ for a list of genome browsers and other resources) and certainly represents a powerful model organism – for other birds and vertebrates.

The genetic map

(Prepared by M. Groenen and R. Crooijmans)

In chicken the consensus linkage map first published in 2000 (Groenen et al., 2000) plays a central role in the alignment of the different types of maps developed for chicken. The consensus linkage map combines the data from the original genetic maps based on the three different mapping populations set up in East Lansing (Crittenden et al., 1993), Compton (Bumstead and Palyga, 1992) and Wageningen (Groenen et al., 1998). Since that time the map has seen a modest increase in the number of markers from 1,889 loci on 51 linkage groups spanning 3,800 cM, to its current size of 4,200 cM with 2,261 loci on 53 linkage groups. Currently the East Lansing and the Wageningen reference populations are the two mapping populations still actively used for linkage mapping. Although, marker information from QTL mapping studies can be used to add additional markers to the consensus map, the exact marker order between markers typed on the different populations is more difficult to estimate. Mapping information from QTL mapping studies nevertheless allowed the further localization of 45 microsatellites on the consensus map (Jacobsson et al., 2004; Rabie, 2004) resulting in a total number of 2,306 markers.

The two major types of marker on the consensus linkage map are microsatellites and AFLPs. However, in the past two years a sharp increase is seen in the number of SNP markers and it is to be expected that this trend will further increase with the SNP marker soon being the dominant type of marker on the consensus map. The main reason for this is the ongoing effort to map markers from unassigned BAC contigs to the consensus linkage map as well as the finemapping of quantitative trait loci (QTL). Because of the lack of detailed RH maps for many of the chicken chromosomes, the consensus linkage map has played a central role in the assembly of the draft sequence of the chicken genome (International Chicken Genome Sequencing Consortium, 2004). Furthermore, the linkage map remains the essential tool for the mapping of QTL. The most up to date version of the chicken consensus linkage map is available through the ChickAce database at https://acedb.asg.wur.nl/ or through the chicken comparative mapping database ChickCmap at http://www.animalsciences.nl/cmap. ChickCmap also allows the alignment of the chicken maps with the human and mouse sequence maps. An example of the aligned linkage, RH and sequence maps for chicken chromosome 5 (GGA5) is shown in Fig. 1.

So far, 31 of the linkage groups have been assigned to a particular chromosome (Masabanda et al., 2004). The remaining 22 linkage groups have an ExxCxxWxx number with reference to the linkage groups of the original three linkage maps. Ongoing experiments are aimed at the development of genetic markers from unassigned FPC contigs and sequence contigs (see The physical map, below) and the mapping of these (SNP) markers on the consensus linkage map. A large proportion of these SNP markers are being mapped both on the East Lansing and the Wageningen reference populations, which should result in a further reduction of the number of linkage groups. Currently, sequence maps are only available for 30 chromosomes and the ultimate aim is to construct linkage and sequence maps for all the 39 individual chromosomes in the chicken genome.

The physical map

(Prepared by R. Crooijmans and M. Groenen)

A considerable number of different BAC libraries have been constructed in chicken (Zoorob et al., 1996; Zimmer et al., 1997; Crooijmans et al., 2000; Ren et al., 2003) but only five of these are predominantly used for the building of BAC contig maps in chicken. Four of these BAC libraries (Ren et al., 2003; Pieter de Jong, unpublished results) are derived from the one inbred Red Jungle fowl bird used in whole genome sequencing and the fifth library was derived from a White Leghorn bird (Crooijmans et al., 2000), a breed phenotype selected for egg production. Using chromosome walking, partial BAC contig maps have been constructed for a number of different chromosomes (Crooijmans et al., 2001; Buitenhuis et al., 2002; Jennen et al., 2002, 2003) but these maps only covered between 10–50% of these chromosomes. The construction of a physical BAC map of a complete vertebrate genome the size of that of the chicken is not feasible using chromosome walking. Fingerprint clone (FPC) maps, based upon complete restriction

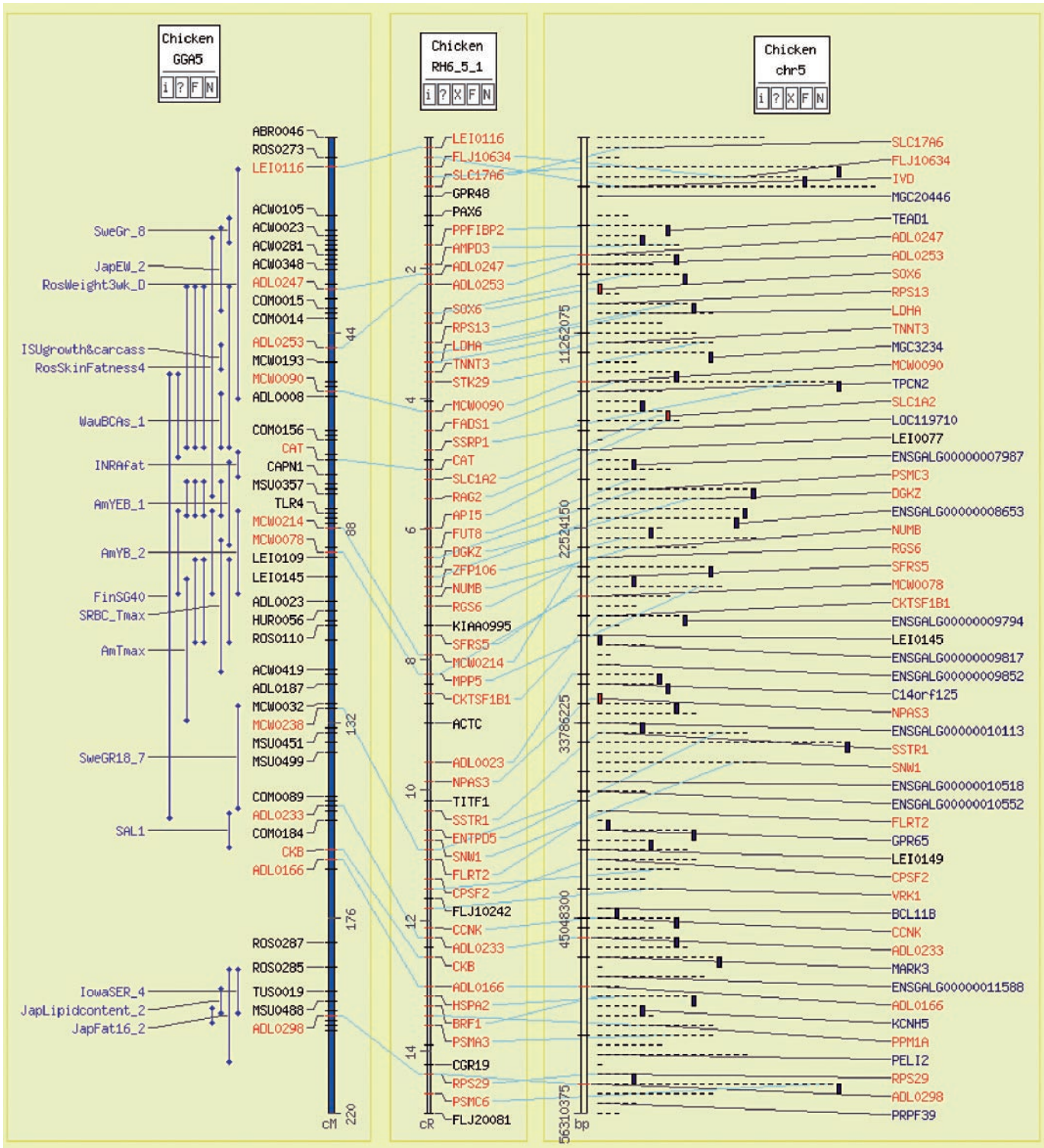


Fig. 1. Alignment of different maps for GGA5 in ChickCmap. Left: the consensus linkage map (Groenen et al., 2000); middle: radiation hybrid map (Pitel et al., 2004); right: sequence map (International Chicken Genome Sequencing Consortium, 2004). The purple bars to the left of the linkage map show the location of QTL that have been mapped on this chromosome. Detailed information of the QTL and the genetic markers are provided through a direct link to the ChickAce database. More detailed information of the chicken genes on the sequence map is provided through a direct link to the Ensemble database at www.ensembl.org/Gallus_gallus/. Microsatellite and STS markers that form the links between the different maps are shown in red. ChickCmap has been built using the open source software package Cmap available at www.gmod.org.

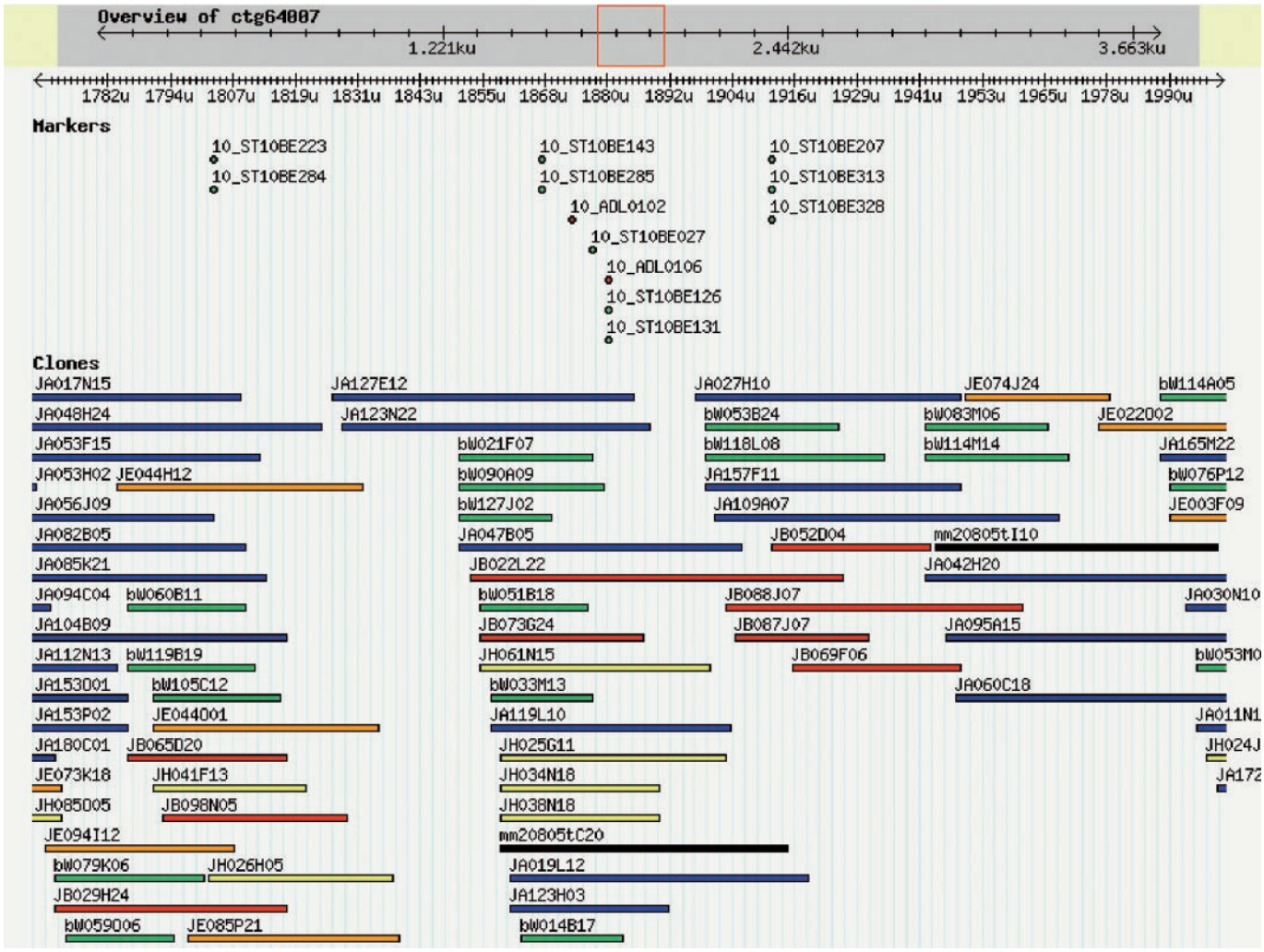


Fig. 2. ChickFPC showing a detail of the central region of FPC BAC contig ctg64007. This BAC contig consists of 1,239 clones and spans the region from 56 to 120 cM of the consensus linkage map of chromosome 10. Color codes of the BACs are as follows: Green, BAC clones from the Wageningen white leghorn library; blue, BAC clones from the Chori-261 library of Pieter de Jong; ochre, BAC clones from the *Eco*RI Texas A&M library; yellow, BAC clones from the *Hin*dIII Texas A&M library; red, BAC clones from the *Bam*HI Texas A&M library. The dots in the upper panel indicate the approximate location of STS markers from chromosome 10.

enzyme digests of clones and the subsequent determination of clone overlap by comparing fragment sizes are the method of choice towards this goal. A physical clone map is a valuable resource to be linked to a sequence to identify local mis-assemblies, supply long-range linking of assembly supercontigs to anchor sequence contigs to the genetic map, and to provide templates for closing gaps in draft sequence assemblies. The first published preliminary chicken BAC based maps of 7.5× and 3.6× genome coverage (Ren et al., 2003; Aerts et al., 2003) lacked the required coverage to obtain a whole genome physical map. Both efforts, however, represented considerable progress in the alignment of the physical and linkage maps by providing a large number of links between BACs and markers that are located on the chicken consensus linkage map. All these five BAC libraries were eventually used in a combined effort to generate a comprehensive BAC contig map covering 95% of the chicken genome (Wallis et al., 2004). This map consists of over 180,000 BAC clones representing 20-fold coverage of the chicken genome. These BACs could eventually be assembled into 260 contigs of which 226 could be assigned to a particular chromosome (Fig. 2). However, 10% of these contigs still have ambiguous links to different chromosomes which currently are being further addressed by the development and mapping of SNP markers on the chicken consensus linkage map. Many of the BACs from the FPC map have been end sequenced also enabling the alignment of the FPC contigs with the chicken sequence map. In total over 140,000 BAC end sequences have been deposited in GenBank. The alignment of the FPC BAC contigs as well as the chromosome walking contigs with the chicken linkage and sequence maps is available at the ChickCmap website. An example showing the available BAC contig maps for chicken chromosome 10 is shown in Fig. 3. Details of the chicken FPC BAC contigs are also available at http://www.animalsciences.nl/ChickFPC/ or http://www.genome.wustl.edu.

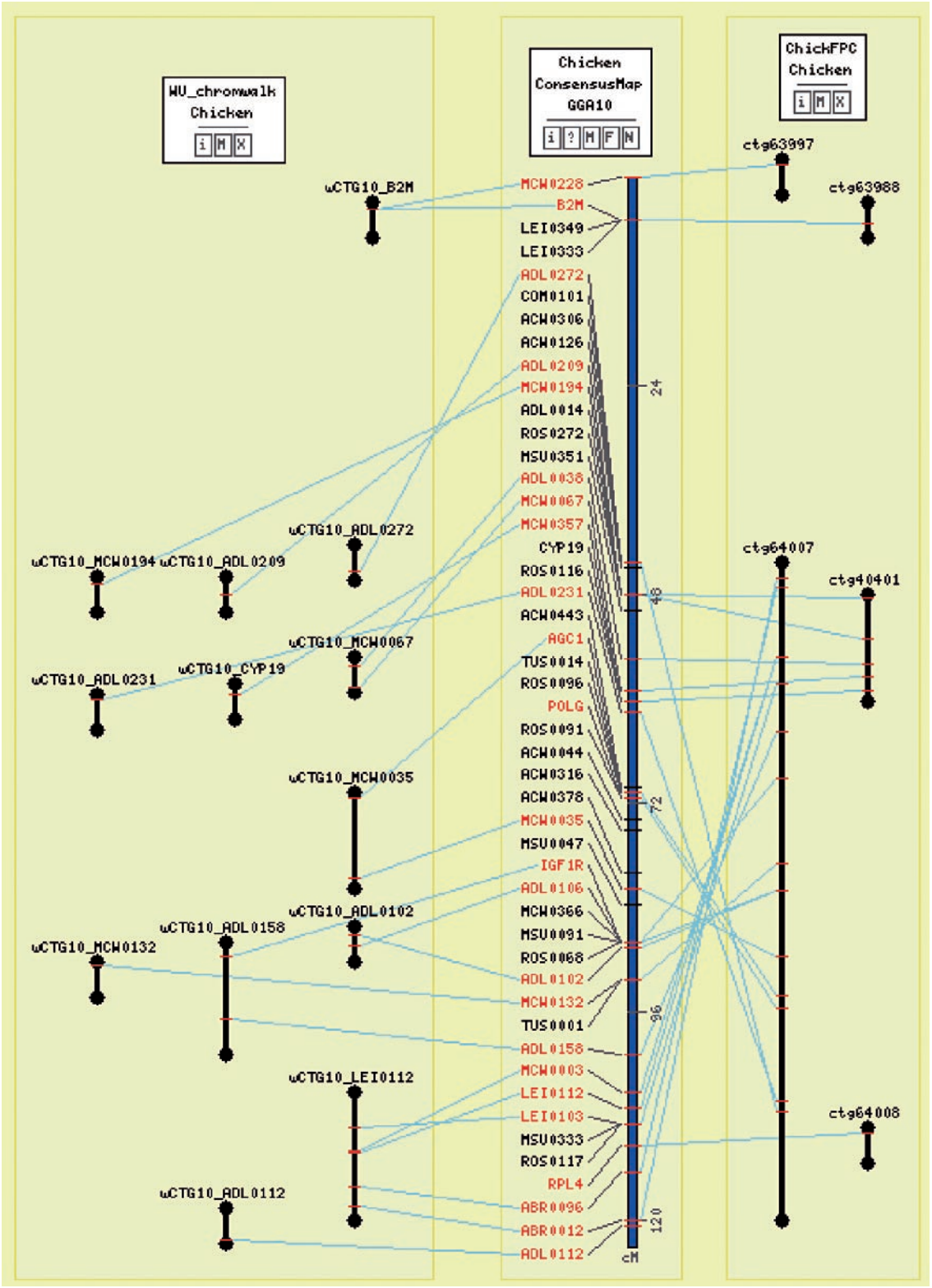


Fig. 3. ChickCmap alignment of the walking (wCTG) and FPC (ctg) BAC contigs on chromosome 10. Markers that are located on the linkage and on the BAC contigs are highlighted in red.

Integration of the genetic and physical maps of the chicken: update

(Prepared by J. Smith and D. Burt)

Here we present an update on the mapping status of loci that have been both physically and genetically mapped in the chicken. Most linkage groups have now been assigned a chromosome number and have a marker available which can be used to identify one microchromosome from another (Masabanda et al., 2004). The clones that have been both genetically and physically mapped for each chromosome (including microchromosomes) are shown in Table 1.

Table 1. Loci that have been both physically and genetically mapped in the chicken

Locus	References	Chr	Linkage group	RBG[a]	FLpter	Consensus (cM)	Error (cM)
MCW0248	Schmid et al., 2000	1	1	p26	0.01	0	
ALVE6A	Schmid et al., 2000	1	1	p26	0.02	0	
GCT0006	Schmid et al., 2000	1	1	p24-p22	0.13	66	
IFNG	Schmid et al., 2000	1	1	p23-p21	0.16	105	6
LYZ	Schmid et al., 2000	1	1	p22-p15	0.20	112	12
HMGIC	Schmid et al., 2000	1	1	p21-p13	0.23	116	20.5
DCN	Schmid et al., 2000	1	1	p21-p13	0.23	116	20.5
GCT0015	Schmid et al., 2000	1	1	p22-p21	0.21	118	
CRADD	Schmid et al., 2000	1	1	p21-p15	0.22	132	7
H1F1	Schmid et al., 2000	1	1	p22-p21	0.25	143	1
ADSL	Schmid et al., 2000	1	1	p21-p13	0.22	151	10
H1F0	Schmid et al., 2000	1	1	p14-p13	0.25	155	10
ASCL1	Schmid et al., 2000	1	1	p15-p11	0.29	160	29.5
IGF1	Schmid et al., 2000	1	1	p12-p11	0.31	172	17
LDHB	Schmid et al., 2000	1	1	p13-p11	0.34	204	3
ALVE1	Schmid et al., 2000	1	1	p12-p11	0.36	204	
CCND2	Schmid et al., 2000	1	1	p11-q11	0.39	230	35
GAPD	Schmid et al., 2000	1	1	q11-q12	0.47	241	13
SCYC1	Morroll et al., 2001	1	1	q11-q13	0.46	266	44
CRYAA	Schmid et al., 2000	1	1	q14-q21	0.57	341	1
OTC	Schmid et al., 2000	1	1	q13-q14	0.57	356	
GCT0013	Schmid et al., 2000	1	1	q14	0.57	362	
EWSR1	Groenen et al., unpublished	1	1	q31	0.75	433	17
GCT0007	Schmid et al., 2000	1	1	q31-q35	0.79	441	
PGR	Schmid et al., 2000	1	1	q42-q44	0.94	520	26
TYR	Schmid et al., 2000	1	1	q42-q44	0.95	556	14.5
LEI0331	Schmid et al., 2000	1	1	qter	0.97	561	
MCW0107	Schmid et al., 2000	1	1	q45	0.99	565	
LEI0332	Schmid et al., 2000	1	1	qter	1	566	
VIM	Schmid et al., 2000	2	2	p24-p21	0.14	76	12
TGFBR1	Schmid et al., 2000	2	2	p12-p11	0.27	179	12
PRL	Schmid et al., 2000	2	2	p11	0.32	186	19
OVY	Schmid et al., 2000	2	2	q11-q12	0.49	226	
GCT0023	Schmid et al., 2000	2	2	q11-q12	0.53	241	
ROS0150	Schmid et al., 2000	2	2	q26-q32	0.75	336	
LYN	Schmid et al., 2000	2	2	q26	0.77	355	6.5
CALB1	Schmid et al., 2000	2	2	q26	0.79	358	1
SDC2	Chen et al., 2002	2	2	q31	0.84	372	7.5
ROS0120	Schmid et al., 2000	2	2	q32-q35	0.89	395	
ADL0146	Schmid et al., 2000	2	2	q36-37	0.98	403	
MCW0261	Schmid et al., 2000	3	3	p12	0	0	
BMP2	Schmid et al., 2000	3	3	q11-q21	0.22	52	14
TGFB2	Schmid et al., 2000	3	3	q22-q23	0.23	77	12
MYB	Schmid et al., 2000	3	3	q24-q26	0.53	170	10
CCNC	Schmid et al., 2000	3	3	q26-q29	0.60	210	10
ROS0119	Schmid et al., 2000	3	3	q27-q29	0.63	210	
ROS0108	Schmid et al., 2000	3	3	q29-q33	0.63	210	
GCT0019	Schmid et al., 2000	3	3	q28-q2.10	0.68	210	
MCW0037	Schmid et al., 2000	3	3	q35	0.98	317	
HPRT1	Schmid et al., 2000	4	4	p14	0.02	–9	17
ADL0143	Schmid et al., 2000	4	4	p14	0.03	0	
ROS0107	Schmid et al., 2000	4	4	p14-p13	0.04	1	
PGK1	Schmid et al., 2000	4	4	p14-p11	0.11	48	24
TLR2	Boyd et al., 2001	4	4	q11	0.30	90	6.5
FGB	Schmid et al., 2000	4	4	q11	0.31	90	6.5
IL8	Schmid et al., 2000	4	4	q12-q13	0.44	118	16
CLOCK	Noakes et al., 2000; Yoshimura et al., 2000	4	4	q13	0.45	131	
ALB	Schmid et al., 2000	4	4	q13-q24	0.61	158	16

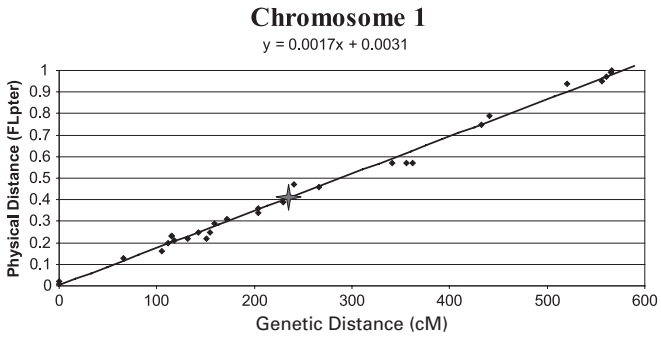
Table 1 (continued)

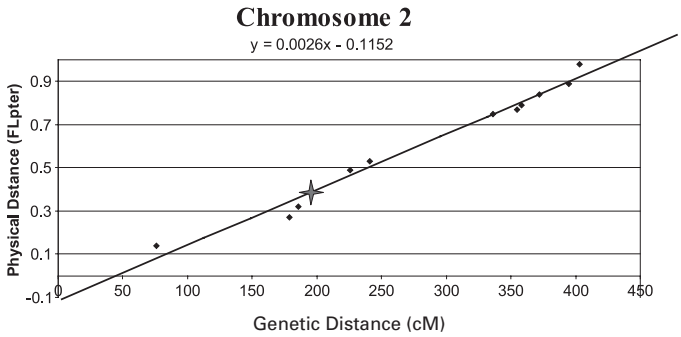
Locus	References	Chr	Linkage group	RBG[a]	FLpter	Consensus (cM)	Error (cM)
GC	Schmid et al., 2000	4	4	q13-q24	0.61	158	16
MCW0240	Schmid et al., 2000	4	4	q21	0.76	201	
LEI0073	Schmid et al., 2000	4	4	q25	0.97	243	
LEI0340	Schmid et al., 2000	4	4	qter	1.00	269	
LEI0341	Schmid et al., 2000	4	4	qter	1.00	270	
CD5	Koskinen et al., 2001	5	5	p11-q11	0.14	19	19
MCW0263	Schmid et al., 2000	5	5	q11	0.17	28	
MYOD1	Schmid et al., 2000	5	5	q11-q12	0.27	44	19
IGF2	Schmid et al., 2000	5	5	q11-q12	0.31	57	12
INS	Schmid et al., 2000	5	5	q12	0.34	57	12
TH	Schmid et al., 2000	5	5	q12	0.34	57	12
CCND1	Schmid et al., 2000	5	5	q11-q12	0.31	61	22.5
RYR3	Schmid et al., 2000	5	5	q13	0.47	106	1
FUT8	Coullin et al., 2002	5	5	q13	0.46	108	2
HTR1D	Schmid et al., 2000	5	5	q21-q22	0.69	110	10
PTAFR	Schmid et al., 2000	5	5	q21-q22	0.69	110	10
UNK	unpublished	5	5	q21-q22	0.69	110	10
AF111167.2	unpublished	5	5	q21-q22	0.69	110	10
TGFB3	Schmid et al., 2000	5	5	q21-q22	0.69	110	10
ADL0298	Schmid et al., 2000	5	5	q25	0.98	198	
LEI0192	Schmid et al., 2000	6	6	q11	0.22	31	
SUPV3L1	Schmid et al., 2000	6	6	q12	0.37	59	6
SCD	Schmid et al., 2000	6	6	q14	0.58	63	5
MCW0326	Schmid et al., 2000	6	6	q14-15	0.72	63	
THRSP	Carre et al., 2001	6	6	q12-q14	0.60	64	4
LEI0064	Schmid et al., 2000	7	7	p12	0.00	0	
ROS00331	Schmid et al., 2000	7	7	p11	0.17	40	
MCW0201	Schmid et al., 2000	7	7	q13-q14	0.66	79	
ROS0128	Schmid et al., 2000	7	7	q13	0.59	80	
RPL37A	Schmid et al., 2000	7	7	q12-q14	0.60	80	
ADL0169	Schmid et al., 2000	7	7	q16	0.94	165	
MCW0275	Schmid et al., 2000	8	8	p12	0.11	6	
AT3	Schmid et al., 2000	8	8	p11	0.33	50	13
RPL5	Schmid et al., 2000	8	8	p11-q11	0.35	56	40
ROS0149	Schmid et al., 2000	8	8	8cen	0.5	66	
LEI0044	Schmid et al., 2000	8	8	q14	0.99	96	
B2M	Schmid et al., 2000	10	E29C09W09	m	N/A	1	1
CSK	Crooijmans et al., 2001	10	E29C09W09	m	N/A	1	1
CYP11A	Crooijmans et al., 2001	10	E29C09W09	m	N/A	1	1
MLSN1	Crooijmans et al., 2001	10	E29C09W09	m	N/A	48	1
NTRK3	Crooijmans et al. 2001	10	E29C09W09	m	N/A	71	1
AGC1	Schmid et al., 2000	10	E29C09W09	m	N/A	71	1
IGF1R	Schmid et al., 2000	10	E29C09W09	m	N/A	88	1
MEF2A	Crooijmans et al., 2001	10	E29C09W09	m	N/A	101	1
UQCRFS1	Smith J. et al., 2002	11	E30C14W10	m	N/A	32	
CCNE	Smith J. et al., 2002	11	E30C14W10	m	N/A	38	1
KIAA0355	Smith J. et al., 2002	11	E30C14W10	m	N/A	44	1
GPI	Smith J. et al., 2002	11	E30C14W10	m	N/A	44	1
GABRA6	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	32–39	
GABRA1	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	32–39	
GABRG2	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	32–39	
KIAA1673	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	39	
MSX2	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	39–47	
FLJ12686	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	47	
KIAA0731	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	47	
C5ORF4	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	51	
CNOT8	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	51	
SPARC	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	51–55	
CDX1	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	55	
FLJ10290	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	55	
MADH5	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	55–67	
CAMLG	Schmid et al., 2000	13	E48C28W13	m	N/A	70	7
UBE2B	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67	
DTR	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67–74	
POU4F3	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67–74	
KIAA0837	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67–74	
IRF1	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67–74	
NR3C1	Buitenhuis et al., 2002	13	E48C28W13	m	N/A	67–74	
HBA1	Schmid et al., 2000	14	E35C18W14	m	N/A	26	30
CRYBB1	Schmid et al., 2000	15	E18W15	m	N/A	31	3

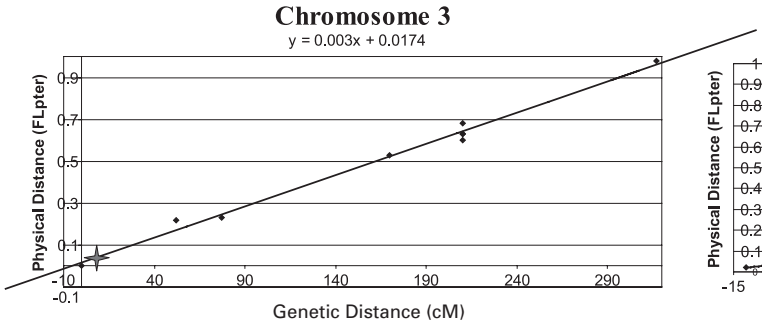
Table 1 (continued)

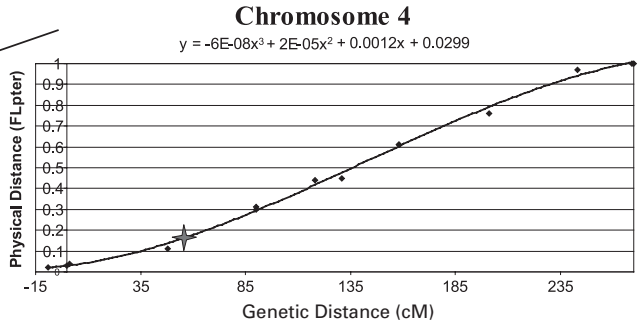
Locus	References	Chr	Linkage group	RBG[a]	FLpter	Consensus (cM)	Error (cM)
IGL@	Schmid et al., 2000	15	E18W15	m	N/A	35	1
HLA	Schmid et al., 2000	16	16	m	N/A	0	20
SURF5	Colombo et al., 1992	17	E41W17	m	N/A	11	1
RPL7A	Schmid et al., 2000	17	E41W17	m	N/A	11	1
SURF1	Colombo et al., 1992	17	E41W17	m	N/A	11	1
SURF2	Colombo et al., 1992	17	E41W17	m	N/A	11	1
SURF4	Colombo et al., 1992	17	E41W17	m	N/A	11	1
ABL1	Schmid et al., 2000	17	E41W17	m	N/A	22	1
AK1	Schmid et al., 2000	17	E41W17	m	N/A	29	1
MYH1	Schmid et al., 2000	18	E31E21C25W12	m	N/A	0	1
H3F3B	Schmid et al., 2000	18	E31E21C25W12	m	N/A	35	5
FASN	Schmid et al., 2000	18	E31E21C25W12	m	N/A	40	12
ACACA	Schmid et al., 2000	19	E52W19	m	N/A	–1	45
HCK	Schmid et al., 2000	20	E32W24	m	N/A	15	6
BMP7	Schmid et al., 2000	20	E32W24	m	N/A	16	6
CDC2L1	Schmid et al., 2000	21	E54	m	N/A	0	1
AGRN	Schmid et al., 2000	21	E54	m	N/A	25	1
ETS1	Jennen et al., 2002	24	E49C20W21	m	N/A	0	1
SEMA3C	Jennen et al., 2002	24	E49C20W21	m	N/A	20	1
HNT	Jennen et al., 2002	24	E49C20W21	m	N/A	20	1
OPCML	Jennen et al., 2002	24	E49C20W21	m	N/A	20	1
CD3E	Jennen et al., 2002	24	E49C20W21	m	N/A	40	20
ZW10	Jennen et al., 2002	24	E49C20W21	m	N/A	40	20
NCAM1	Jennen et al., 2002	24	E49C20W21	m	N/A	40	20
APOA1	Jennen et al., 2002	24	E49C20W21	m	N/A	59	10
KIAA1532	Smith J. et al., 2002	28	E53C34W16	m	N/A	2	5
hnRNP-M4	Smith J. et al., 2002	28	E53C34W16	m	N/A	2	
SF3A2	Smith J. et al., 2002	28	E53C34W16	m	N/A	21	
AMH	Smith J. et al., 2002	28	E53C34W16	m	N/A	21	1
PTB	Smith J. et al., 2002	28	E53C34W16	m	N/A	30	
TRAP95	Smith J. et al., 2002	28	E53C34W16	m	N/A	30	
ROD1	Smith J. et al., 2002	28	E53C34W16	m	N/A	30	
ABCA7	Smith J. et al., 2002	28	E53C34W16	m	N/A	46	
JUND	Smith J. et al., 2002	28	E53C34W16	m	N/A	48	
GDF1	Smith J. et al., 2002	28	E53C34W16	m	N/A	48	
RENT1	Smith J. et al., 2002	28	E53C34W16	m	N/A	48	
COMP	Smith J. et al., 2002	28	E53C34W16	m	N/A	48	
INSR	Smith J. et al., 2002	28	E53C34W16	m	N/A	58	
PTPRS	Smith J. et al., 2002	28	E53C34W16	m	N/A	60	
CALR	Smith J. et al., 2002	30	E65	m	N/A	0	
GCDH	Smith J. et al., 2002	30	E65	m	N/A	0	
RAD23A	Smith J. et al., 2002	30	E65	m	N/A	0	
FARSL	Smith J. et al., 2002	30	E65	m	N/A	0	
LIG1	Smith J. et al., 2002	31	E64	m	N/A	0	
EZFIT	Smith J. et al., 2002	31	E64	m	N/A	0	
SNRPD2	Smith J. et al., 2002	32	E25C31	m	N/A	–2	
TGFB1	Smith J. et al., 2002	32	E25C31	m	N/A	–1	
RYR1	Smith J. et al., 2002	32	E25C31	m	N/A	0	
CAPN4	Smith J. et al., 2002	32	E25C31	m	N/A	16	
CKM	Smith J. et al., 2002	32	E25C31	m	N/A	20	
ATP5A1	Schmid et al., 2000	Z	Z	p24-p23	0.06	–1	15
ADL0022	Schmid et al., 2000	Z	Z	p24-p23	0.03	0	
PRLR	Schmid et al., 2000	Z	Z	p23-p22	0.13	24	4
GHR	Schmid et al., 2000	Z	Z	p23-p22	0.13	28	1
DMRT1	Schmid et al., 2000	Z	Z	p21-p13	0.24	37	8
VLDLR	Schmid et al., 2000	Z	Z	p13-p12	0.28	73	5
NTRK2	Schmid et al., 2000	Z	Z	p12-p11	0.36	125	5
CHD1	Schmid et al., 2000	Z	Z	q21-q22	0.57	131	6
CHRNB3	Schmid et al., 2000	Z	Z	q12-q13	0.68	139	4
ALDOB	Schmid et al., 2000	Z	Z	q13-q22	0.78	160	10
LEI0075	Schmid et al., 2000	Z	Z	q14-q21	0.75	165	
ACO1	Schmid et al., 2000	Z	Z	q13-q22	0.80	187	6
B4GALT1	Schmid et al., 2000	Z	Z	q13-q22	0.80	187	5

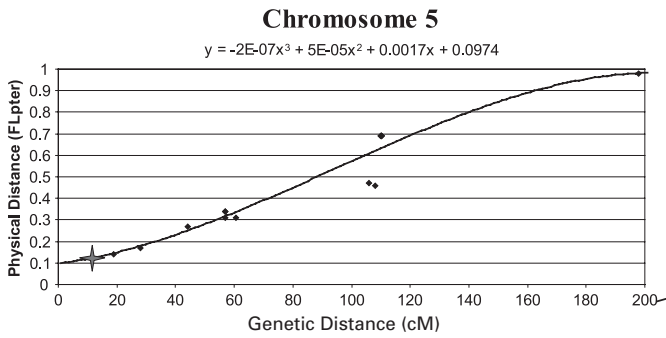
[a] RBG positions were estimated in cases where GTG positions were published and FLpters estimated from given band positions; m = microchromosome

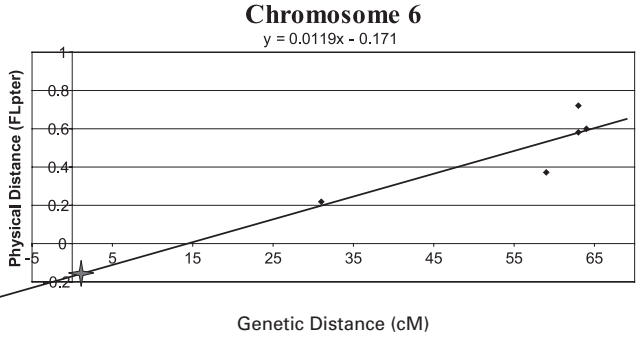

Chromosome 1
y = 0.0017x + 0.0031
Physical Distance (FLpter)
Genetic Distance (cM)

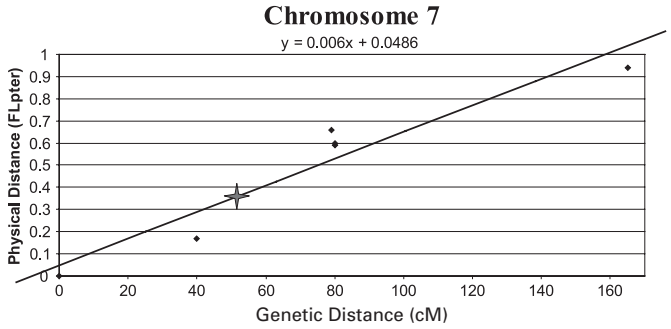

Chromosome 2
y = 0.0026x - 0.1152
Physical Dstance (FLpter)
Genetic Distance (cM)

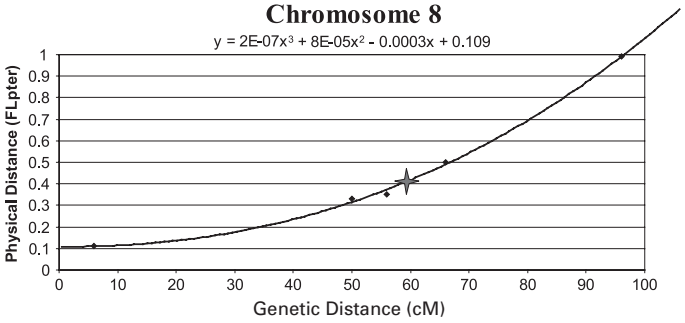

Chromosome 3
y = 0.003x + 0.0174
Physical Distance (FLpter)
Genetic Distance (cM)


Chromosome 4
y = -6E-08x³ + 2E-05x² + 0.0012x + 0.0299
Physical Distance (FLpter)
Genetic Distance (cM)


Chromosome 5
y = -2E-07x³ + 5E-05x² + 0.0017x + 0.0974
Physical Distance (FLpter)
Genetic Distance (cM)


Chromosome 6
y = 0.0119x - 0.171
Physical Distance (FLpter)
Genetic Distance (cM)


Chromosome 7
y = 0.006x + 0.0486
Physical Distance (FLpter)
Genetic Distance (cM)


Chromosome 8
y = 2E-07x³ + 8E-05x² - 0.0003x + 0.109
Physical Distance (FLpter)
Genetic Distance (cM)

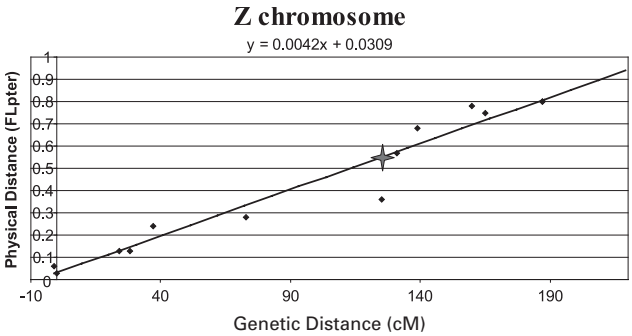

Z chromosome
y = 0.0042x + 0.0309
Physical Distance (FLpter)
Genetic Distance (cM)

Table 2. Summary of chromosome probes identifying each chromosome. Markers and linkage groups are indicated where known (Masabanda et al., 2004; adapted and expanded upon from Fillon et al., 1998).

Group	Chromosome number	Chromosome paint isolated?	BAC (or cosmid) isolated?	Linkage group[a]	Marker[b]
A	1	+	+	N/A	
	2	+	+	N/A	
	3	+	+	N/A	
	4	+	+	N/A	
	5	+	+	N/A	
	6	+	+	N/A	
	7	+	+	N/A	
	8	+	+	N/A	
	9	+	+	E36C06W08	MCW0134
	10	+	+	E29C09W09	ADL0112
B	11	+	+	E30C14W10	LEI0143
	12	+	+	E16C17W22	MCW332
	13	+	+	E48C28W13W27	GCT907
	14	–	+	E35C18W14 + C37	GCT903
	15	–	+	E18C15W15 + C37	GCT14
	16	–	+	NOR chromosome	MCW371
C	17	–	+	E41W17 + C24	ADL0293
	18	+	+	E31E21C25W12	FASN
	19	+	+	E52W19	ACACA
	20	–	+	E47W24 + E32	ADL324
	21	–	+	E54	PLOD
	22	–	+	E38	TVSB3
	23	+	+	E27C36W25W26	MCW249
	24	+	+	E49C20W21	GCT905
	25	+	–	–	*
	26	+	+	E60E04W23	GCT906
	27	+	+	E59C35W20	GCT22
	28	+	+	E53C34W16	GCT904
	29	+	+ (cosmid)	E62	ROS0257
	30	+	+ (cosmid)	E65	ROS0263
	31	+	+ (cosmid)	E64	ROS0264
	32	–	+ (cosmid)	E25C31	–
D	33	+	–	–	–
	34	+	–	–	–
	35	+	–	–	–
	36	+	–	–	–
	37	+	–	–	–
	38	+	–	–	–
A	Z	+	–	N/A	
A	W	+	–	N/A	

[a] N/A: not applicable.
[b] *: Chromosome 25 paint was isolated 11 times following microdissection and flow sorting experiments. Each time the paint was bright and specific leading us to conclude that this chromosome mostly consists of highly repetitive sequences.

We have correlated genetic (cM) to physical distance (FLpter) for the macrochromosomes as shown in Fig. 4, which allows estimation of genetic position from physical data and vice versa. Where no direct FLpter data was available, we estimated fractions from the given cytogenetic band positions. The increase in mapping information since the First Report was published in 2000 (Schmid et al., 2000), means that we can draw more detailed conclusions about how physical distance relates to genetic position for each of the nine largest chromosomes. Current physical and genetic mapping data on the chicken is available at http://www.thearkdb.org/browser?species=chicken.

Fig. 4. Correlation of genetic distance with physical distance for each of the chicken macrochromosomes. Centromere positions are marked by a star.

Note: When presenting cytogenetic data, standardization is important. FLpter measurements must be given so that results can be assigned to the correct band on either the GTG or RBG ideograms.

The last major karyotype?

(Prepared by D.K. Griffin and J.S. Masabanda)

A karyotype of any organism can be thought of as a low-resolution map of the genome of that species. Each gene reproducibly resides in the same place on the same chromosome in every cell and physical evidence of genetic linkage can be provided by the co-localization of two or more loci on the same chromosome. Thus both physical and genetic maps are drawn with respect to a chromosomal assignment. Indeed, in humans and many other species, "chromosomology" was the first step in the mapping of the genome and allowed genes to be given

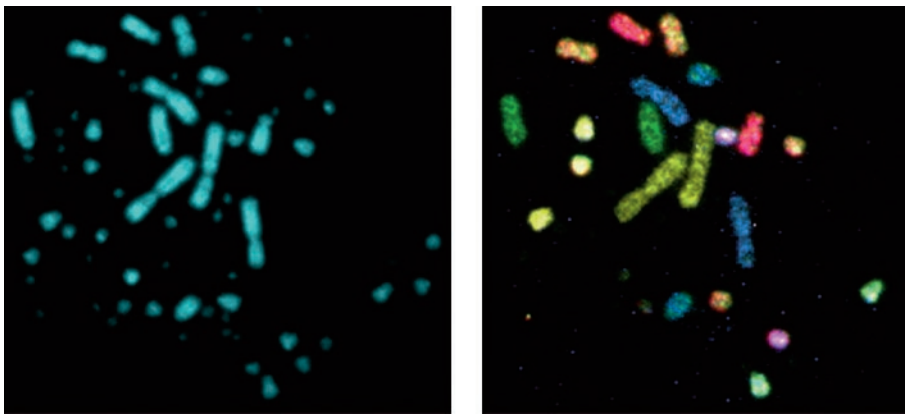


Fig. 5. Eleven-color painting of chromosomes 1–10 and Z.

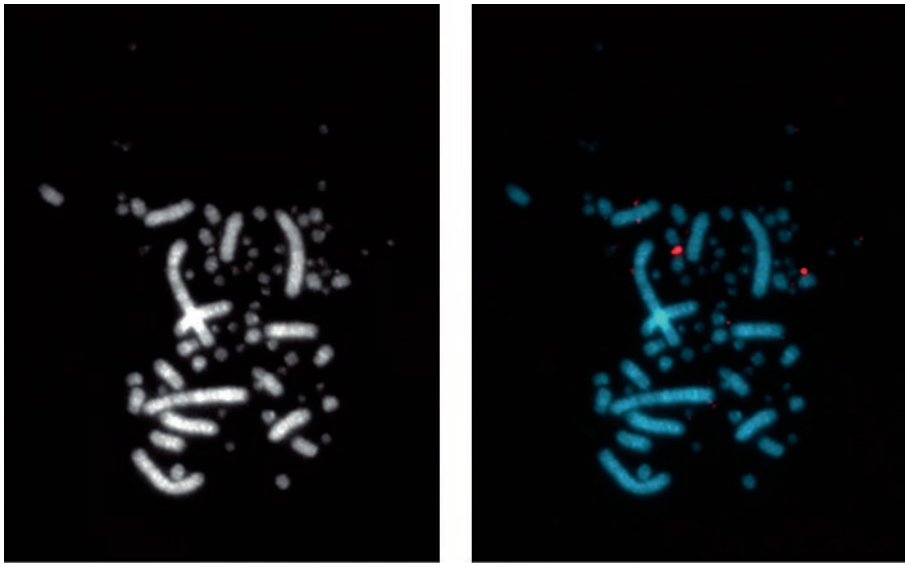


Fig. 6. Single color painting of chromosome 35.

proper assignments in terms of a convention agreed by the scientific community. In the case of humans, the Paris Conference (1971) finally agreed on a standard nomenclature for human chromosomes following the work of Sumner et al. (1971) that permitted reproducible identification of each human chromosome. This preceded the announcement of the completion of the human genome map and sequence by over 30 years. In the case of chickens however the completion of the karyotype and of the genome sequence has occurred almost simultaneously. This is largely due to the fact that chicken chromosomes belie assignment by conventional means a) because there are so many of them (2n = 78) and b) because the majority of them are tiny microchromosomes. Nevertheless, in issue 166 of the Journal "Genetics" we reported the accurate assignment of each individual chromosome by molecular cytogenetic means (Masabanda et al., 2004). A collaboration of the skills of a large number of groups made this possible and the work has taken approximately seven years to complete. Table 2 gives the assignment of each chromosome and indicates the means through which each chromosome was identified. In essence, two approaches permitted chromosomal assignment namely a) chromosome painting and b) mapping of known clones and use of them as landmarks for the chromosomes. Of course, for many chromosomes, both individual landmark clones and chromosome paints were generated (Masabanda et al., 2004).

Strategies for probe generation

In the case of chromosome paints, essentially four approaches were used; all involved isolation of individual chromosomes and subsequent amplification and labeling by degenerate oligonucleotide primed (DOP) PCR. Two approaches involved flow-cytometry and two involved chromosome microdissection (Griffin et al., 1999). In the case of the former, the larger chromosomes tractable in a flow-karyotype were sorted in multiple copies (Fig. 5). For chromosomes that were not easily resolved however (including some of the larger microchromosomes) it was necessary to isolate single microchromosomes and perform amplification and labeling from this single chromosomal template (Griffin et al., 1999; Masabanda et al., 2004). While this was not as reliable in generating paints as performing it from multiple copies (nor were the paints as bright or specific), this proved a successful strategy in some

cases and we are indebted to the Ferguson-Smith lab (particularly Patricia O'Brien) for performing the flow-cytometry. For the microdissection-based approaches, the amplification of single templates met with some success (Fig. 6), however, for the very smallest of the microchromosomes we adopted an approach of pre-hybridization of DOP-PCR amplified DNA to the chromosomes prior to microdissection, amplification and labeling (Masabanda et al., 2004).

For individual clones, BACs or cosmids (once isolated and mapped to a microchromosome by FISH) were used as landmarks for that chromosome. In the use of these clones, we followed the convention set down by Fillon, Vignal and colleagues (Schmid et al., 2000) for assignment of chromosomes 9–30 (Table 2). Given that many of these clones had been previously assigned to known linkage groups, co-hybridization experiments permitted integration of genetic and physical maps.

Classification of chromosomes

In the past, avian chromosomes have been classified as either macro- or microchromosomes. The definition of the borderline between the two has, however, varied in the literature with between 6 and 10 pairs of macrochromosomes including the Z and W in the heterogametic female reported (e.g. Kaelbling and Fechheimer, 1983; Fritschi and Stranzinger, 1985; Auer et al., 1987; Schmid et al., 1989; Ponce de Leon et al., 1992). We however propose a system that does not make that distinction since there is no clear size-based separation between macro- and microchromosomes. Instead, we propose that chromosomes 1–10 (plus the sex chromosomes) should be called group A and include all those that are tractable in a flow-karyotype. Conveniently, this includes chromosome 10 which, although slightly smaller than 11 and 12 had already been assigned to linkage group E29C09W09 and also can be isolated individually in a flow karyotype whereas 11 and 12 sort together in the same peak. Group D (chromosomes 33–38) are the smallest of the chromosomes and were classified as such as they had yet to be assigned to a known linkage group. At time of writing we are in the process of sequencing DNA from these paints to try and establish matches with recently established data. We took the decision to split the remaining chromosomes either side of the NOR chromosome. A change of assignment of this chromosome was suggested as size measurements clearly indicate that it is much smaller than the designation 16 would suggest (with the current 22 being called 19 and the current 19 taking up the 16 assignment). This was met with some support but was, overall, rejected by the wider chicken genome mapping community and thus the designation 16 will be retained for the NOR chromosome. Therefore group B includes chromosomes 11–16, leaving the remainder (17–32) designated as group C. Interestingly, our studies uncovered a chromosome (chromosome 25) that produced a paint that was bright, specific and was generated 11 times despite there being no means of prior selection of which chromosome was being isolated. In other words there was no means of knowing which chromosome was being isolated until the paint was made and co-hybridized with a pre-existing one. We feel that the most likely explanation for this is that the chromosome contains a large number of repeats. Fillon et al. (1998) reported that one BAC painted the whole of one microchromosome and half of another suggesting a highly repeated sequence shared across two chromosomes; in our studies we have found no evidence for this but have not yet been able to use this BAC in our own laboratory to confirm these results.

Utility of the resource

The ability to identify each chicken chromosome opens up a range of possibilities for scientific research. The first is comparative genomics. The ability to distinguish each chicken chromosome is key to the development of genome maps in other birds as cross species FISH is an efficient means of generating low-resolution physical gene maps (Wienberg and Stanyon, 1995; O'Brien et al., 1997). This commonly makes use of human chromosome paints on the metaphases of other mammals but to date has been applied relatively rarely to non-mammalian vertebrates (Suzuki et al., 1999; Nanda et al., 2000; Suchyta et al., 2001). Priority avian species for comparative gene mapping are turkey, goose, duck and quail. Less common species however are under investigation and chromosome painting strategies have established that, in contrast to mammals, bird chromosomes are remarkably conserved throughout evolution (Shetty et al., 1999). Similar experiments with microchromosomes will now ensue.

It is becoming increasingly apparent that three- and four-dimensional genome organization in the interphase nucleus is central to development and disease. Assaying for the position of chromosome territories is a well-established assay for establishing genome organization and our chromosome probes have made it possible to begin to study structure and arrangements of chromosome territories in chicken cells (Habermann et al., 2001). It seems likely that these investigations will be extended to a range of developmental processes and tissue types.

Chicken chromosome probes are essential for the characterization of aberrant avian karyotypes such as chromosomally rearranged (e.g. infertile) individuals or aberrant cell lines. They have been used in the characterization of functional domains in the *BRCA2* gene (Warren et al., 2002) and for DT40 karyotyping (Fukagawa et al., 1999). Detecting chromosome aberrations in DT40 can be a fundamental step in addressing questions of genome stability, DNA repair, gene expression, cell death, cell division and non-disjunction.

In conclusion, we have provided an essential step for the completion of genetic and physical maps of birds and developed a resource that is applicable in a wide range of investigations. Researchers are encouraged to contact us if they wish to make use of this resource.

Comparative mapping – fluorescent in situ hybridization of BAC clones reveals strong homologies between Galliformes and Anseriformes

(Prepared by V. Fillon, M. Vignoles, A. Garrigues, R. Crooijmans, M. Groenen, J. Gellin and A. Vignal)

During the last few years, international collaborative efforts led to a consensus molecular linkage map of the chicken genome (Groenen et al., 2000; Schmid et al., 2000) of high

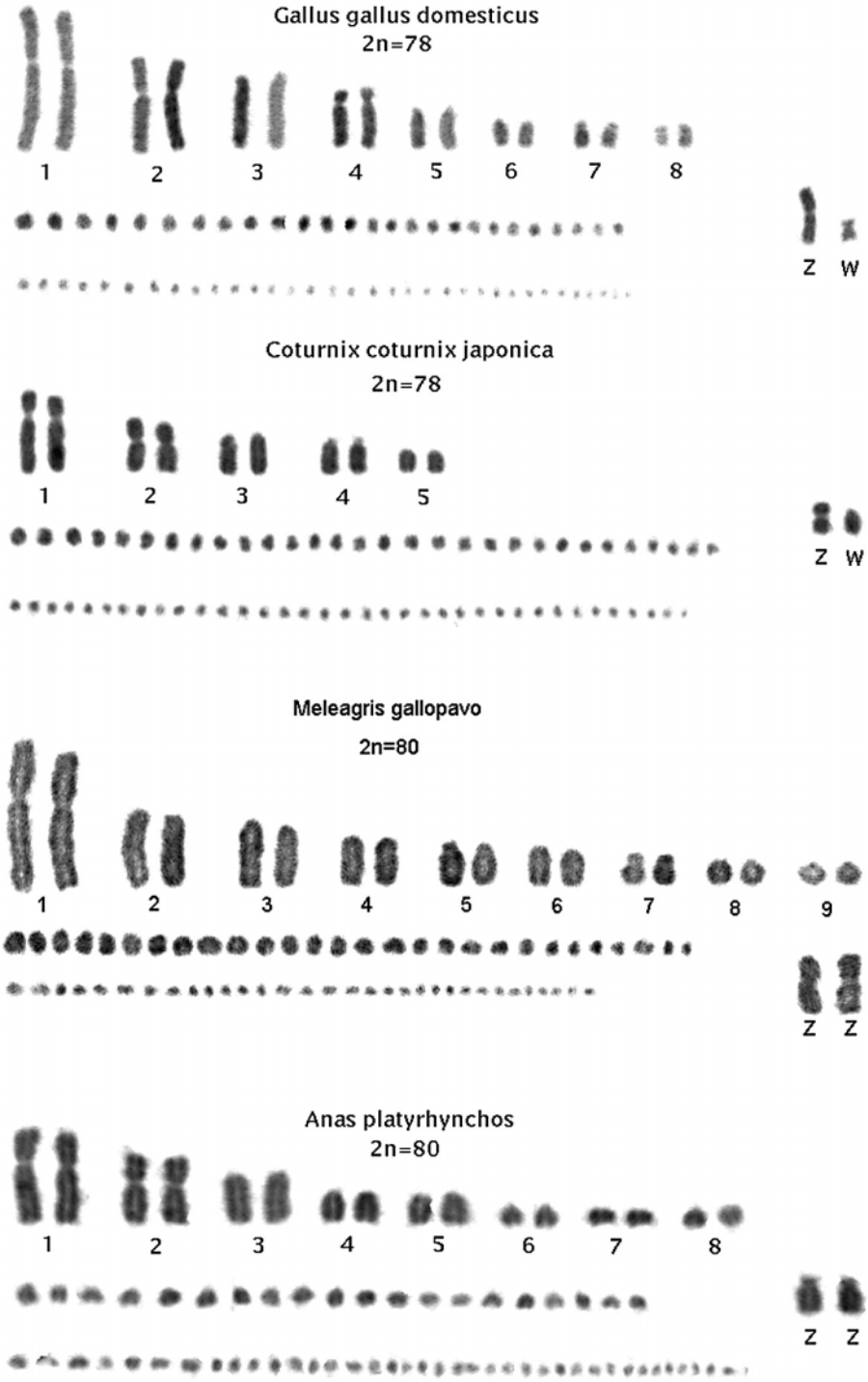


Fig. 7. The typical organization of avian karyotypes comprising a few macrochromosome pairs and a lot of tiny microchromosomes. Chicken *(Gallus gallus domesticus)* 2n = 78, quail *(Coturnix coturnix japonica)* 2n = 78, turkey *(Meleagris gallopavo)* 2n = 80, duck *(Anas platyrhynchos)* 2n = 80.

interest for the mapping of quantitative trait loci (QTL). This knowledge on the chicken genome could be of direct benefit for other poultry species and therefore comparative mapping studies were developed.

Most of the avian karyotypes share the same typical organization comprising a few macrochromosome pairs and many microchromosomes, too small to be identified unambiguously and usually classified approximately by size (Stock and Bunch, 1982) (Fig. 7). In chicken, a standard for the eight macrochromosome pairs, plus the Z and W gonosomes has been described (Ladjali-Mohammedi et al., 1999) and 22 of the thirty microchromosome pairs have been identified by FISH markers, the others still remaining indistinguishable (Fillon et al., 1998; Schmid et al., 2000).

This presence of microchromosomes complicated greatly classical cytogenetics, as compared to mammals. The order

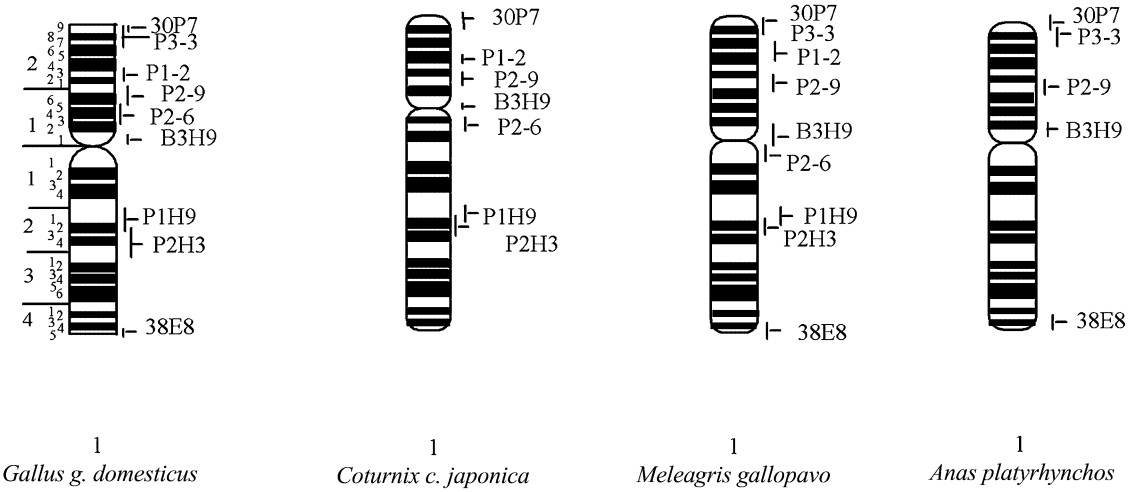


Fig. 8. Localization of different chicken BAC clones along chromosomes 1 of chicken, quail, turkey and duck. The order in each chromosome is quite similar to that of chicken, demonstrating the high degree of homology.

Galliformes is one of the best studied, and many homologies have been shown between poultry species (Stock and Bunch, 1982). Therefore, despite of this lack of banding studies, the conservation of avian macrochromosomes was supposed to be high. Using Zoo-FISH, Shetty et al. (1999) demonstrated the remarkable homology between the chromosomes of chicken and emu *(Dromaius novaehollandiae)* over 80 million years of bird evolution. The occurrence of homologous chromosome segments in the genome of several bird species was also evidenced by Guttenbach et al., using chromosome painting (see Schmid et al., 2000). Moreover, a fine comparative cytogenetic study by FISH of chicken cosmid clones has shown that the macrochromosome locations of DNA clones and the gene order were quite similar in Japanese quail *(Coturnix japonica)* and chicken (Suzuki et al., 1999; Shibusawa et al., 2001), with a very low number of internal rearrangements.

Considering the chicken standard karyotype as a reference (Ladjali-Mohammedi et al., 1999) idiograms based on banding patterns previously described have been established for the duck (*Anas platyrhynchos*, 2n = 80; Denjean et al., 1997), the quail (*Coturnix coturnix*, 2n = 78; Stock and Bunch, 1982; Suzuki et al., 1999) and the turkey (*Meleagris gallopavo*, 2n = 80; Stock and Bunch, 1982) (summary in Schmid et al., 2000).

In order to establish precise chromosome correspondences between the poultry species, with an insight on internal rearrangements, we hybridized BAC (Bacterial Artificial Chromosome) clones (Zoorob et al., 1996; Crooijmans et al., 2000) in heterologous conditions on quail, turkey and duck metaphases. All the BACs used had been previously mapped on chicken chromosomes (Fillon et al., 1998; Morisson et al., 1998; Schmid et al., 2000). Although most results showed similar localizations in all four species, several intrachromosomal rearrangements were detected on macrochromosomes (Table 3, Fig. 8).

Table 3. Correspondences between macrochromosomes in chicken (*Gallus gallus domesticus*, GGA), quail (*Coturnix coturnix*, CCO), turkey (*Meleagris gallopavo*, MGA) and duck (*Anas platyrhynchos*, APL). Only intrachromosomal rearrangements, fusions or fissions were detected.

GGA	CCO[a]	MGA[a]	APL[a]
1	1 (inv)	1 (rea)	1
2	2 (inv)	3 + 6	2
3	3	2	3
4	4 (inv)	4 + micro	4 (rea) + micro
5	5	5 (with p-arm)	5 (rea)
6	6	8	6 (rea)
7	7 (rea)	7	7 (rea)
8	8 (inv)	9	8 (rea)
Z	Z	Z (inv)	Z (rea)
No. of probes[b]	28/29	43/48	25/26

[a] inv: inversion, rotation around the centromere; rea: rearrangement, the order of the markers is not the same between species because of an intrachromosomal rearrangement.
[b] Number of markers hybridized in heterologous conditions. Only a few of them gave no hybridization signal.

Most BAC clones from chicken microchromosomes gave hybridization signals on microchromosomes in duck (29 out of 34 probes gave a result), turkey (23 out of 28) and quail (20 out of 20), demonstrating a very low rate of rearrangements. Surprisingly, rearrangements between macro- and microchromosomes were almost never detected, except for the chicken chromosome 4 that separated into chromosome 4 plus a pair of microchromosomes in duck and turkey.

From the evolutionary point of view, it appears that bird karyotypes have evolved slower in time than those of mammals for which more drastic rearrangements have been described.

Table 4. Sequence tagged sites (STSs): Primer sequences, PCR product length and BAC clone name

STS	Oligo1	Oligo2	Size	BAC-end	Contig
T10BE004	CAGCCGTAGTTCTGTTTGGG	CACCACATCTGAAGTGCCGG	145	bW092I14Q	ADL0231
T10BE007	TGATACGGTACTGAAGAAAC	CTCTGAATAACACAGGACAG	163	bW071J05R	ADL0209/COM0101
T10BE008	GCACTAGAAAGCAGTTTGAG	CACTGCAGGTTATCGTTGTC	141	bW098C14Q	ADL0112
T10BE009	AGGGTGTGGGGAGAAAGAGG	GCTCGACTTCATGAGAAGGG	270	bW098C14R	ADL0112
T10BE014	ACAGTGTAGACACCATGTTC	CTTCAGCTTCAACATTACGG	199	bW046J07Q	MCW0194
T10BE016	GACTGAAAGCACTGACTGTC	TCAGCAGGTGTAAGTAGCTG	137	bW033O18Q	MCW0357
T10BE030	CCCCCTCAAATACCACAAAG	TTAGAAGAGTGGCCCGGAAC	156	bW071I20R	LEI0112
T10BE035	GCTTACATGATGCTGTTTGG	TAAGGGGTTACCAGTACAAG	194	bW045D14Q	LEI0112
T10BE071	CAGAATTCTCATACCCTGGG	CATGTGTTAGGGAGCTCATG	239	bW038M03R	AGC1
T10BE075	CCAGAGCATCTTGCATCATG	ATCAGGACTGCAAAGACAGC	217	bW033L04R	CRABP1
T10BE078	GCTTGTTGTCCCTTTACAAG	ATCACGCTTTGCTCTTTCAG	197	bW009F14Q	ANXA2
T10BE085	GTGTTCCAGACTTGCGTGACC	TGCTGAGTGTCCTTCTCACC	218	bW025K19Q	NEO1
T10BE086	AATCGTAAGGGGTGGAAGAG	GGGCATATCTCCTTATCTGG	132	bW025K10R	NEO1
T10BE104	AATGGAGCGAGGACAAAAGC	CCCTTCTCTTCTCCAACATG	140	bW014H05Q	LEI0112
T10BE108	CAAGCAATTGTACATCTGAG	AGGAATGAAAGAGTAGCTTG	240	bW028E16Q	ADL0038
T10BE111	CACAAAGTAAGATCCTTGGC	GTATCAGCTTTATCCGTTGG	115	bW030D08Q	ADL0038
T10BE116	TCTGCTGCTCTGCTCATGAC	TTAGGTCCCACAGTTGGAGC	155	bW033G17Q	ADL0272
T10BE119	CAATGGCAACTGACATACTC	TAGCTGTAAACATGTGTCCC	180	bW041G13R	ADL0209/COM0101
T10BE122	AAGCTTGGCAGCTCGCAGAC	CACAGTCTGCAGCCAAACTC	138	bW072B05Q	ADL0209/COM0101
T10BE140	CAACATCCAGTTCTTCTCAG	CTAACTCAGCAGTGCTCTAC	182	bW011O02R	MCW0067
T10BE141	CCACTGGCTAGTGACAAATC	TAAGGAATGGCTGGAACTGC	262	bW037E07R	MCW0357
T10BE143	ATTAGGTCAAACCATGTGCC	AGATTACCAGAATTGAGACC	166	bW021F07Q	ADL0106
T10BE145	CTTGTCCTTCGTAGCGTTGG	GGAATCTGGTGCCTGTTTGG	242	bW028F24Q	CRABP1
T10BE148	CTGGAGACAACAGCAGAGTC	TTTGCTATGCAGAACCGGTC	130	bW014A09Q	AGC1
T10BE150	TTCATCTCGGTCCAATCTGC	AACTTGGAGGAGCACGATTC	239	bW017L04Q	ADL0272
T10BE153	CCACAACTAAGTAGCACGAC	ATTCCATGCCTTTTCTTCCC	223	bW081N11Q	MCW0035
T10BE156	AAGGATCTGCTGCCTCTAAC	ATGGGACTGAGTGATGCTTG	249	bW011D17R	IGF1R
T10BE157	ACATCATCCAGTAACAGTGC	AAACCCTACGATGAGTTGCC	179	bW008G10Q	MCW0132
T10BE158	AAGCTTGATGAGAGAGACCC	TTGGGCCAGTTTCAGTTCAC	263	bW030I03Q	CYP11A
T10BE159	TCATTTGCATGCTTGCCATG	TTCTACCTAACCTATTCAGG	157	bW066K13Q	CHRNA7
T10BE160	CAACCATAGAGATGGCATGG	AGTAGCTGCAGAATAAGGCG	282	bW066K13R	CHRNA7
T10BE167	ATCAGTGGCAGTAGGAAGTC	TGTGGTTCAGTCCTCTAATG	199	bW043H13R	ADL0158
T10BE169	CCCAAGAAAGCAAACTCAAC	CCCCAATGGACAACGATAAG	200	bW038A10R	MCW0035
T10BE170	TATCTTGAGAGCCCTCACTG	ACCTCGAAGAATACAAGTGG	278	bW038K03R	MCW0132
T10BE171	GCAGAACACTTCTGAAGATC	AAATGGGTGACTCAACACAG	330	bW012C22R	MCW0356
T10BE172	CTAAAGCCAGCATATCCTGC	AGCCATCCATCCACAGATAC	256	bW006E19R	LEI0112
T10BE177	GGAGGAAACCTGAAATGCTG	GAAGCTCTAGATGGCAATTC	241	bW026J07R	LEI0112
T10BE178	ACAATCCAGGCAGTCTGACC	CATCCTGGTATCTAACACAG	235	bW003G13R	ADL0158
T10BE185	AAGAGGCTTTCTGCACACAG	CAAGAAGCCTACCTACCAAG	195	bW112M15R	B2M
T10BE187	TCATTGTGGAGGCTGTTAGG	AACGCATCTGAGTGCACAAG	194	bW116J21R	B2M
T10BE188	TCAGTAGTCCTTGTTTTCGG	GAGGAAGAGAGGGCATAATC	219	bW044O14R	MCW0194
T10BE189	ACTCAACCTGCCTCAATTTC	GCTTGTTTCATCCAAAAGCC	219	bW029L15R	ADL0272
T10BE196	ACTGGAAGCTCAGAACTCAG	CCTGCCTCAGTTTGACGTTG	261	bW006H24R	IGF1R
T10BE199	AATCGGATAGGACAGCTCTG	GTGACAGATTCCTTCCATAC	213	bW086J14R	N2RF2
T10BE201	CCTGTCTGTCTCCTGCATTG	ACTCCGAGATACCAGCATGC	248	bW010O09R	ANXA2
T10BE207	GCAACTGTTGAAGCAGAAGG	ATGACCTTGGTAAGCTCCAG	230	bW085M07Q	ADL0102
T10BE208	CTTTGCCACTAACAGCACAG	CCTCATTCATCTTCAACCCG	165	bW039P22Q	ADL0038
T10BE231	GACCTGCCAACAAGCTGTTG	GGGAAAGCGAATTATGCTTC	190	bW023K12Q	LEI0112
T10BE234	GAGTCCAGGGAGAGTCAAGG	GAGCCGTGGGGATGTGATAC	250	bW096N16Q	NTRK3
T10BE241	AGGTCTGGATGTGCTGACTC	ACAGAGCTCTCAGGGCTTTC	142	bW020P08Q	MYO5A
T10BE242	CAAAGGATAATTGAGGGCAC	AATGAAGGTGAAATCCAGCC	155	bW005N24Q	ADL0158
T10BE245	TGATCTGGCTTAGGGGCAGG	TGCTTTGTGCTCCCACTGGG	300	bW086J14Q	N2RF2

These data also suggest that the evolution of bird karyotypes could be the result of many fusion/fission processes and/or inversions instead of reciprocal translocations. Thus, microchromosomes could represent the conservation of ancestral syntenies (Burt, 2002).

This high conservation of poultry genomes will facilitate to build comparative maps and will be of great help for the study of genes with zootechnical interest in other poultry species using the chicken genetic map as reference.

A radiation hybrid map of GGA10 as a test case for the resolution power of ChickRH6

(Prepared by M. Morisson, R.P.M.A. Crooijmans, F. Pitel, M.A.M. Groenen and A. Vignal)

As a first step towards the development of radiation hybrid maps, we produced chicken whole genome radiation hybrids by fusing female chicken fibroblasts irradiated at 6,000 rad to a HPRT-deficient hamster cell line (Morisson et al., 2002). To confirm that the achieved resolution is higher than recombinant mapping, we selected 72 of the hybrids for their high

microchromosome retention rate and genotyped 65 markers chosen on microchromosome GGA10.

Selection of a panel of 72 microchromosome-rich hybrids

The whole genome retention rate of each of the 452 hybrids was assessed by genotyping markers chosen across the genome. Due to the particularities of the chicken genome structure, care was taken in the choice of markers to represent the two chromosome types. Among the 45 markers used, 22 were located on macrochromosomes, 16 were on identified microchromosomes, two were on small linkage groups and five were genetically unlinked. These seven markers were assumed to be located on microchromosomes and we analyzed the results considering two classes of markers: 22 localized on macrochromosomes and 23 localized on microchromosomes.

We selected 72 hybrids for their high microchromosome retention rate. The average retention rates of this panel were 21.2% for the whole genome, 15.9% for the macrochromosomes and 25.7% for the microchromosomes.

Development of sequence tagged sites (STSs)

Fifty-two STS markers were generated for physical mapping of GGA10 using two different approaches.

The first consists of chromosome walking from BAC clones isolated with microsatellite markers that were previously mapped to GGA10. BAC clones were end-sequenced with either the forward (Q) or reverse (R) M13 universal sequence primers. STS markers were made in the BAC-end sequences, generating PCR products in the size range of 120 to 400 bp. These were subsequently used for screening the chicken BAC library (Crooijmans et al., 2000).

The other approach consists of designing specific PCR primers in chicken gene sequences homologous to genes from regions of conserved syntenies on human chromosome 15 (Crooijmans et al., 2001). The primers were also used to screen the chicken BAC library. The BAC clones and therefore also the genes, were mapped by FISH or mapped in the RH panel (this paper) and used as starting points for chromosome walking if they were mapped to GGA10 (as described above).

The list of 52 STS markers derived from the different BAC-end sequences, including primer sequences, PCR product length and BAC clone names is shown in Table 4.

Construction of the GGA10 RH map

The 52 contig-end STS markers, 12 microsatellite markers (ADL0158, LEI0112, MCW0132, MCW0035, ADL0106, MCW0067, ADL0272, MCW0194, LEI0103, LEI0333, ADL0231 and MCW0003) as well as one gene tag *(FBNI)* were genotyped in duplicate and scored as present, absent or ambiguous when positive in only one of the PCR typings. The estimated retention frequency for the data set was 20.7%.

Pairwise and multipoint data analyses were completed using RH2PT and RHMAXLIK programs in the RHMAP3.0 software package (Boehnke et al., 1991; Lunetta and Boehnke, 1994). We assumed random breakage along the chromosomes and equiprobable retention of fragments.

We used a lod score threshold of 6 to define linkage groups and that resulted in a large group including 47 markers, a smaller one including seven markers, and three very small ones including only two markers. The five following markers were unlinked: LEI0112, T10BE086, T10BE035, T10BE078, T10BE201, of which T10BE078 and T10BE201 belong to the same contig as well as T10BE035 and LEI0112. Among them, LEI0112 showed a very low overall retention (0.070) and should be genotyped again.

Multipoint analyses were performed using the RHMAXLIK program. For each of the two larger linkage groups, a framework map was built with a threshold likelihood ratio >1:1,000 (minimum log10 likelihood difference of 3). Eleven markers mapped on the framework in the large linkage group and five markers mapped on the framework in the small one. A global framework of 16 markers (Fig. 9) was assembled by testing the four possible orientations of the two previous frameworks using the RHMAXLIK program. The global framework was checked again by removing one marker at a time and analyzing the likelihood of all its possible locations on the map compared to the other markers, and T10BE111 was added to the global framework. This framework was validated when log10 likelihood difference for one order compared to the other orders was superior to 3. A comprehensive map was established by adding the remaining markers at their most likely locations to the right of the framework map (Fig. 9). The map design was created by using MapChart, a software for the graphical presentation of linkage maps and QTL developed by Voorrips (2002).

Microsatellite markers localized on the RH map are indicated in bold in Fig. 9. They facilitate integration of the genetic and RH maps. The radiation map is 562.6 cR_{6000} long and the genetic map is 120 cM long, which resulted in a distance (genetic vs RH) ratio for GGA10 of 1 cM = 4.7 cR_{6000}. The order of the shared markers is the same for the two maps except for ADL0272 which is mapped below marker ADL0231 on the RH map which is in agreement with BAC contig data (R.P.M.A. Crooijmans, personal communication).

The nine STS markers linked to genes (on the same BAC contig), T10BE156 to *IGF1R*; T10BE071 to *AGCI*; T10BE160 to *CHRNA7*; T10BE145 to *CRABP1*; T10BE085 to *NEO1*; T10BE199 to *NR2F2*; T10BE158 to *CYP11A*; T10BE241 to *MYO5A* and T10BE185 to *B2M* appear underlined in Fig. 9. Together with the gene *FBNI*, which is localized on the comprehensive map, we could map ten genes on GGA10.

The primary goal of this work was to test the validity of the radiation dose applied to the chicken fibroblasts used to build the ChickRH6 panel. It was therefore performed before completion of the definitive panel.

The results show that the radiation dose chosen is low enough to allow the construction of linkage groups and high enough to refine the genetic map. In this preliminary work we could map ten genes on microchromosome GGA10. Thus the definitive panel, ChickRH6 composed of 90 clones, which is now available to the academic community, provides a valuable complementary approach to the other chicken genome mapping techniques. Data thus obtained can be of great help for building the more detailed maps based on large insert BAC clone contigs (Lee et al., 2001; Jennen et al., 2004). The mapping of genes is also greatly facilitated, as compared to genetic mapping, an important point to be taken into consideration

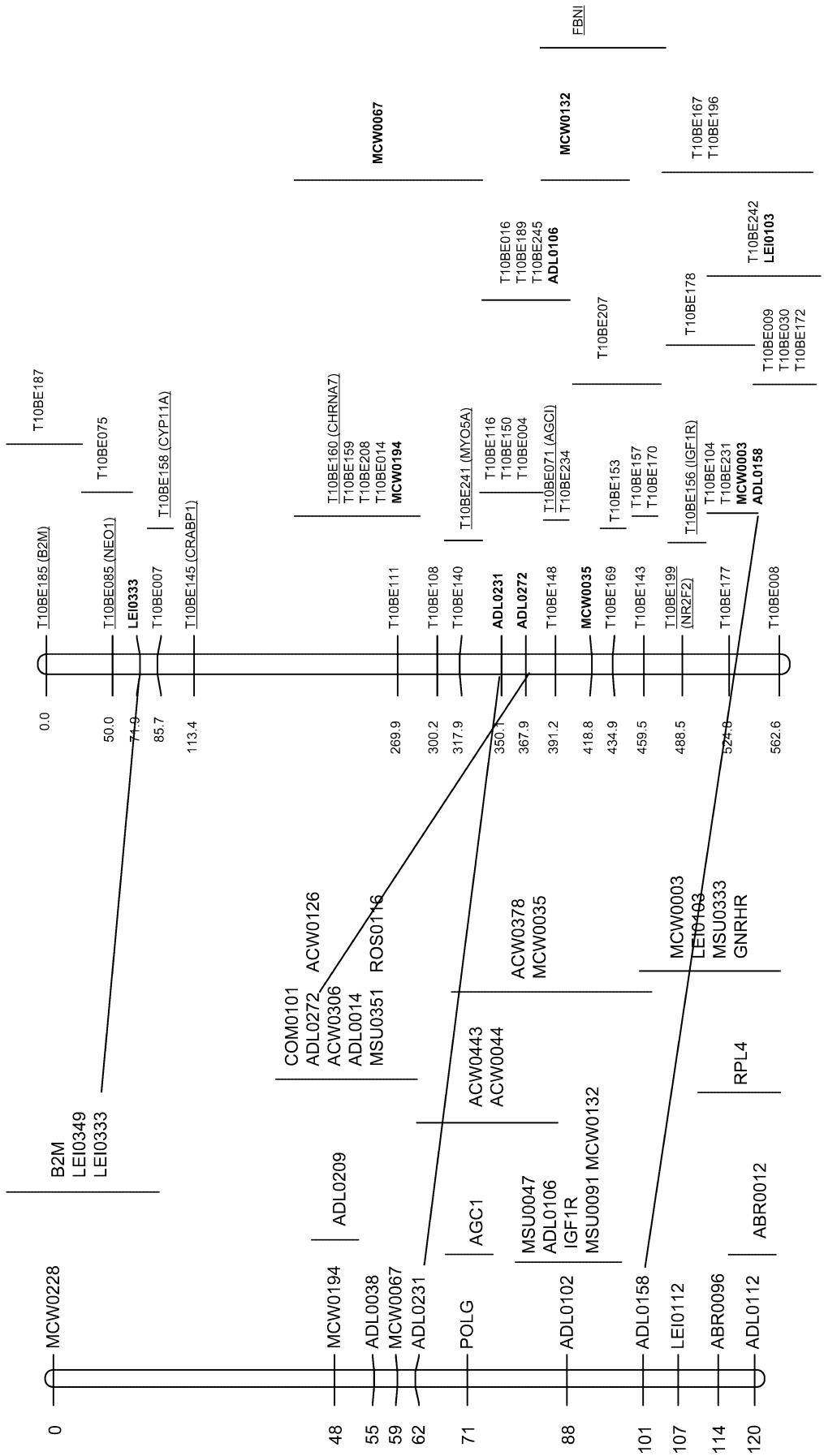


Fig. 9. Genetic and radiation maps of microchromosome GGA10.

with the large amount of EST data now available (Abdrakhmanov et al., 2000; Tirunagaru et al., 2000; Carre et al., 2001; Boardman et al., 2002; Buerstedde et al., 2002).

Moreover, although human/chicken comparative mapping has been well studied so far (Schmid et al., 2000; Crooijmans et al., 2001; Ladjali-Mohammedi et al., 2001; Buitenhuis et al., 2002; Jennen et al., 2002, 2003; Nanda et al., 2002a; Smith J. et al., 2002), RH-mapping data will increase the resolution of the gene maps within the conserved syntenies. It will thus help in refining the synteny breakpoints as well as detecting internal rearrangements, as it has been done in other species (Barbazuk et al., 2000; Goureau et al., 2001; Smith S.F. et al., 2002; Sun et al., 2002; Lahbib-Mansais et al., 2003; Martins-Wess et al., 2003).

Using the ChickRH6 definitive panel, composed of 90 clones, will enhance the precision of comparative mapping and will extend the number of positional candidates in QTL mapping approaches.

Single nucleotide polymorphism (SNPs) in chicken: resources and possible applications

(Prepared by G. Ben-Ari, L. David, S. Blum, T. Twito, A. Vignal, S. Weigend, M.W. Feldman, U. Lavi and J. Hillel)

Preface

This review was invited on February 2003 and submitted on August 2003. Its publication at the present date should not diminish from its significance. Meanwhile, the chicken genome draft has been released on March 2004. On December 2004, three important articles on the chicken genome have been published in Nature: (1) Sequence and comparative analysis of the chicken genome (International Chicken Genome Sequencing Consortium, 2004), (2) 2.8 million SNPs in the chicken genome (International Chicken Polymorphism Map Consortium, 2004), and (3) physical map of the chicken genome (Wallis et al., 2004). These revolutionary events point out the necessity to further study the chicken genetic variation at the level of single nucleotide polymorphism and face serious scientific challenge for the chicken geneticists.

SNPs – the latest type of DNA markers

Single nucleotide polymorphisms (SNPs) are the latest generation of DNA markers (Jin et al., 1995; Collins et al., 1997; McKenzie et al., 1998; Wang et al., 1998). SNPs are single nucleotide differences between individuals that are found in functional regions (e.g. promoters and exons) and in non-functional regions of the genome. Almost all SNPs constitute biallelic polymorphisms. They are the most common polymorphisms in all genomes that have been examined, and probably account for most of the genotypic contributions to phenotypic variation (Botstein and Risch, 2003). Other types of sequence variation such as chromosome aberrations and variation in number of tandem repeats are less common throughout the genome, and their distribution does not seem to be as random as SNPs (Cargill et al., 1999; Hacia et al., 1999).

A major effort is currently being devoted to research and utilization of SNPs in the human genome. NCBI's dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) currently contains 4,540,241 validated human entries, out of which 45,896 are non-synonymous coding SNPs (Reumers et al., 2005). An important goal of the human genome project is to generate a nearly complete catalog of the common genetic variants (Kriglysh and Nikerse, 2001).

So far, only a few studies have focused on the characterization and application of SNPs in chicken but, similarly to humans, this type of polymorphism has the potential for future interesting discoveries. The current status of SNP discovery and utilization in chickens is presented below.

Sources of information

Chicken databases. Several research centers have facilitated SNP research in chickens by maintaining public databases of chicken ESTs and SNPs. The University of Delaware – UD (http://www.chickest.udel.edu/) provides data on SNPs in chicken genes using a diverse collection of chicken EST libraries. The database is based on 18 cDNA libraries generated from various tissues including liver, oviduct, fat, pituitary, reproductive tract, muscle, and lymphoid cells. Over 40,000 EST sequences were generated from these libraries. The information includes the construction of each library and protocols for dealing with the inserts. Approximately 3,000 contigs were assembled from more than 23,000 chicken ESTs from various individuals, populations and tissues. A total of 12,019 SNPs are currently available in this EST data set, discovered by alignment of homologous ESTs. The average SNP within transcriptional regions of this data set occurs every 2,119 bp, which is rather low relative to other reports on the chicken genome. The UD chicken SNPs homepage (http://chicksnps.afs.udel.edu/) contains a searchable database of the chicken cSNPs (Emara and Kim, 2003).

In the UK, a collaborative effort of UMIST (University of Manchester Institute of Science and Technology), Nottingham University, the University of Dundee, and BBSRC (Biotechnology and Biological Sciences Research Council) has yielded 340,000 ESTs (http://chick.umist.ac.uk). These were sequenced from 21 different fetal and adult chicken tissues. All tissues originated from the White Leghorn breed (Boardman et al., 2002).

The chicken gene index at the Institute for Genome Research (TIGR), combines research data from all international *Gallus gallus* EST sequences and genetic research projects. The goal is to generate a non-redundant database of all chicken genes, their expression patterns, functions and evolutionary relationships. The current "release 5" (5 January 2003) shows 71,395 singleton ESTs out of a total of almost 108,000 ESTs. A total of 35,790 "tentative consensus" genes (EST clusters) and 684 "mature transcripts" (ETs) were identified. The database can be searched for sequences and genes of interest (http://www.tigr.org/tdb/tgi/gggi).

SNP characterization. SNP studies have typically involved two main phases: SNP discovery and SNP genotyping. The main approach for SNP discovery is sequence comparison of specific regions between various individuals (Fig. 10). This approach is costly and time consuming. In addition, when two alleles are present at a locus of an individual, it is hard to distin-

guish between sequencing artifacts and an actual heterozygote for the genotyped site. SNPs identified on the basis of EST data are of special interest since they have a greater chance of resulting in amino acid change. However, it has been claimed that the number of SNPs of this kind is limited due to selection pressure that coding sequences have undergone (Vignal et al., 2002). Bacterial artificial chromosome (BAC) libraries can be used for SNP discovery. However, the distribution of these SNPs is uneven across the genome and depends on the number of overlapping BAC clones along the genome (Sachidanandam et al., 2001). In addition, in silico comparison of DNA sequences (ESTs and genes) can reveal SNPs. Identification of SNPs has been improved by the development of computer programs with better quality of base-calling such as PHRED (Ewing and Green, 1998; Ewing et al., 1998), and polymorphism detection using POLYPHRED (Nickerson et al., 1997) and POLYBAYES (Marth et al., 1999).

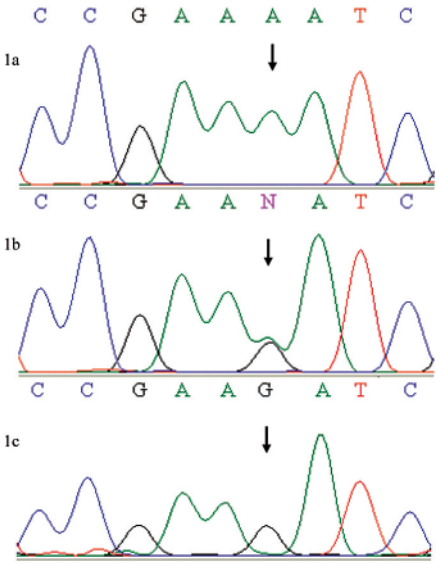


Fig. 10. SNP discovery by sequence alignment of three individuals. The SNP position is marked with arrows. 1a: homozygous AA; 1b: heterozygous AG; 1c: homozygous GG.

SNP genotyping can be done using several methodologies and instruments. The genotyping methods are based on the discrimination between different alleles and signal-detection technologies. The different methodologies are chosen according to their accuracy, throughput and price. The major techniques of SNP genotyping are: (a) Oligonucleotide chips, in which fluorescently labeled PCR products are hybridized to sets of oligonucleotides complementary to the allelic sequences placed on a microarray. The fluorescence pattern is analyzed to determine which allelic set of probes gives a positive hybridization signal (Wang et al., 1998). (b) MALDI-TOF Mass Spectrometry is based on differential extension of a short primer through the polymorphic region. The specific amplification products are immobilized on a magnetic solid phase and subsequently denatured. A specific primer is annealed close to the SNP site and a limited primer extension reaction is performed in the presence of at least one dideoxynucleotide triphosphate. Specific termination products are generated and then analyzed by MALDI-TOF mass spectrometry (O'Donnell et al., 1997). (c) Pyrosequencing is based on the detection of pyrophosphate (PPi) during the incorporation of dNTP into a newly synthesized DNA. The released pyrophosphate allows the synthesis of ATP, which serves to release light in the presence of luciferase (Ronaghi et al., 1998). Several other methods have been developed for SNP genotyping (Twyman and Primrose, 2003).

SNP frequencies and their association with phenotypes

Frequency and distribution. The private sequencing endeavor of the human genome has so far reported 2.4 million SNPs and the public SNP consortium 1.8 million SNPs; the total number of SNPs was estimated by Botstein and Risch (2003) to be at least 15 million. In the avian genome however, we are just at the very first steps; at the time of writing, there have been only a few reports regarding frequencies and distribution of SNPs (Table 5). Sequencing of 21 fragments from a cDNA

Table 5. Literature summary of frequencies and distribution of SNPs in fowls

Population	SNP frequency	Source of DNA	% Transition SNPs	Number of individuals	% of non-synonymous SNPs	Reference
Chicken Guinea fowl Pigeon Quail	1:277 bp 1:1029 bp 1:159 bp 1:1632 bp	EST		10 10 10 10		Smith E.J. et al., 2000[a]
White leghorn	1:470 bp		64	1		Smith E.J. et al., 2001[b]
Between layers Between broilers	1:138 bp 1:180 bp		40 40	10 10		Smith E.J. et al., 2002[c]
Between layers and broilers	1:640 bp 1:144 bp	EST Introns and promoters	85 53	1 from each	35	Twito et al., 2002

[a] SNPs within species.
[b] SNPs within white leghorn. Heterozygotes were identified as SNPs.
[c] SNPs between broilers and between layers. Six layer lines and three broiler lines were used. Heterozygotes were identified as SNPs.

library of turkey in five species resulted in SNP rates ranging from 1:159 bp in pigeon to 1:1,632 bp in quail (Smith et al., 2000). Heterozygosity estimation based on sequencing of 50 ESTs from one embryo of a White Leghorn chicken suggested a SNP frequency of 1:470 bp (Smith et al., 2001). Comparing 12 ESTs among six lines of layers resulted in SNP every 138 bp. The same sequences yielded a SNP every 180 bp among three broiler lines. Only 33% of these SNPs were common between layers and broilers (Smith E.J. et al., 2002). The common SNPs were C↔T and A↔G transitions.

In a follow up study conducted in our labs in Israel aimed at the identification of type-specific SNPs (broilers and layers), primers were synthesized on the basis of EST data. Liver cDNA fragments of one broiler and one layer were compared for SNP discovery. Each detected SNP was validated by reverse-primer sequencing. A total of 16,630 bp (from 37 coding regions) revealed 26 cDNA SNPs (cSNPs), ranging from 0 to 5 per sequence. The average was 1 SNP in 640 bp (Table 5).

Thirty-five percent of the cSNPs were found to be non-synonymous (causing a change of amino acid). In addition, we searched for chicken genes in the NCBI database (http://www.ncbi.nlm.nih.gov/) and designed primers for non-coding regions. A total of 7,930 bp (from 18 genes) revealed 55 SNPs, ranging from 0 to 9 per sequence, an average of one SNP at every 144 bp. In both, coding and non-coding regions, most of the SNPs were transitions. In the coding sequences the percentage of transitions was 85% compared to 53% in the non-coding regions (P[t] = 0.0012).

In a collaborative project – AVIANDIV (EC project: Development of Strategy and Application of Molecular Tools to Assess Biodiversity in Chicken Genetic Resources; EC Contract No. BIO4-CT98-0342: Weigend S. [Coordinator], Groenen M.A.M., Tixier-Boichard M., Vignal A., Hillel J., Wimmers K., Burke T. and Mäki-Tanila A.), ten individuals from each of ten chicken populations of various origins were genotyped at SNP loci. Sequencing of 6,952 bp from 15 genomic DNA fragments yielded 145 genomic DNA SNPs (gSNPs), giving an average of 1 per 50 bp, which is much higher than the reported frequencies in chickens, and in other species, particularly in human (~ 1 per 1,000 bp; Marth et al., 2001). This high frequency presumably reflects the wide genetic spectrum among the tested populations, including the wild Red Jungle Fowl. The average frequency of the rare allele was 0.13, which is similar to humans where no SNP with rare allele frequency below 4% was observed (Hillel et al., 2002; Vignal et al., 2002).

In a study performed in our laboratories in Israel, ten individuals from each of twenty populations of the AVIANDIV populations (the ten populations mentioned above are included) were genotyped at 25 cSNP loci of 25 genes (one SNP per gene). Genotyping was carried out by the MassArray (mass spectrometry) technology. All SNPs were found to be biallelic. Frequencies of the rare alleles were 0.29, 0.29 and 0.095 in the untranslated regions, synonymous and non-synonymous SNPs, respectively (Fig. 11).

The rare allele frequency at the non-synonymous SNPs is significantly lower than in the untranslated regions ($P < 0.0001$) and in the synonymous SNPs ($P < 0.0001$). Similar results have been reported in humans (Cargill et al., 1999). In the chicken

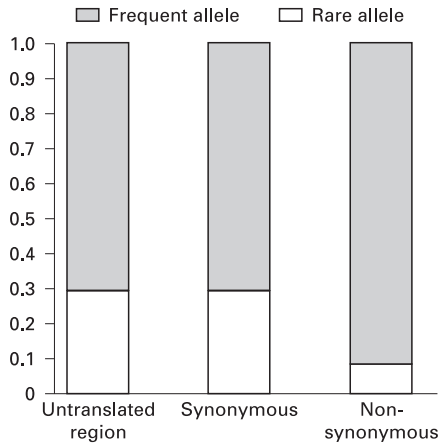


Fig. 11. Frequencies of the rare and the frequent alleles of various SNPs used in the biodiversity analysis.

cSNPs mentioned above, the average frequency of the rarest allele was 0.28 and the average observed frequency of the heterozygotes, 0.23, was significantly different from the expected frequency – 0.4, assuming Hardy-Weinberg equilibrium (P[t] = 0.0009).

Association between SNPs and phenotypes. More than 1,200 human disease genes have been identified over the past two decades (Botstein and Risch, 2003). As of March 2003, the Human Gene Mutation Database (HGMD) has reported on 39,145 mutations in 1,516 genes, which are associated with human diseases and traits (Stenson et al., 2003). The relative frequencies of the various types of these mutations are as follows: the majority originated from in-frame amino acid substitutions, including missense and nonsense mutations (57.5%), deletions (22.2%), insertions/duplications (7.4%), splice-site mutations (9.6%), indels (1%), and less than 1% are in regulatory regions. At this stage, the rate at which annual increase entries in this database accumulate is more than 5,000.

In order to narrow the genomic interval containing genes of interest, one needs a saturated map of markers and a large resource population. In chickens, there is a need to increase the marker density of the genetic map, especially in the microchromosomes. Currently, genome scans for linkage between markers and genes of interest are within the range of about 10–20 cM or more (Groenen et al., 2000). Efforts should be made to benefit from the abundance of SNPs in the chicken genome and map them in intervals of less than 5 cM apart, which will facilitate "fine-mapping" of chromosome fragments.

Both synonymous and non-synonymous SNPs are valuable genetic markers to map QTL. Indeed, high-resolution genetic maps based on SNPs are under development for many human chromosomes to facilitate fine-mapping studies (Mullikin et al., 2000; Roberts, 2000; Taillon-Miller and Kwok, 2000; Shastry, 2002; Akey et al., 2003). Although many SNPs are randomly distributed along the genome, some are clustered within certain genes (Feder et al., 1996). These clusters can actually be

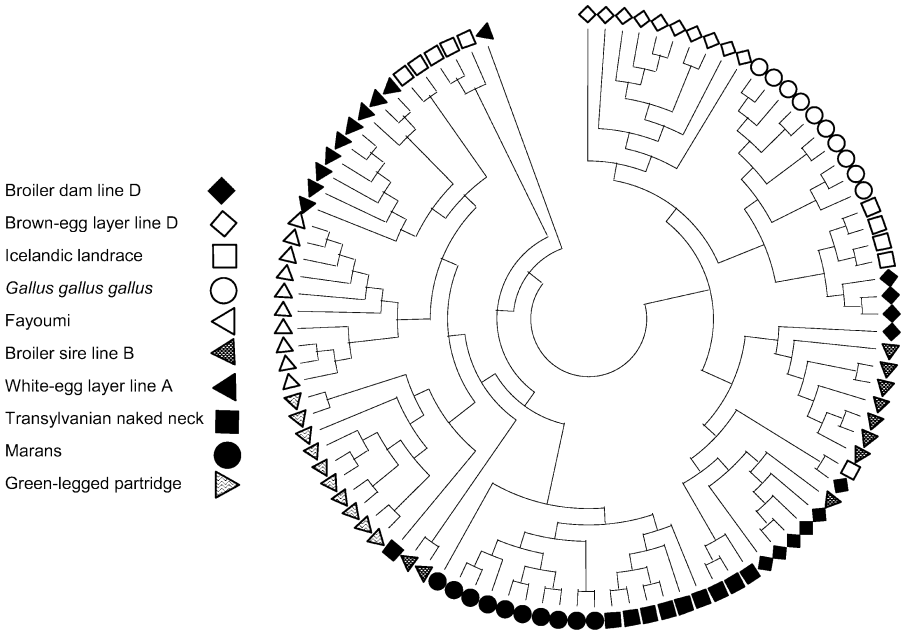


Fig. 12. Hierarchical clustering of 100 individuals. Labels designate the ten populations.

used to generate a molecular haplotype across a gene (Judson and Stephens, 2001).

Many SNPs were found to be fully informative in crosses between inbred lines of mouse (Lindblad-Toh et al., 2000). This is relevant to chickens, where several inbred lines are available and some of the resource populations for mapping complex traits are derived from crosses between these inbred lines (Vallejo et al., 1997; Yonash et al., 1999, 2001; Deeb and Lamont, 2003).

Interest in the discovery and analysis of SNPs grows with the increase in genome projects resulting in full sequence of more and more species. Associations between SNPs and complex phenotypes have already been characterized in a few cases such as schizophrenia (Chumakov et al., 2002; Stefansson et al., 2003) and cancer in humans (Bonnen et al., 2002), and the ability of *Saccharomyces cerevisiae* to grow at high temperature (Steinmetz et al., 2002). A few studies in chicken have identified association between SNPs and phenotypes. Liu et al. (2003) found SNP polymorphism in a highly conserved region of Natural Resistance-Associated Macrophage Protein 1 gene *(NRAMP1)* associated with *S. enteritidis* vaccine and pathogen challenge response in young chicks. An SNP within *NRAMP1* was found to be associated with the control of resistance to *Salmonella* carrier state in hens by Beaumont et al. (2003). Zhou and Lamont (2003) detected associations of single nucleotide polymorphisms in MHC class I and II genes with antibody response parameters (Liu et al., 2002, 2003). A non-synonymous SNP resulting in a specific amino acid substitution (Ser to Asn) in the chicken *Mx* gene was considered to determine the antivirally positive or negative *Mx* gene for responses to influenza virus and vesicular stomatitis virus (Ko et al., 2002).

Biodiversity and phylogenetic analyses

The species gene pool of chickens consists of many breeds and strains. The pattern of polymorphism in each of these populations results from mutation, genetic drift and selection (Hillel et al., 2002).

Assessments of genetic diversity between and within chicken populations have been carried out using various DNA markers (Dunnington et al., 1994; Zhou and Lamont, 1999; Romanov and Weigend, 2001; Rosenberg et al., 2001; Hillel et al., 2002, 2003). These studies contribute to the assessment of genetic relationship between these populations. SNPs are a very promising class of markers for use in biodiversity studies because of their abundance and their amenability to high throughput analysis. SNPs are applicable to biodiversity studies using both coding and non-coding regions. These markers can be used as single loci as well as haplotypes which are quite stable on an evolutionary time scale. On the other hand, SNPs are slower evolving markers than microsatellites. Thus, it is predicted that more recent changes in the history of populations would be hard to detect using SNPs.

Nuclear SNPs. Eight European laboratories collaborated in a research project (AVIANDIV) to study biodiversity among 52 chicken populations, ten of which were genotyped at SNP loci (ten individuals per population). Sequencing of 6,952 bp from genomic DNA fragments yielded 145 gSNPs. Based on these 145 gSNP data, clustering analysis assigned most of the individuals to their original population (Fig. 12).

When genotype frequencies of these 145 SNPs were used to construct hierarchical clusters of the ten populations, bootstrap values of the resulting SNP tree were high (data not shown). Thus, in this case, the high value of the bootstraps for the unrooted SNPs tree is in good agreement with the high proportion of correct assignments of the individual birds to their original populations. Rosenberg et al. (2001) used data on 22 micro-

satellite markers to cluster 600 birds, 30 individuals from each of 20 populations (the ten populations mentioned above constitute a subset of these 20 populations, which in turn is a subset of the 52 AVIANDIV samples). About 98% of the 600 individuals were correctly assigned to their populations from just their genotypes. However, when hierarchical clustering of these 20 populations was done, bootstrap values were low. From these particular sets of data one may conclude that when SNP data are used, high bootstrap values for trees correlate well with correct affiliation of individuals to their original populations. Microsatellite data, on the other hand, provide very good affiliation of the individuals but produce a poor population tree. It is not clear whether this result was obtained by chance or whether it is a rule, due to the different mutation rate of the two marker types and the relatively few generations (at most, hundreds) of separation between most of the analyzed populations.

This dilemma could also be relevant to the following experiment. In biodiversity studies where populations are represented by a given sample size of individuals and our resources are limited, we often struggle with the question: what is the best tradeoff between number of individuals per population, and number of loci for genotyping. It is obvious that there is no definite single rule, since it depends on the genetic variability within the assessed populations, on the genetic distances between population pairs and on the polymorphism level of the marker loci being used. To partially address this question on a given gene pool described above, we first analyzed the data on the 145 SNP loci for two sample sizes of the ten populations: (1) three individuals per population and (2) ten individuals per population. The two resulting trees were similar with quite high bootstrap values. A similar examination was performed for the number of loci to be used; we compared the ten individuals from each of the ten populations, once with 145 loci located on 14 fragments, and again with 50 loci (3–4 SNP loci at each of the fragments). The smaller sample of loci provided different tree patterns with much lower bootstrap values. Improved bootstrap values for the sample size of ten individuals per population were achieved when the number of loci was about 100. This examination indicates that when SNP data are used for phylogenetic studies, sampling variation among markers is higher than among individuals. Similar results were obtained for microsatellite data (data not shown). At this point and for this particular set of samples and markers it was evident that sample size can be quite small (< 10) while the number of loci should be large (> 50). An interesting and probably unexpected result was obtained with regard to the effect of number of loci within DNA fragments (about 460 bp each). From the above detailed examination and from other particular tests it was detected that increasing the number of SNP loci within fragments significantly improved the reliability (bootstrap values) of the tree in spite of the expected linkage disequilibrium between loci. Interestingly, similar results were obtained when data were analyzed as 145 non-linked markers or as haplotypes from 14 fragments.

SNPs in the mitochondria. The chicken mitochondrial DNA is a circular molecule, containing 16,775 base pairs. This mitochondrial sequence was fully described by Desjardins and Morais (1990), and has the genetic code common to all vertebrates.

SNPs in the mitochondrial DNA (mtDNA) are widely used in molecular evolutionary studies. These sequences have proven helpful for estimating times of species and population divergence, and for comparison of evolutionary rates and phylogenetic relationships (Nei, 1987). The mutation rate of the mitochondrial DNA is 10–20 times higher than that of the nuclear sites (Merriwether et al., 1991).

The displacement loop (D-loop) region of the mtDNA contains the elements that control the replication of the molecule. This region is characterized by a high mutation rate. Therefore, this region has frequently been used to study matriarchal evolutionary and phylogenetic aspects of species and populations. Ancestry of the domesticated fowls has been described using sequence variation and restriction patterns from the control region (Fumihito et al., 1994, 1996). In these SNP studies, mtDNA of wild species and subspecies of *Gallus* were compared with mtDNA of domesticated populations. The authors suggest that the origin of the domestic fowls is monophyletic. Most of the variation found in the domesticated specimens was common to *Gallus gallus gallus* and *Gallus gallus spadiceus*, both of which are subspecies of the Red Jungle fowl. Some variation patterns, however, were common also to *Gallus gallus bankiva*. The wild chicken samples were obtained mainly from Thailand and therefore, this region was suggested as the geographic origin of domestication. It would be interesting to test the ancestry of domesticated fowls based on nuclear SNPs and with broader geographical sampling, in order to see if these findings are true for the patriarchal ancestry and for other possible origins of domestication as well. SNP analysis of the D-loop region in some native Chinese breeds suggests that they originated in Thailand as well (Niu et al., 2002).

Chicken SNPs in mitochondrial regions other than the D-loop have also been used in phylogenetic studies. For instance, the sequence of the *Cyt-b* gene has proven useful in distinguishing between meat-type chickens (chunky broiler from Japan) and egg-type chickens (White Leghorn, Fayoumi, and Black Minorca breeds). The chunky broiler was much more polymorphic than the egg-type breeds (Shen et al., 2002).

Concluding remarks

In summary, SNPs can be considered as a good tool for the identification of genes controlling important traits as well as for the assessment and analysis of biodiversity and phylogenetic relationships. Either associations between SNP alleles and phenotypes or the allelic variants themselves are expected to be used to improve chicken breeding programs. Detection of allelic variants in agriculturally important genes will improve our understanding of the connection between sequence variation and phenotypes. In addition, SNPs can be used to study the genetic structure of wild and domesticated populations in order to both preserve and utilize the existing genetic resources. For that, we need to develop SNP-specific strategies for accurate and reliable estimation of genetic diversity and to use optimal approaches for its conservation. Recently, the sequence draft of the chicken genome was released in March 2004, and a relevant article in December 2004 (International Chicken Genome Sequencing Consortium, 2004). At the same time the International Chicken Polymorphism Map Consortium (2004) re-

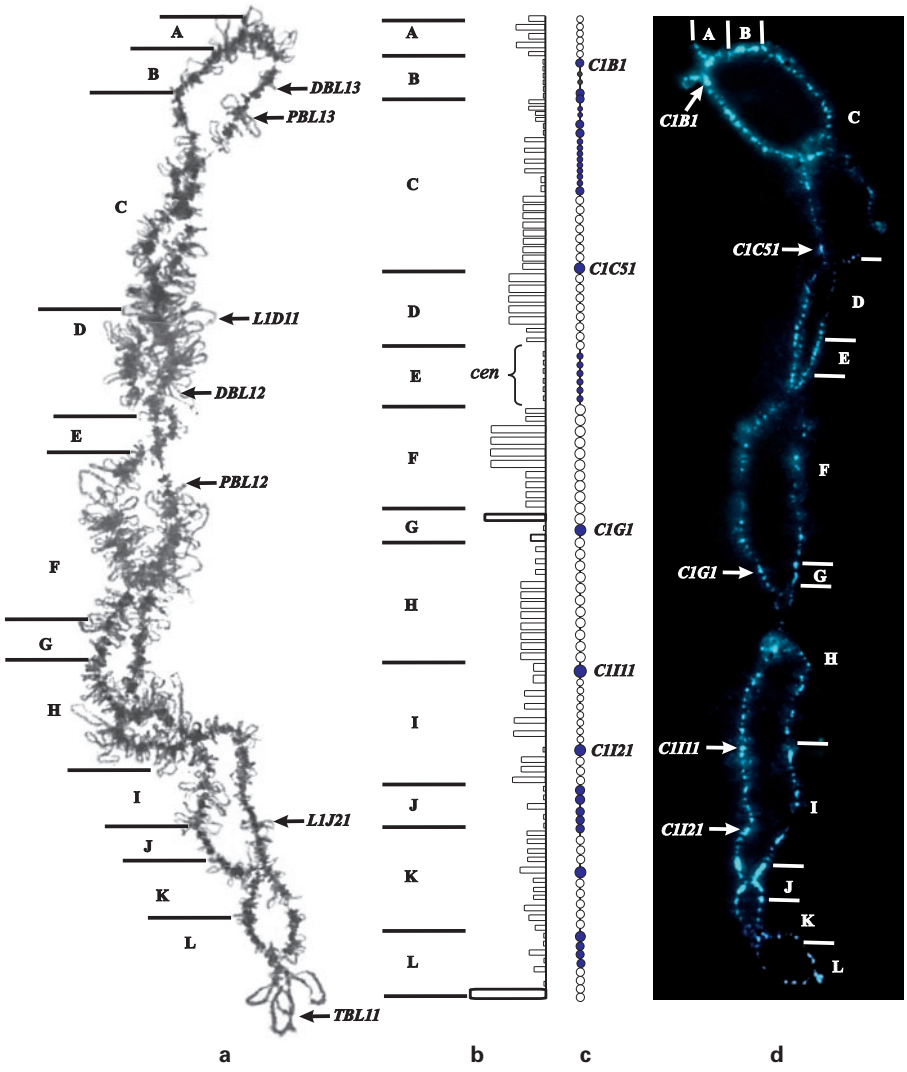


Fig. 13. Chicken lampbrush chromosome 1. (**a**) Coomassie blue R250 staining. The landmarks of chromosome regions A–L and some marker loops are shown. DBL, distal border loops of loopless regions; PBL, loops on the proximal border of loopless regions; TBL, telomeric bow-like loop on qter; L1D11, L1J21, marker loops; *cen*, centromeric region. (**b**) Cytological map. The map shows the landmarks of chromosome regions. (**c**) Chromomere map. Blue axial dots symbolize DAPI-positive chromomeres. (**d**) DAPI staining. The landmarks of chromosome regions A–L and some marker chromomeres are shown. C1B1–C1I21, marker chromomeres.

ported on the chicken variation database (http://chicken.genomics.org.cn/index.jsp) containing 2.8 million SNPs. These important milestones will significantly improve the two main areas of chicken SNP research: (1) detecting genes involved in the genetic variation of important traits and understanding their role and function, and (2) better assessment of chicken biodiversity, including phylogenetic issues related to the time of locations and species that were involved in the process of the chicken domestication.

Maps of the lampbrush macrochromosomes of the chicken and Japanese quail

(Prepared by A.V. Rodionov, S.A. Galkina and N.A. Lukina)

Lampbrush chromosomes (LBCs) in oocyte nuclei of the chicken were first observed by Holl in 1890. Later they were described by Loyez (1906), who also studied the lampbrush stage of oogenesis in some other birds. Then Koltzoff (1938) studying LBCs of the chicken, pigeon, and tritons arrived at the conclusion that an LBC is a giant chain molecule, a so-called genonema. In his opinion it consists of elementary chromomeres, which reproduce by a template copying mechanism and, by a similar mechanism, induce the formation of gene products in the form of either granular strands or lampbrush loops. Kropotova and Gaginskaya (1984) and Hutchison (1987) introduced the bird LBCs as a new model in current cytogenetics. They believed, and we hold this viewpoint, that bird LBCs can be a fruitful tool for poultry cytogenetics and genome research. In particular, the use of these extended, looped chromosomes can facilitate high-resolution gene mapping and preparation of locus-specific DNA sequence libraries by microdissection. Because each lampbrush bivalent possesses one or few chiasmata, which are a reliable indicator of reciprocal recombination events, they can also be used in the study of the chromosome control of crossing over in the chicken and other poultry species.

Chromosomes in the form of lampbrushes appear in the chicken growing oocytes at 2–3 weeks of posthatching development (Gaginskaya and Chin, 1980). In practice, well-developed

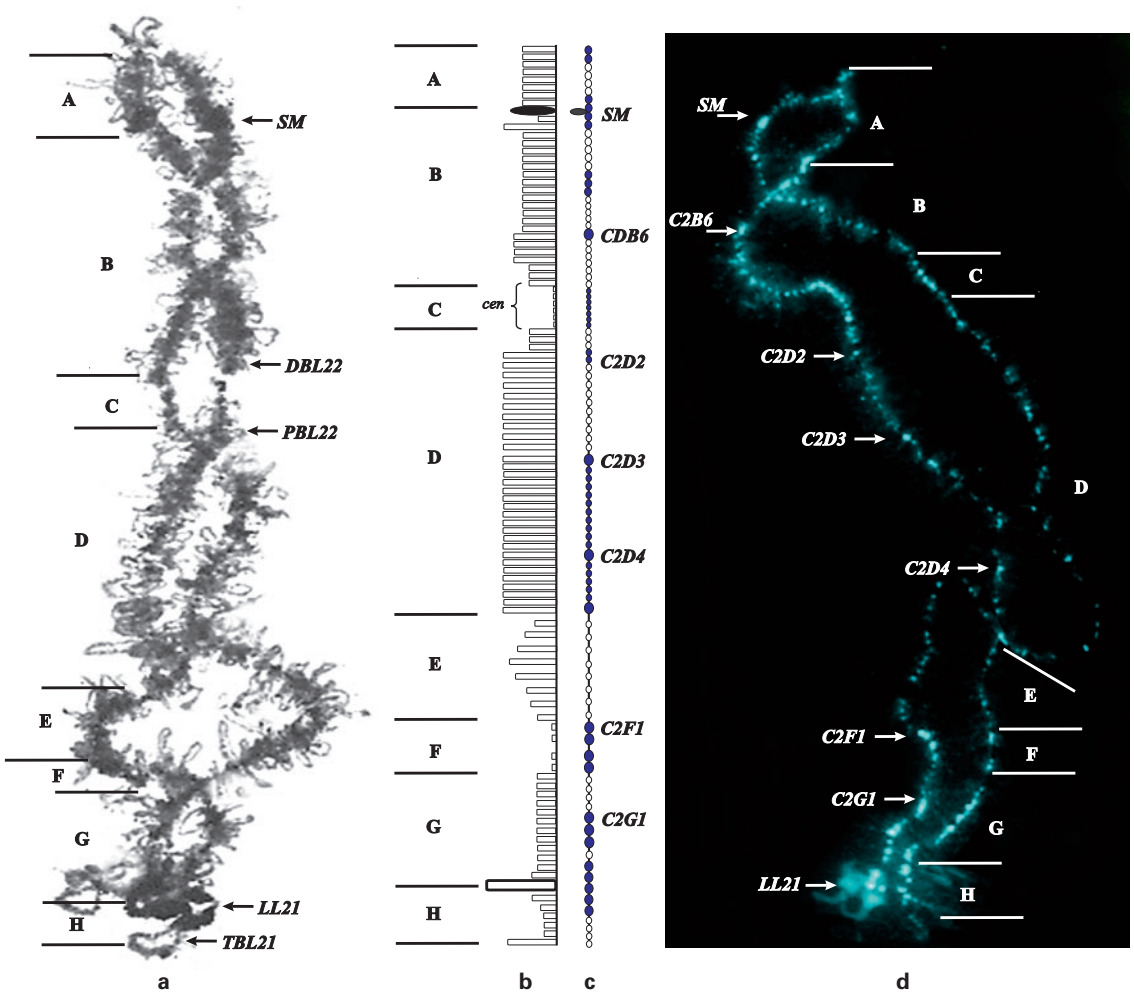


Fig. 14. Chicken lampbrush chromosome 2. All indications as for Fig. 13. SM, spaghetti marker.

LBCs are prepared by manual isolation from the oocyte nuclei of previtellogenic white follicules with diameters of 0.5–1.5 mm from sexually mature hens. Isolation of oocyte nuclei and chromosomes from smaller oocytes is more difficult. In more advanced vitellogenic yellow oocytes chromosomes are much shorter and condensed, their lateral loops are already contracted (Chelysheva et al., 1990). The technique for working with bird LBCs was described by Solovei et al. (1992) and Rodionov and Chechik (2002).

Figures 13a–23a show chicken and Japanese quail LBCs stained with Coomassie blue R250. In each LBC the homologous chromosomes are held together by few or single chiasmata. Each homologue consists of a linear array of numerous compact chromatin granules or chromomeres from which laterally projecting loops arise. According to their length the macrobivalents are numbered from 1 to 5. ZW describes the sex bivalent. A characteristic serpentine form of the sex bivalent is due to a single terminal chiasma.

Electron microscope studies showed that each chromomere of the avian LBCs is composed of both numerous miniature and few extended lateral loops with a contour length of about 10–15 μm or more (Kropotova and Gaginskaya, 1984; Hutchison, 1987; Tsvetkov, 1987; Solovei et al., 1992). Thus the extended loops contain about 30–40 kb. The longest ever observed loop, the telomeric loop of turkey chromosome 4, measures 215 μm corresponding to 688 kb of fully extended DNA (Myakoshina and Rodionov, 1994). In light microscope preparations the chromomeres without extended loops look like loop-less.

Each lateral loop contains a single DNP axis with almost fully extended DNA, which carries numerous RNA polymerases and their associated RNP transcripts. There are lateral loops consisting of a single transcription unit or few transcription units of different lengths and polarities (Kropotova and Gaginskaya, 1984; Hutchison, 1987; Tsvetkov, 1987). The fact that each extended lateral loop carries a bulk of RNA transcripts provides a way to study the genetic content and transcription pattern of individual loops by in situ hybridization (Pukkila, 1975; Tarantul et al., 1989; Solovei et al., 1993). Note also, that numerous RNA transcripts on the lateral loops should considerably amplify the hybridization signal (Weber et al., 1984).

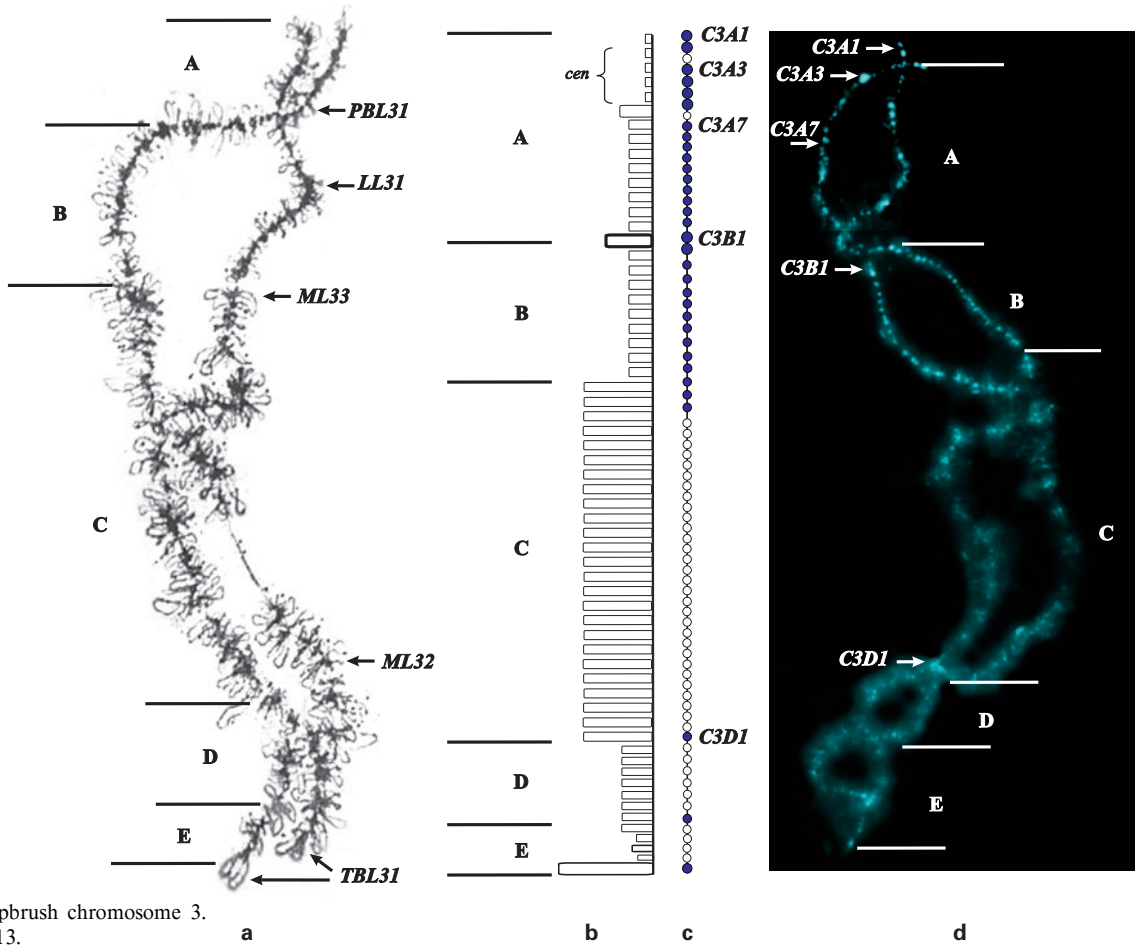


Fig. 15. Chicken lampbrush chromosome 3. All indications as for Fig. 13.

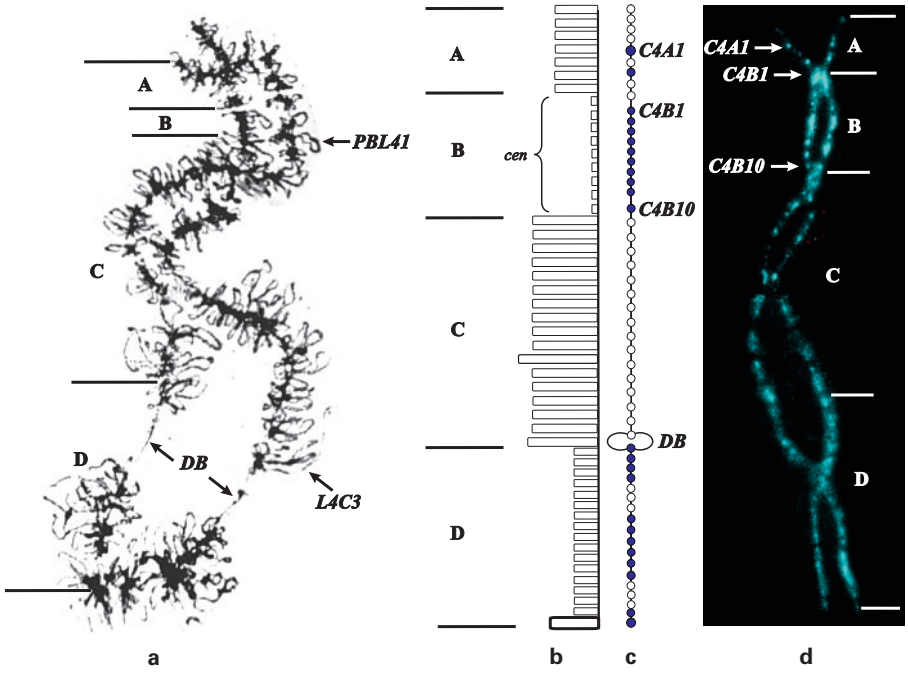


Fig. 16. Chicken lampbrush chromosome 4. All indications as for Fig. 13. DB, double bridge.

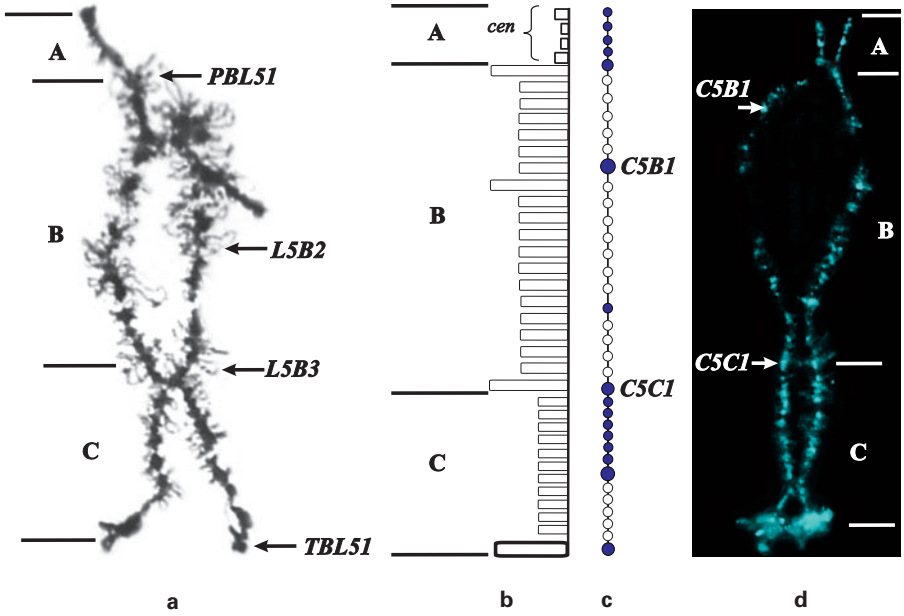


Fig. 17. Chicken lampbrush chromosome 5. All indications as for Fig. 13.

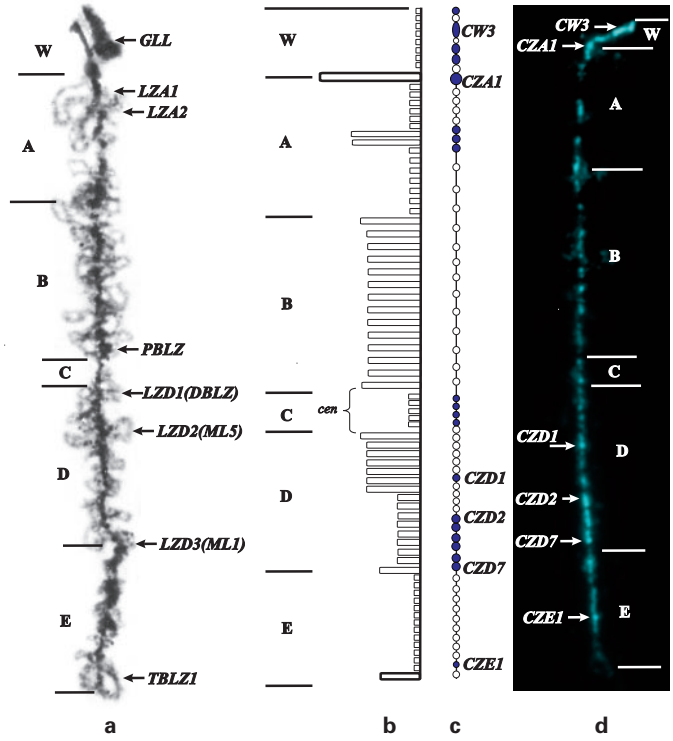


Fig. 18. Chicken lampbrush ZW-bivalent. All indications as for Fig. 13.

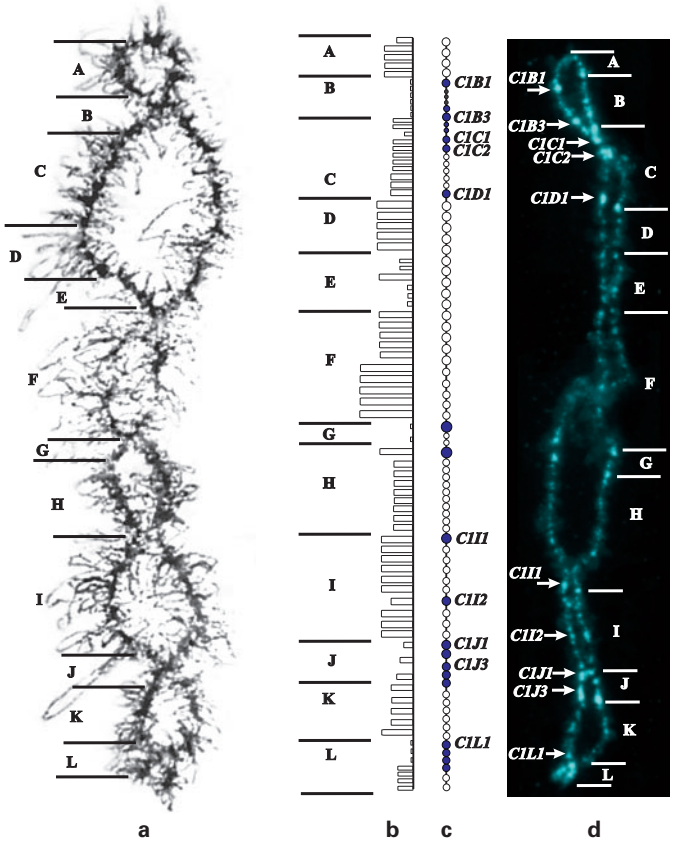


Fig. 19. Japanese quail lampbrush chromosome 1. All indications as for Fig. 13.

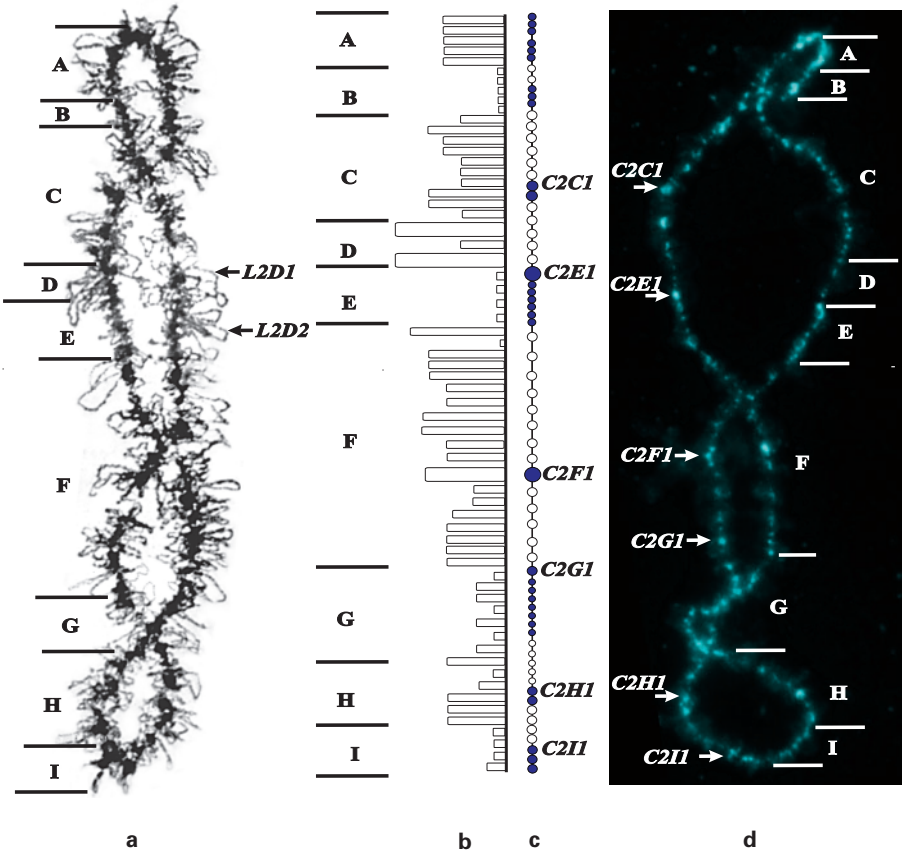


Fig. 20. Japanese quail lampbrush chromosome 2. All indications as for Fig. 13.

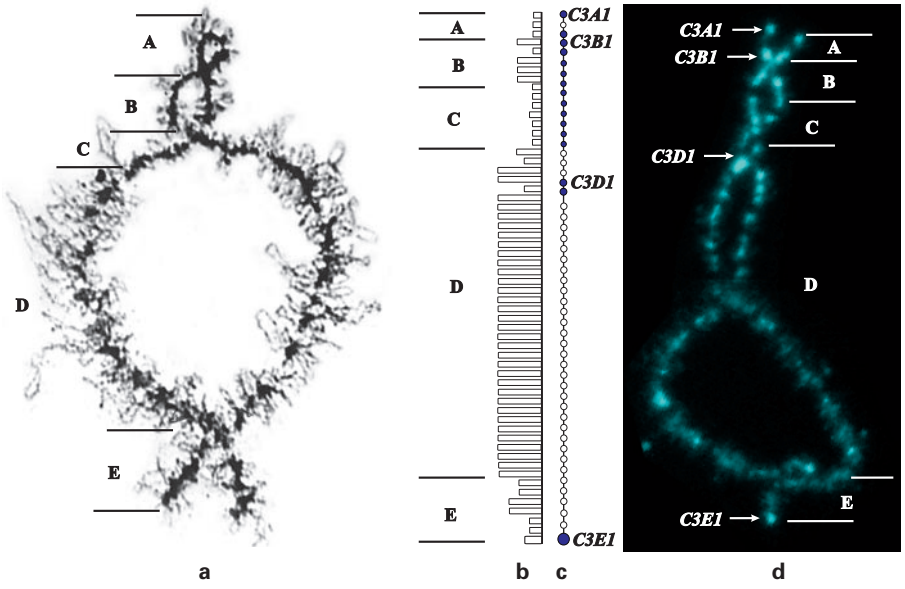


Fig. 21. Japanese quail lampbrush chromosome 3. All indications as for Fig. 13.

The staining of the chicken LBCs with the dA-dT-specific dye DAPI and the dG-dC-specific dye chromomycin A_3 (CMA_3) shows a distinction between two types of chromomeres. In contrast to amphibian LBCs (see Sims et al., 1984), the axis of bird macro-LBCs consists of single chromomeres arranged in linear arrays, and the clusters of chromomeres stuck together. Some of them fluoresce very strongly with DAPI, other ones fluoresce dull after DAPI-staining but they are bright after CMA-staining (Fig. 24). Each macro-LBC is composed of a chromosome-specific number of chromomeres (Table 6).

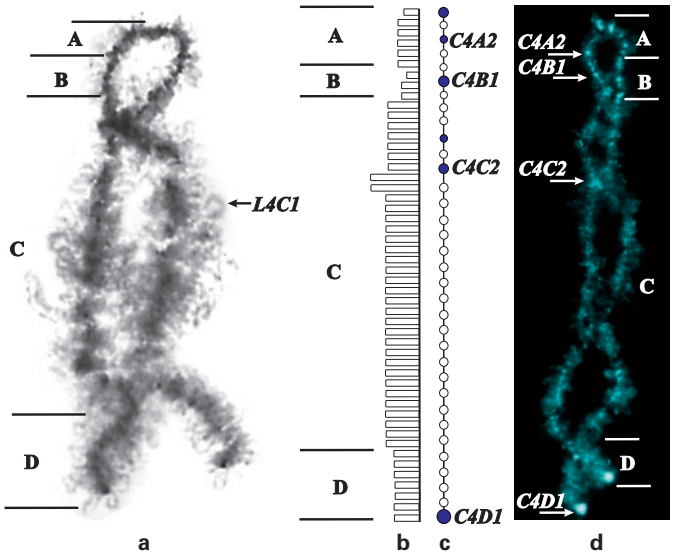


Fig. 22. Japanese quail lampbrush chromosome 4. All indications as for Fig. 13.

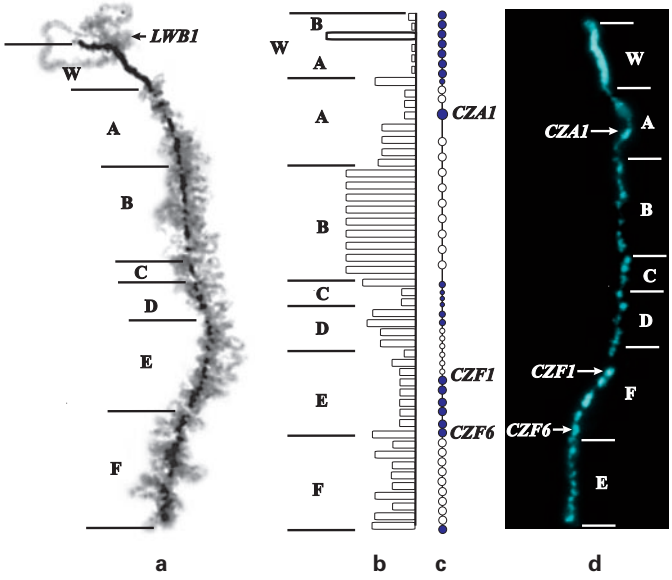


Fig. 23. Japanese quail lampbrush ZW-bivalent. All indications as for Fig. 13.

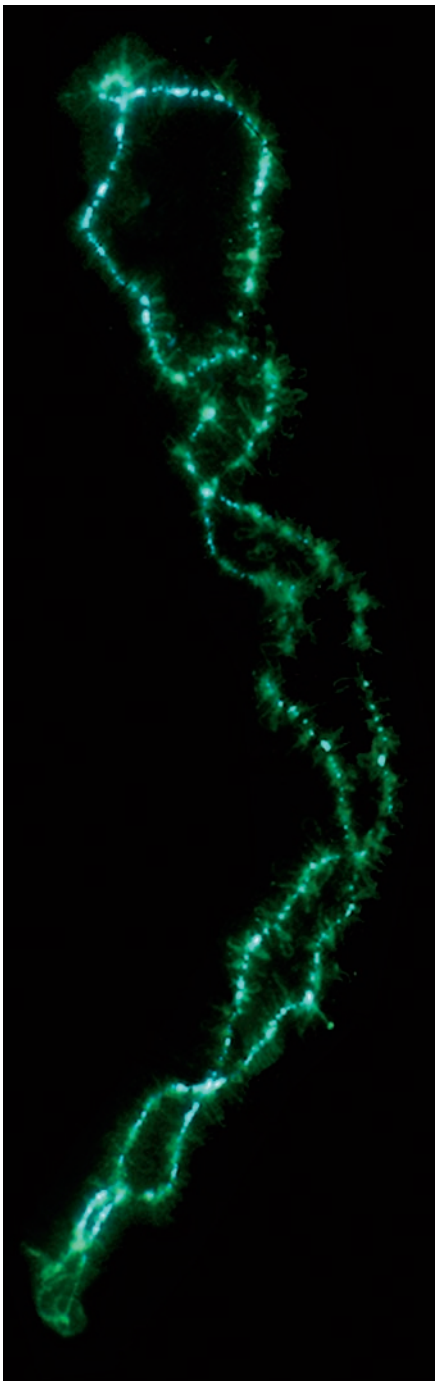


Fig. 24. CMA_3 (green fluorescence) and DAPI (blue fluorescence) positive chromomeres on chicken lampbrush chromosome 1.

LBCs of the chicken and Japanese quail may be identified by their length and by morphological characteristics such as marker loops and DAPI-positive chromomeres. Table 6 gives the mean value of the chicken LBC lengths. Different types of marker loops used for chromosome identification in the chicken and Japanese quail are presented in Figs. 13–23. In the chicken, there are the specific telomeric bow-like loops (TBLs) and interstitial lumpy loops (LLs). The ZW-bivalent carries characteristic giant lumpy loops (GLLs) (Chelysheva et al., 1990). A nucleolus-like protein structure, the spaghetti-marker (SM), is located in the subtelomeric region of the 2p-arm of the chicken (Solovei et al., 1992). In spite of the varying size of the lateral loops during the different phases of oocyte development, the marker loops can usually be identified in each chromosome set.

Table 6. Average chromomere number and DNA content in chicken macrochromosomes 1–5, Z and W

LBC	LBC length (μm)	DNA content per chromosome[a] (Mb)	Chromomere number per LBC[b]	DNA content per chromomere (Mb)	G- and R-bands number[c]	Chromomere number per band
1	185.2 ± 6.3	250	115 ± 6	2.1 ± 0.11	34	3.5
2	150.6 ± 1.3	181	103 ± 4	1.8 ± 0.07	28	3.6
3	128.0 ± 3.1	138	83 ± 6	1.7 ± 0.13	24	3.3
4	107.2 ± 3.4	109	50 ± 1	2.3 ± 0.05	20	2.4
5	71.3 ± 3.2	64	28 ± 1	2.3 ± 0.08	12	2.3
Z	62.3 ± 1.1	101	53 ± 3	1.9 ± 0.11	18	2.9
W	5.0 ± 0.2	34	7[d]	4.8	8	0.9

[a] Smith and Burt, 1998.
[b] Our data.
[c] Ladjali-Mohammedi et al., 1999.
[d] Solovei et al., 1993.

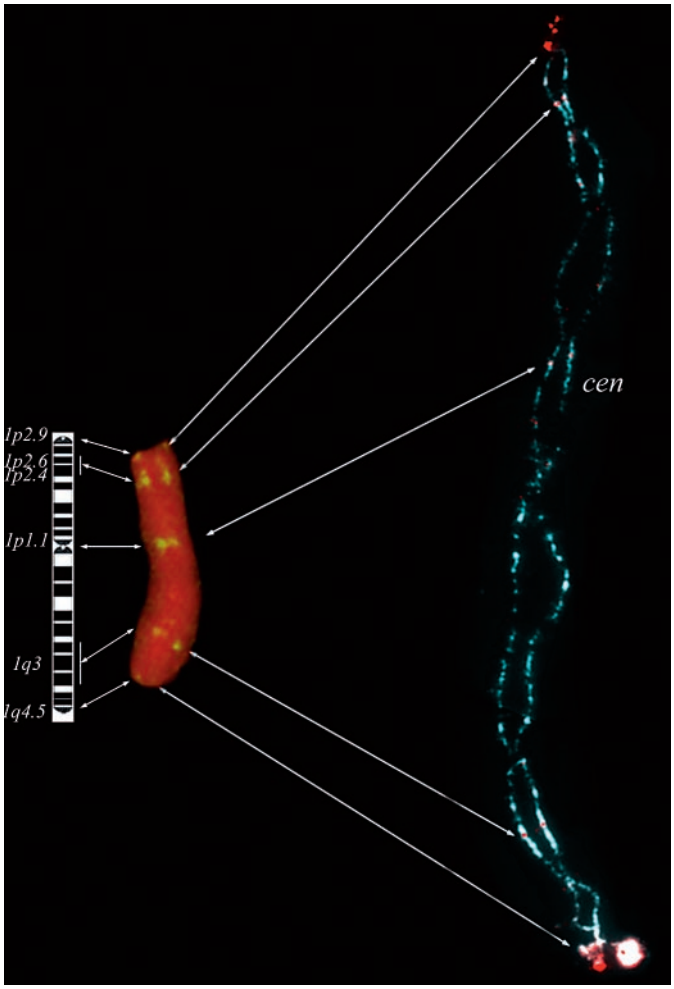


Fig. 25. The position of TTAGGG-positive sites on the chicken mitotic and lampbrush chromosome 1.

Working maps exhibiting the positions of landmark loops and other features have been published earlier by Chelysheva et al. (1990) and Rodionov and Chechik (2002). Figures 13–23 present a new version of these maps that are adapted to FISH. The chromosome axes are represented as a line corresponding to the mean length of the bivalent. Blue axial dots symbolize DAPI-positive chromomeres. On the map, the letters L and C designate marker loops and chromomeres, respectively. The next character of the marker symbols shows the chromosome number, then a letter referring to the chromosome region name, and then a number of subregions. A final character in each LBC marker symbol is an individual number of either the loop or chromomere within the chromosome subregion. For example, L1K21 is the first loop of subregion 2 of region K of the chicken LBC1. For a few marker loops that were identified and designated earlier (Chelysheva et al., 1990) we used traditional names: e.g. PBLs and DBLs for the proximal and distal border loops of the loop-less regions, respectively. DB (double bridge) marks the positions of weak sites of the chromosome axis that look usually as a pair of long interchromomere fibrils, the so-called double-loop bridges (Callan, 1986). Lateral loops are drawn on one side of the axis only according to their respective mean length. Note that the relative modal length of lateral loops is a characteristic feature of individual chromosome regions.

In the chicken we established a correlation between LBCs and mitotic chromosomes by comparative mapping of TTAGGG repeats. An alignment of the mitotic and LBC 1 of the chicken are presented in Fig. 25.

The bird LBCs are diplotene bivalents with easily identifiable chiasmata (Rodionov et al., 1992, 2002). Since chiasmata in diplotene chromosomes correspond in position to the sites of crossovers (Tease and Jones, 1995), estimation of the crossover-determined distances between chromosome markers and construction of a chiasma-based map of chromosome marker linkages is possible (Fig. 26, Table 7). Note that the lengths of chiasma-based linkages in all cases are lower than the distances estimated in molecular genetic experiments (Rodionov et al., 2002).

As the chicken and Japanese quail are useful genetic and laboratory animals, an investigation of theirs LBCs is a worthwhile approach for poultry cytogenetics and genomics.

Table 7. Chiasma frequency and linkage length in chicken and quail female

Chromosome of the standard galliform karyotype[a]	Chiasma frequency $\bar{x} \pm SE$		Chiasma-based genetic length (cM), $\bar{x} \pm SE$	
	Japanese quail	Chicken	Japanese quail	Chicken
Chromosome 1	6.3 ± 0.94	7.7 ± 0.11	313 ± 47.2	386 ± 5.3
Chromosome 2	4.8 ± 0.7	6.1 ± 0.09	238 ± 34.9	304 ± 4.3
Chromosome 3	3.1 ± 0.67	4.5 ± 0.09	154 ± 33.4	227 ± 4.7
Chromosome 4	2.8 ± 0.38	3.9 ± 0.13	143 ± 18.9	195 ± 6.5
Chromosome 5	about 2	2.9 ± 0.11	about 100	144 ± 5.5
Bivalent ZW	1.0 ± 0.0	1.0 ± 0.0	50	50
Macro 1–5	19	25.1	948	1256
Micro 6–10	about 10	8.5	about 500	625
Total per oocyte genome	55.34 ± 2.69	61.6	2650–2900	2950–3200

[a] Ladjali-Mohammedi et al., 1999; Schmid et al., 2000.

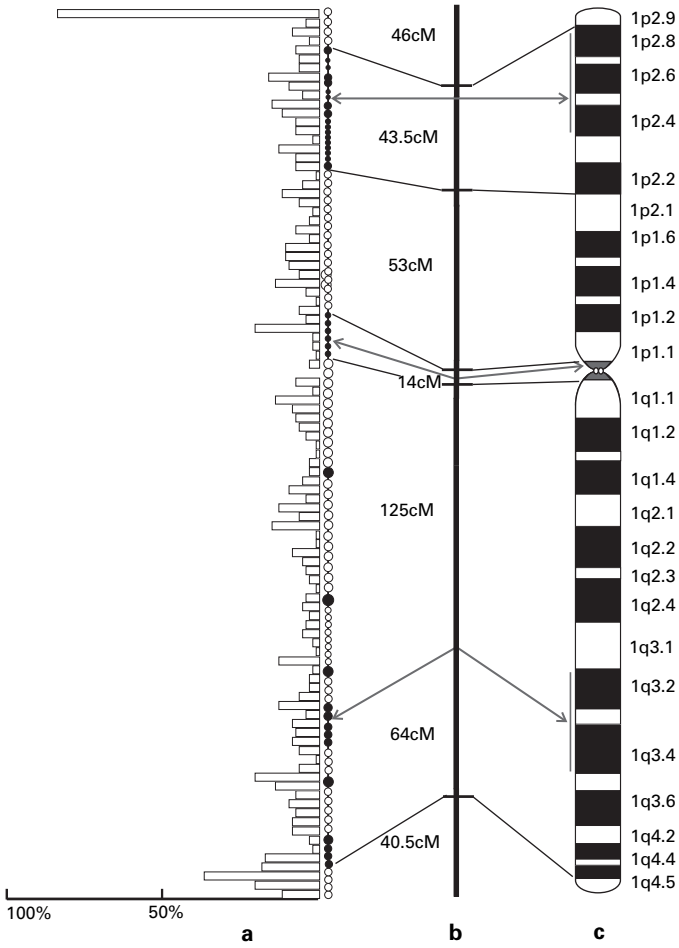


Fig. 26. Integration of the physical maps of the chicken lampbrush and mitotic chromosome 1 and chiasma-based genetic distances between chromosomes markers. (**a**) Chiasma distribution on the chicken lampbrush chromosome 1. Percent bivalents with chiasmata. (**b**) Chiasma-based genetic distance. (**c**) Ideogram of the banded mitotic chromosome 1 (Ladjali-Mohammedi et al., 1999).

Sex chromosomes and sex determination in birds

(Prepared by I. Nanda, T. Haaf, M. Schartl, H. Hoehn and M. Schmid)

Although in both birds and mammals sex is determined during fertilization by the inheritance of specific sex chromosomes, there are striking differences between the avian ZZ/ZW and the mammalian XX/XY sex chromosome systems. Unlike mammals, male birds are the homogametic (ZZ) and females the heterogametic (ZW) sex. The function(s) of avian sex chromosomes during sex determination and sex differentiation is likely to be different from that of the mammalian X and Y chromosomes. In addition, the Z and W chromosomes harbor different sex-determining genes than the X and Y.

In mammals, heterogametic (XY) individuals develop into males because they express the *SRY* gene from the Y chromosome (Koopman et al., 1991). Since no avian *SRY* homolog was found, it has been speculated that a W-linked gene functions as a dominant ovary-determining factor in female birds. However, none of the few known W-linked genes seems to perform a genuine female-specific role. Alternatively, it is possible that the two copies of the Z chromosome in male birds, compared with only one copy in females determine sex by a dosage-dependent mechanism. A somewhat contradictory report by Arit et al. (2004) calls the role of Z chromosome dosage in sex determination in question. Genes on the Z chromosome may have a selective advantage because of extreme forms of sexual selection in many birds. In contrast to the situation in mammals in which males do not receive a paternal X chromosome, in birds Z-linked loci involved in sexual selection could be beneficial to homogametic ZZ males (antagonistic alleles).

In the light of excellent and comprehensive reviews on avian sex chromosomes and sex determination (e.g., Graves, 2003; Handley et al., 2004; Schartl, 2004; Smith and Sinclair, 2004), here we will provide only a brief overview over some recent research advances.

Structural organization and evolution of avian Z and W sex chromosomes from an ancestral chromosome pair

In modern birds (Neognathae) both Z and W sex chromosomes are easily recognized by conventional cytogenetic methods. The Z is usually a medium-sized macrochromosome, representing 7–10% of the entire genome. The highly variable morphology of the Z chromosome in different bird karyotypes suggests that the Z has undergone extensive structural changes during avian evolution. Therefore, the gene order can be expected to differ significantly between the Z chromosomes of different bird species. The average W chromosome is much smaller than the Z and sometimes only slightly larger than the microchromosomes. It is gene-poor and largely heterochromatic. However, because of the accumulation of constitutive heterochromatin in some species the W chromosome became even larger than most macrochromosomes (Fig. 27). In extant paleognathous birds (Ratitae) the W chromosome resembles the Z in size and morphology and most likely also in gene content

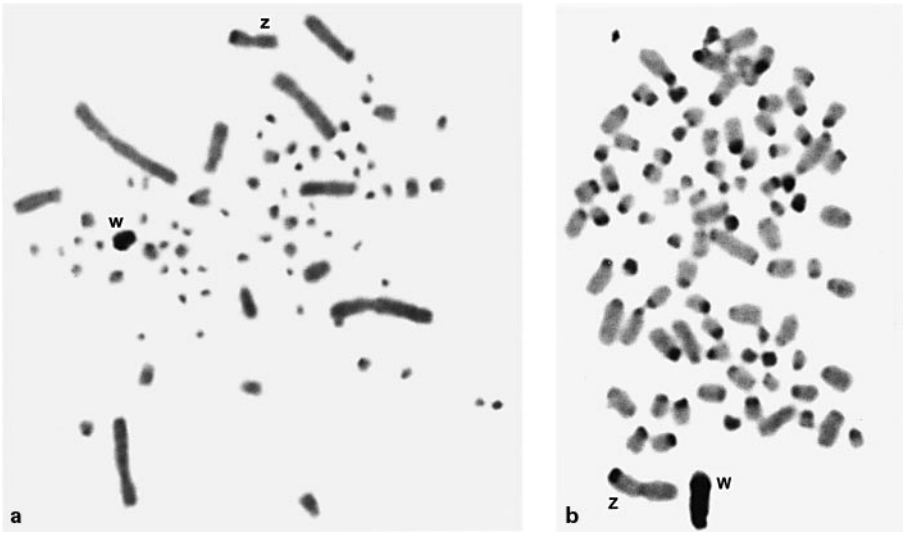


Fig. 27. Female metaphases of chicken (**a**) and barn owl (**b**), displaying C-band positive heterochromatin in the sex chromosomes. Note the large amounts of constitutive heterochromatin in both small (**a**) and extremely large W chromosomes (**b**).

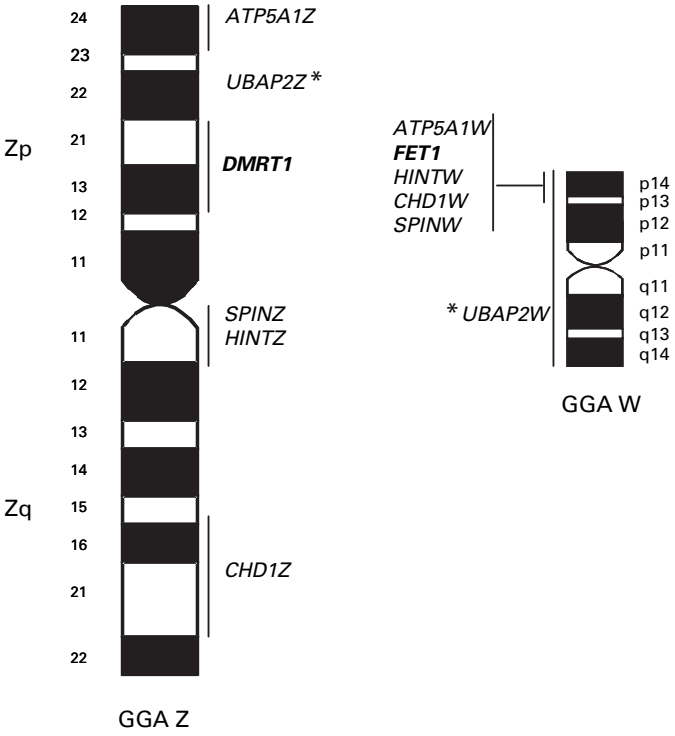


Fig. 28. G-banded ideogram of chicken sex chromosomes showing cytological location of sex determining candidate genes (bold) as well as the genes shared between Z and W. (* Unpublished observation of Handley et al., 2004).

(Fridolfsson et al., 1998; Ogawa et al., 1998). During meiosis in most birds the Z and W are paired to some extent and at least one recombination nodule is formed in the synapsed region(s). Meiotic and chromosome painting studies suggest that the Z and W sex chromosomes have a common ancestry. Chicken Z painting probes labeled both the Z and W chromosomes of representative carinate and ratite birds (Shetty et al., 1999; Raudsepp et al., 2002). Differentiation of the Z and W chromosomes in carinates and other modern birds is thought to result from cessation of meiotic recombination.

Data published by the research groups of S. Mizuno in Japan and H. Ellegren in Sweden have provided new insights into the gene content and evolution of the W chromosome. Five of the six known W-linked genes have homologs on the Z (Fig. 28; Hori et al., 2000; Itoh et al., 2001; Handley et al., 2004), reflecting their common origin from an ancestral homologous chromosome pair. Phylogenetic analysis of intron sequences from five gametologous genes (*CHD1*, *HINT*, *SPIN*, *UBAP2*, *ATP5A1*) in different avian lineages suggests that avian sex chromosomes evolved in two steps. The initial step probably involved suppression of Z-W recombination around 102–170 million years ago (Mya) through at least one mutational event, i.e. an inversion on the W (Handley et al., 2004). Molecular analysis of four gametologous gene loci in a paleognathous bird, *Tinamous*, revealed only a single gene, *SPIN*, that had differentiated into Z and W forms. This is consistent with the view that suppression of recombination did not occur simultaneously for all gametologous loci but in different steps, maybe involving different genes in different species (de Kloet and de Kloet, 2003). Female ostriches possess a single copy of the Z-linked marker *IREBP*, whereas in emus *IREBP* is present on both the Z and W chromosomes. This promotes the idea that a small deletion involving the *IREBP* locus has generated an active Z copy and a degenerated W copy in primitive sex chromosomes (Ogawa et al., 1998). Following the initial differentiation event(s), which may be "frozen" in the primitive ratite sex chromosomes, subsequent rounds of heterochromatinization and deletions of genetically inert material on the W, as well as extensive intrachromosomal rearrangements of the Z, may have formed the highly differentiated sex chromosomes in extant birds.

In many respects the avian ZW sex chromosomes exhibit remarkable parallels to the mammalian XY pair. Therefore it has been assumed for a long time that both types of sex chromosome pairs originated from a common ancestral chromosome pair (Ohno, 1967). However, our previous comparative mapping data of Z-linked genes in the human genome (Nanda et al., 2000, 2002a) as well as the current assembly of the chicken genome draft sequence failed to detect orthologous loci on the avian Z and mammalian X chromosome. On the contrary, most Z-linked genes have signatures on human chromosome 9 and chromosome 5, implying that the Z chromosome is largely homologous to HSA9 and HSA5. Three additional human chromosomes, HSA2, HSA4, and HSA8 share smaller segments of conserved synteny with the chicken Z. These segments were most likely inserted into a Z chromosome ancestor after the split of the avian and mammalian lineages. Evidently, most of the avian Z chromosome and human chromosomes 9 and 5 evolved from the same ancestral chromosome. This implies that avian and mammalian sex chromosome systems evolved independently from each other.

Candidate sex-determining genes on the Z and W chromosomes

Since avian sex is thought to be controlled by either Z chromosome dosage or by a W-linked dominant gene, several research groups have searched for candidate sex-determining genes on both Z and W chromosomes. Five of the six known W-linked genes have Z chromosome homologs and lack a female sex-specific expression profile consistent with an ovary-determining function. The most interesting candidate for an ovary-determining gene is *FET1* (female expressed transcript 1), which was isolated through differential screening of genes that are expressed in chicken gonads. It lies in the euchromatic region of the W short arm and lacks a Z homolog (Fig. 28). Its expression is restricted to females and up-regulated in the cortex of the left gonad during the critical sex-determining period (Reed and Sinclair, 2002). The encoded protein, which contains a signal peptide and a transmembrane domain, has no orthologs in mammals. Although *FET1* displays some features of a dominant sex-determining gene in birds, its female sex-specific expression and localization on the W but not the Z needs to be confirmed in paleognathous birds. In addition, the precise role of *FET1* and the characterization of its downstream target genes remain to be elucidated.

The homology between the chicken Z chromosome and HSA9 is of particular interest, because deletion of a critical region on the short arm of HSA9 leads to gonadal dysgenesis and XY sex reversal in humans. Several *DMRT* (Doublesex and Mab3-related transcription factor) genes, which share significant structural homology with male sex-regulatory genes of nematode and fly, are localized in the critical region HSA9p24 and expressed in adult testis (Raymond et al., 1998; Ottolenghi et al., 2002). Based on this information, an ortholog of mammalian *DMRT1* was isolated from the short arm of the chicken Z (Nanda et al., 1999). During early embryonic development, chicken *DMRT1* is expressed in the genital ridge of both sexes with a higher expression in male chicken embryos; however, after the onset of sexual differentiation *DMRT1* expression becomes confined to the testes (Raymond et al., 1999; Shan et al., 2000). As expected from a dosage-sensitive sex-determining gene, *DMRT1* has no homolog on the W and is conserved on the Z chromosomes of both neognathous and paleognathous birds (Nanda et al., 2000; Shetty et al., 2002). Its exclusive Z-chromosomal location, its expression during gonadal development, and its conserved testis-specific expression pattern in different vertebrate orders all support a role for *DMRT1* in avian sex determination. In this model, avian sex is determined by a gene dosage effect: two *DMRT1* dosages are required for testis formation in ZZ males, whereas a single dosage in ZW females leads to differentiation of an ovary.

Both experimental sex reversal and natural sex chromosome aneuploidy can provide important clues as to the importance of Z chromosome dosage in avian sex determination. Induction of female-to-male sex reversal in chicken embryos treated with an aromatase inhibitor leads to elevated *DMRT1* expression levels in the sex-reversed male embryos (Smith et al., 2003). This argues in favor of the notion that up-regulation of *DMRT1* rather than Z chromosome dosage is the key event in male sex determination. Nevertheless, in the normal situation an increased expression of *DMRT1* may require the presence of two Z chromosomes, which then lead to male sexual differentiation. Very recently, an unusual case of female 2A:ZZW aneuploidy was reported in a natural population of great reed warblers (Arit et al., 2004). This aneuploid female bird reproduced regularly, although it had two Z chromosomes, as revealed by heterozygosity for two Z-linked microsatellite loci. The female phenotype of this ZZW bird is not consistent with the view that two Z chromosome dosages trigger male sexual differentiation. Surprisingly, all male offspring from this ZZW female inherited only the alleles from one particular Z chromosome. This unusual inheritance could be explained by the fact that the other Z was truncated. In this situation it might be implied that the truncated Z did not possess a functional *DMRT1* copy. Unfortunately, the presence of two Z chromosomes in the aneuploid ZZW female was not confirmed by cytogenetic analysis.

The mechanism by which *DMRT1* is down-regulated on the single Z chromosome of female embryos remains to be elucidated. There seems to be a complete cessation of *DMRT1* expression in the developing ovaries. Teranishi et al. (2001) hypothesized that this downregulation of *DMRT1* in the female gonad depends on an interaction between Z and W chromosomes. In females a non-coding RNA is expressed from a region on the single Z in close proximity to *DMRT1*. This locus (MHM) remains perpetually hypermethylated in males and can only be transcribed in the presence of a W chromosome. Interestingly, the MHM region is transcribed in ZZW triploid birds, but it remains inactive in ZZZ males (Teranishi et al., 2001). A similar phenomenon can also explain the female phenotype of the reported ZZW aneuploid great reed warbler. Thus, the W chromosome may exert an effect on a Z-linked region that is directly or indirectly involved in the regulation of the putative sex-determining gene, *DMRT1*. Graves (2003) proposed the existence of an as yet unknown locus *(ZUF)* on the W chromosome that activates the MHM region on the Z chromosome(s) in females.

Table 8. Putative germ cells associated functions of Z linked orthologs in mammals. Orthologs with ubiquitous expression are not included. Many of the cited orthologs may have somatic functions.

Genes	Chicken[a]	Mammals (mouse, human)
DMRT1 (HSA9)	Testis	Testis
VASA (HSA5)	Testis, Ovary	Testis, ovary
FGF10 (HSA5)	Limb development	Accessory sex organs/prostrate
SCOT (HSA5)	n.s.	Sperm, Leydig & Sertoli cells
CDK7 (HSA5)	n.s.	Testis (meiotic function)
MADH2 (HSA5)	Ovarian granulosa	Meiotic germ cells
PDE4D (HSA5)	n..s	Spermatid
HEXB (HSA5)	n.s.	Testis (epididymis)
HMGCR (HSA5)	n.s.	Gonadal function (via cholesterol)
POLK (HSA5)	n.s.	Testis (spermatogenesis)
ALDH1 (HSA9)	n.s.	Testis (Leydig cells)
ALDH5 (HSA9)	n.s.	Testis (cytosolic)
TRKB (HSA9)	Ovarian follicle	Germ cells and peritubular cells
DAZAP1 (HSA19)	n.s.	Spermatogenesis
PCSK4 (HSA19)	n.s.	Spermatocytes, round spermatids
GAK (HSA4)	n.s.	Ubiquitous (testis high)
PRLR (HSA5)	Testis	Testis (spermatogenesis)
SPTRX3 (HSA9)	n.s.	Testis (spermatocytes, acrosome)
CAMK4 (HSA5)	n.s.	Chromatin remodeling during nuclear condensation of spermatids
FST (HSA5)	Ovary	Germ cell survival in ovary
GP130 (HSA5)	n.s.	Ovary
RAD17 (HSA5)	DNA damage	Testis (high expression)
MSH3 (HSA5)	n.s.	Testis (pachytene spermatocytes)
VLDLR (HSA9)	Ovary	Ovary, testis
RAD23B (HSA9)	n.s.	Testis
PAM (HSA5)	n.s.	Testis
SPIN (HSA9)	Ovary (high expression)	Gametogenesis
PCSK5 (HSA9)	n.s.	Testis (activates MIS)

[a] n.s.: not studied.

The Z chromosome was shaped to execute male specific functions

Secondary sexual characters are very conspicuous in birds. Considering the degenerate nature of the W chromosome in modern birds, it is plausible that sex-specific traits involved in the mating system are encoded on the Z chromosome. For example, Z-linked genes seem to control male plumage characters (Saetre et al., 2003). Similarly, zebrafinch males, but not females, sing a courtship song. Recent studies revealed that sex chromosomal genes are expressed differently in brain cells of males versus females. These genes act in a cell-autonomous fashion and are responsible for sex-specific differences in the development of the song system (Agate et al., 2003). The Z chromosome may not only be important for sex determination but also plays a role in several other aspects of reproduction. In this context, it is interesting to note that the mammalian X chromosome is enriched with genes related to sexuality and reproduction (Saifi and Chandra, 1999; Zechner et al., 2001). A systematic study of the functions of Z-linked genes which are available in the public database (http://www.ensembl.org/Gallus_gallus) may reveal a similar enrichment of reproductive genes on the Z. Indeed we already identified a number of Z-linked genes with mammalian orthologs that are expressed in testis and/or exert a specific function(s) during spermatogenesis. Although it is not known whether these genes are also expressed in the avian testis or ovary, their evolutionary conservation may be considered a good indicator for comparable functions in mammals and birds. One important example is the location of *VASA* on the Z chromosome. This gene is required for both germ line and oocyte differentiation in various animal groups including mammals (Noce et al., 2001). However, a knock-out study in mice suggests that *Vasa* is required for spermatogenesis and may be dispensable in germ line formation (Tanaka et al., 2000). Another interesting Z-linked gene is *PCSK5* (proprotein convertase), which is required for bioactivation of Mullerian inhibiting substance (MIS). On the other hand, there are a number of Z-linked genes *(VLDLR*, *AVIDIN*, *FST* and *ZOV3)* that are important for ovarian functions and, therefore, may be down-regulated in ZZ males. It is possible that the very early expression of *DMRT1* and other male-specific genes (e.g. *MIS*) interferes with the expression of female-specific Z-linked genes in males. As seen in Table 8, there are many HSA5 and HSA9 orthologs with testis-related functions on the chicken Z chromosome. This supports the simplistic view that the Z chromosome was shaped during evolution to carry out male-specific functions in birds. However, a more comprehensive analysis of the gene content of the avian Z and W chromosomes is necessary to prove this hypothesis.

Conclusions

Sex chromosomes and sex determination will undoubtedly continue to excite biologists. Recently, it was reported that the sex chromosomes of the platypus form multivalent meiotic chains which share homology with the human X and bird Z chromosomes. Because monotremes represent the earliest mammalian lineage, an evolutionary link between mammalian and avian sex chromosomes has been suggested (Grützner et al., 2004). However our growing information on the gene content of the chicken Z chromosome is not consistent with this view and strongly supports an independent evolution of the avian and mammalian sex determination systems. We may be able to learn more about the specific functions of candidate genes for avian sex determination such as *FET1* and *DMRT1* by over-expression and knock-down experiments. Although the differentiation of the heteromorphic avian Z and W sex chromosomes from an ancestral autosome pair resembles the evolution of the mammalian X and Y, the master control genes in avian and mammalian sex determination are clearly different (i.e., *DMRT1* vs. *SRY*). If a control gene confers sexual differentiation as a dominant factor it may evolve as one allele of a gene on opposite sex chromosomes as illustrated by the Y-borne *SRY* and X-linked *SOX3* in mammals. However, in this respect avian sex determination may differ from the mammalian situation.

Sex chromosome-linked genes and DNA sequences of chickens and their relevance to the process of sex determination or gonadal sex differentiation

(Prepared by S. Mizuno)

The aim of this article is to review briefly newly described genes and DNA sequences and to further characterize previously identified genes on the chicken sex chromosomes, which may

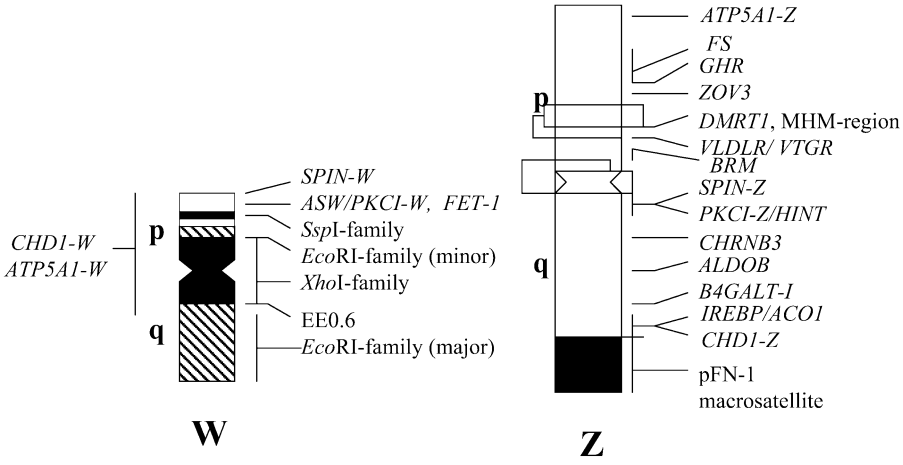


Fig. 29. Approximate positions of genes and DNA sequences pertinent to this article on the W and Z chromosomes of chicken.

be relevant to the process of sex determination or gonadal sex differentiation, since the publication of the First Report in 2000 (Schmid et al., 2000). Approximate map positions of these genes and sequences are illustrated in Fig. 29.

Genes on the W and Z chromosomes

spindlin gene. The experimental approach which led to the identification of *spindlin* genes on the W chromosome *(SPIN-W)* and the Z chromosome *(SPIN-Z)* was described in the First Report in 2000. It is unlikely that these genes are directly involved in sex determination because their cDNA sequences are nearly identical except for slight differences in their sequences of 3′-untranslated regions (UTRs). One region in the 3′ UTR showing sequence difference of about 20% was utilized as a probe to distinguish mRNA molecules transcribed from the W- or Z-linked gene under stringent hybridization conditions. The results suggest that *SPIN-W* is expressed in embryonic gonads and in the somatic tissues of the ovary of adult chickens, whereas *SPIN-Z* seems to be expressed ubiquitously. Thus, the W- and Z-linked *spindlin* genes seem to be controlled under different transcriptional regulatory mechanisms. When FLAG-tagged *SPIN-W* was expressed in chicken embryonic fibroblasts, it was largely accumulated in ND10 (nuclear dots) with SUMO-1 during interphase, while it became associated with entire chromosomes during mitosis. It is notable that the potential sites of PKC phosphorylation and tyrosine phosphorylation and the RNA-binding motif, RNP-1, are well conserved among spindlins of chicken, human and mouse, human DXF34 and mouse Ssty (Itoh et al., 2001). The active *SPIN-W* on the W chromosome may have a role in ovarian differentiation in females.

MHM region and DMRT1 gene. The presence of MHM (male hypermethylated) region adjacent to the *DMRT1* gene on the short arm of the chicken Z chromosome was mentioned in the First Report 2000. The detailed description of this interesting region was published subsequently (Teranishi et al., 2001). The MHM region consists of about 210 tandem repeats of a *Bam*HI 2.2-kb sequence (the genomic and cDNA sequences are deposited with DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers AB046698 and AB046699). This region was first noticed as a female-specific cDNA clone from the cDNA library of the lampbrush-stage chicken oocytes. Fluorescence in situ hybridization (FISH) to the lampbrush ZW bivalent revealed that RNA transcripts from the MHM region accumulated on a pair of loops and that the *DMRT1* gene was located at the base of each of these particular loops on the Z chromosome. The high level methylation of cytosines in CpG sequences of MHM regions on the two Z chromosomes in male cells is established after fertilization and before the one-day embryonic stage. Similarly, hypomethylation of the MHM region on a sperm-derived Z chromosome in female cells is established after fertilization and before the one-day embryonic stage. These situations are different from those of genomic imprinting in mammals, because the allele-specific differential CpG methylation patterns related to genomic imprinting are established during the later stage of gametogenesis (Constancia et al., 1998). In ZZW triploid intersex chickens, MHM regions are hypomethylated and transcribed on both Z chromosomes, whereas MHM regions are hypermethylated and not transcribed on the three Z chromosomes in ZZZ triploid males. It thus suggests that the state of methylation of the MHM region is affected by the presence or absence of the W chromosome.

In female cells, the MHM region is transcribed into non-coding RNA (MHM-RNA) molecules of approximately 10 kb but heterogeneous in size, which are accumulated in the nucleus at or in close vicinity of the site of transcription on the Z chromosome. When female chicken embryonic fibroblasts were subjected simultaneously to RNA-FISH, to MHM-RNA and to DNA-FISH to either MHM region or *DMRT1* gene, the accumulation of MHM-RNA was observed very closely to the MHM region and also to the *DMRT1* gene locus. These observations imply the possibility that the accumulation of MHM-RNA in the female cell nucleus may be a factor contributing to the inactivation of *DMRT1* gene expression. However, this notion has not been proved at a biochemical or a molecular level. Chromatin structures of the MHM region and the adjacent *DMRT1* locus in male and female chicken cells are now being investigated in our laboratory.

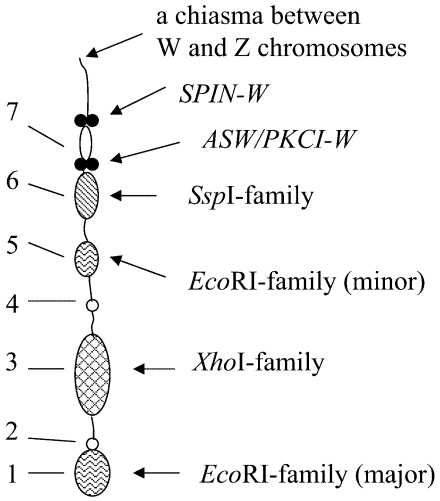


Fig. 30. Locations of three repetitive families and two genes on the W lampbrush chromosome of chicken. Results of high-resolution FISH mapping (Solovei et al., 1998; Itoh and Mizuno, 2002) are illustrated. Chromomeres are numbered (1 to 7) as indicated. DNA sequences constituting chromomeres 2, 4, and 7 are unknown.

FET1 gene. A new W-linked gene, *FET1* (female expressed transcript 1), was discovered recently by a differential expression screening of male and female chicken embryonic gonads during ~4.5–6.5 days of incubation (Reed and Sinclair, 2002). This gene was located by FISH in close vicinity of the *ASW/PKCI-W* gene locus in the terminal region of the W short arm. The nucleotide sequence of *FET1* cDNA (accession number AY113681 in DDBJ/EMBL/GenBank nucleotide sequence databases) revealed that it was a novel gene. The deduced sequence of FET1 contains a putative signal sequence and a trans-membrane domain. The mRNA-level expression of *FET1* was low but similar in the left and right embryonic gonads between day 3 (Hamburger-Hamilton stage 20) and day 4 (stage 24). The expression became conspicuous in the cortex of the left developing gonad at day 4.5 (stage 25) and continued through day 6 (stage 29) in female embryos but the expression declined sharply at day 6.5 (stage 30). The mRNA-level expression of *FET1* was also observed in the caudal portions of developing somites and the neural tube and in the waste collection ducts in early female embryos. These results suggest that *FET1* may play a role in the early process of the ovarian development.

Although the counter-part gene of *FET1* on the Z chromosome has not been identified, the four published W-linked genes, *CHD1-W*, *ATP5A1-W*, *ASW/PKCI-W* and *SPIN-W*, have their respective counterpart genes on the Z chromosome (Fig. 29). The latter facts are in accordance with the suggestion that the present day W and Z chromosomes of birds have evolved from a pair of homologous chromosomes that existed before the divergence of Carinatae and Ratitae birds (Ogawa et al., 1998; Nishida-Umehara et al., 1999).

SspI-family: an additional repetitive sequence family on the W chromosome

About 60 % of DNA of the chicken W chromosome consists of *Xho*I- and *Eco*RI-family repetitive sequences. The *Xho*I-family exists in the pericentric region and the major fraction of the *Eco*RI-family occupies a large area of the long arm and the minor fraction of the *Eco*RI-family is present at a middle region of the short arm (Fig. 29). Recently, *Ssp*I-family, in which a 0.5-kb *Ssp*I fragment is tandemly repeated about 11,300 times, was found on the chicken W chromosome and the family was located in the terminal region of the short arm of the mitotic W chromosome by FISH (Fig. 29) (Itoh and Mizuno, 2002). A unique feature of the *Ssp*I-family is that its 0.5-kb repeating unit contains a 120-bp stretch of polypurine/polypyrimidine sequence (GGAGA repeats) at its 3′ end region. The total amount of *Ssp*I-family DNA is about 6 Mb. Together with 11 Mb of *Eco*RI-family DNA, 21 Mb of *Xho*I-family DNA and 6 Mb of uncharacterized repetitive DNA, the sum of repetitive DNA in the W chromosome is about 44 Mb, leaving only about 10 Mb as non-repetitive DNA (Itoh and Mizuno, 2002). About 20 active genes are estimated to be present in the 10-Mb non-repetitive region (Mizuno et al., 2002).

The *Ssp*I-family itself does not seem to be directly related to the process of sex determination or sex differentiation. However, this family does have some relevance to the study and the state of active genes on the W chromosome in two different aspects. First, this family is a useful positional marker in FISH mapping of active genes on the W chromosome, because the *Ssp*I-family is located at the heterochromatin/euchromatin boundary in the terminal region of the short arm. High resolution FISH mapping on the lampbrush ZW bivalent prepared from a germinal vesicle of a diplotene-stage oocyte (Mizuno and Macgregor, 1998; Solovei et al., 1998) with the digoxigenin-labeled 0.5-kb *Ssp*I-family repeating unit and the biotinylated BAC clone containing a genomic sequence of *SPIN-W* or *PKCI-W* indicated a precise location of each gene relative to the position of the *Ssp*I-family as illustrated in Fig. 30. The second aspect may have some biological significance; that is, the *Ssp*I-family sequence forms a much more diffused chromatin structure in nuclei than *Xho*I- and *Eco*RI-family sequences (Itoh and Mizuno, 2002). This is probably caused by the presence of a polypurine/polypyrimidine stretch in each of the *Ssp*I-family repeating units. It has been shown by non-denaturing FISH to RNase-pretreated nuclei that polypurine/polypyrimidine sequences form triple-stranded DNA clusters in nuclei (Ohno et al., 2002), where nucleosome-based chromatin structure should be interrupted. The presence of *Ssp*I-family sequences at the heterochromatin/euchromatin boundary on the W short arm may be effective in preventing long-range spreading of heterochromatin into the terminal euchromatic region (Suka et al., 1993), where most W-linked active genes are expected to be located.

Dosage compensation of Z-linked genes may be achieved in the absence of Z chromosome inactivation in males

A randomly chosen X chromosome (Xi) in female cells of mammals forms facultative heterochromatin in which transcription of the majority of genes is virtually silenced with con-

comitant epigenetic modifications of its DNA and chromatin: high-level methylation of cytosines in CpG sequences, chromosome-wide spreading of Xist RNA, methylation of lysine 9 of histone H3, accumulation of macrohistone H2A1 and BRCA1, and Xi shows the typical late replication pattern (Heard et al., 2001; Ganesan et al., 2002). This mechanism, i.e. X inactivation, is the basis of dosage compensation of X-linked genes between male and female mammals.

It has been suggested that a Z inactivation mechanism is unlikely to operate in male birds with different experimental approaches. A late replicating Z chromosome was not observed in male cells of the chicken, the Japanese quail and the griffon vulture by the technique of DNA replication banding after successive incorporation of 5-bromo-deoxyuridine and deoxythymidine (Schmid et al., 1989). The enzymatic activity of the Z-linked cytoplasmic aconitase (ACO1 or iron-responsive element-binding protein, IREBP) in liver was 1.4 to 2.4 times higher in males than in females of the house sparrow, the chicken and the spotted turtle-dove (Baverstock et al., 1982). The mRNA-level expression of the Z-linked *DMRT1* gene in the genital ridge of chicken embryos at stages 25 to 31 detected by whole mount RNA-FISH was about twofold higher in males than in females (Raymond et al., 1999).

The above results are suggestive but not conclusive for the absence of Z inactivation at the level of transcription of alleles on the two Z chromosomes. More direct evidences were obtained recently with two different approaches. The first approach utilized RNA-FISH on nascent transcripts in a nucleus (Kuroda et al., 2001). Antisense riboprobes for an intron region of each of the five Z-linked chicken genes, growth hormone receptor *(GHR)*, nicotinic acetylcholine receptor β3 *(CHRNB3)*, aldolase B *(ALDOB)*, β1,4-galactosyltransferase 1 *(B4GALT-1)*, iron responsive element-binding protein *(IREBP* or *ACO1)*, all hybridized to two spots of nascent RNA in a nucleus of male cells but to a single spot in a nucleus of female cells. These cells were derived from a tissue in which the respective gene was expressed in both males and females. The two spots detected in the nucleus of male cells coincided with the corresponding gene loci which were detected by DNA-FISH. These results suggest strongly that both alleles of the five Z-linked genes are transcribed in male cells.

The other approach made use of a single nucleotide polymorphism (SNP) found in an exon region between alleles on the two Z chromosomes in male cells (Kuroiwa et al., 2002). Transcripts from each allele could be distinguished by the primer extension with a single radioactive NTP (for *CHD1-Z*) or by differential digestion with *Rsa*I (for *B4GALT-1*). It was demonstrated with this approach that the two Z-linked genes, *B4GALT-1* and *CHD1-Z*, were transcribed from both alleles in a single cell of the male chicken. These results show unequivocally that a Z inactivation mechanism does not operate on the two Z-linked genes examined. Considering the recent and previous results mentioned above, it is very likely that a Z inactivation mechanism which leads to a virtual shut-off of transcription of genes on one of the two Z chromosomes in males is absent in birds.

On the other hand, studies with the real-time quantitative PCR demonstrated that relative numbers of transcripts in male and female chicken embryos were near equivalent for six *(FS*, *VLDLR*, *BRM*, *CHRNB3*, *ALDOB, IREBP)* out of nine Z-linked genes examined (McQueen et al., 2001). The six genes included *CNRNB3*, *ALDOB* and *ACO1/IREBP* which were shown not to be subjected to Z inactivation as mentioned above. The same procedure applied to male and female chicken embryos also demonstrated that the dosage compensation was likely attained for transcripts of *CHD1-Z* but not for those of *B4GALT1* in spite of the biallelic expression of both genes (Kuroiwa et al., 2002). It is thus conceivable that a dosage compensation mechanism to yield near equivalent levels of steady-state mRNA concentrations between males and females may be present in chickens for certain Z-linked genes but in the absence of Z inactivation. If dosage compensation is attained by a single mechanism or by combination of events such as allelic difference in the efficiency of transcription, efficiency of pre-mRNA splicing and mRNA export to the cytoplasm, and stability of mRNA remains to be elucidated.

Perspectives

Sex-determining gene. The chicken *DMRT1* gene, a candidate for the male-determining gene on the Z chromosome, shows characteristic biphasic expression patterns: approximately male 2:female 1 mRNA levels in undifferentiated or developing gonads in embryos of stages 25 to 31 and testis-specific expression after hatching (Raymond et al., 1999; Shan et al., 2000). The twofold higher level of *DMRT1* mRNA in early male embryos has been implicated to cause the male determination in chickens (Raymond et al., 1999; Shan et al., 2000), because in humans, XY sex reversal takes place when the distal region containing *DMRT1* and *DMRT2* genes on the short arm of one of the chromosomes 9 is deleted (Guioli et al., 1998; Ottolenghi et al., 2000). On the other hand, the testis-specific expression of *DMRT1* in post-hatched male chickens implies its role in the differentiation or functions of the testis. If the half dosage of *DMRT1* mRNA in early embryos initiates the cascade of gene expression toward female development, then the key event in female development that is expression of the aromatase gene (*CYP19*) by the time of stage 30 to 31 (Mizuno et al., 2002) must be explained as its consequence. Clearly, we need to know the dosage-dependent protein function of DMRT1 and its molecular target.

Among the four W-linked genes listed above, *ASW/PKCI-W* is unique in that protein-coding sequences of W- and Z-linked genes are substantially different; i.e., overall identity of the deduced amino acid sequences is about 60% (Hori et al., 2000). Both *PKCI*-W and *PKCI-Z* are transcribed actively in female embryos of stages 20 to 29 in several tissues including the left and right genital ridges. The Z-linked *PKCI-Z* is the chicken ortholog of mammalian *PKCI/HINT*. HINT (histidine triad nucleotide-binding protein) forms a branch in the HIT (histidine triad) superfamily of nucleotide hydrolases and transferases and shows adenosine 5′-monophosphoramidase activity (Brenner, 2002). The deduced sequence of ASW/PKCI-W does not contain the HIT motif, that is essential for the enzymatic function of HINT, but it contains a unique Leu, Arg-rich region, implying that proteins produced from the W- and Z-linked genes exhibit different functions (Hori et al.,

2000). This is a situation resembling the functional differentiation of an original pair of homologous genes on the present-day X and Y chromosomes in mammals; i.e., *SOX3* on the X chromosome mainly functioning in the brain development and *SRY* on the Y chromosome functioning in triggering the testis differentiation (Collignon et al., 1996; Katoh and Miyata, 1999; Graves, 2002).

It has been shown that the mammalian PKCI/HINT exists as a homodimer and that the α-helix region and the C-terminal region encompassing the HIT domain are involved in dimerization (Lima et al., 1996; Brenner et al., 1997). As the latter two regions are well conserved between PKCI-W and PKCI-Z, it has been proposed that PKCI-W would form a heterodimer with PKCI-Z and thereby inhibits function(s) of PKCI/HINT in females, which may be a key step in the sex determination in chickens (Hori et al., 2000; O'Neill et al., 2000). Recently, we prepared fusion and tagged forms of PKCI-W and PKCI-Z and demonstrated that PKCI-W formed a heterodimer with PKCI-Z and inhibited the adenosine 5′-monophosphoramidase activity of PKCI-Z in a dominant-negative manner in vitro (Moriyama et al., in preparation).

The immediate question is how the adenosine 5′-monophosphoramidase activity of PKCI-Z and its inhibition by PKCI-W are related to their putative sex-determining functions. However, it has also been demonstrated that PKCI/HINT interacts with microphthalmia *(mi)* and inhibits its activity as a transcription factor (Razin et al., 1999) and that PKCI/HINT interacts with human Cdk7, a catalytic subunit of the Cdc2 activating kinase, or its yeast homologue Kin28 (Korsisaari and Mäkelä, 2000). The loss of HINT1 enzyme activity in yeast caused hypersensitivity to mutations in *Ccl1*, *Tfb3* and *Kin28*, whose products constitute the TFIIK kinase subcomplex in TFIIH (Bieganowski et al., 2002). These results may imply in vivo protein-protein interacting functions of PKCI/HINT. It has been speculated that PKCI/HINT may exhibit a protein adenylation activity under such circumstances (Brenner, 2002). It is essential to examine and demonstrate in vivo function and target of PKCI-Z and in vivo functional interaction of PKCI-W with PKCI-Z in early chicken embryos.

Search for more genes on the W chromosome. Based on the gene density around *SPIN-W* we estimated about 20 active genes on the chicken W chromosome (Mizuno et al., 2002). However, this number could be about 50 if the average gene density of the human Y chromosome, whose size is about the same as that of the chicken W chromosome, is applied (Graves, 2002). More accurate estimation will be obtained from the results of the chicken whole genome sequencing project. In any case, a substantial number of hitherto unknown genes are expected to be found on the W chromosome and we may find a new gene involved in the process of female sex determination among them. Recently, this subject has been approached by screening cDNA macroarrays in the author's laboratory. cDNA macroarrays carrying about 20,000 clones which were randomly picked up from female-minus-male subtraction cDNA libraries from 2- to 5-day chicken embryos were hybridized successively with male-minus-female cDNA, female-minus-male cDNA, total male cDNA, total female cDNA preparations from each developmental stage as probes. About 1,300 clones exhibiting female-specific or substantially female-dominant expression patterns were selected. Southern blot hybridization to female and male genomic DNAs digested with appropriate restriction enzymes with each of these clones as a probe selected a few candidates of clones derived from W-linked genes, interestingly mostly from the two-day (stages 12 to 13) embryos (Yamada et al., in preparation).

Molecular genetics of gonadal sex differentiation and analysis of candidate sex-determining genes

(Prepared by C. Smith, Q. Hudson and A. Sinclair)

As in other vertebrates, the gonads of birds develop from mesodermal tissue on the ventral surface of the mesonephric kidneys during embryogenesis. In the chicken embryo, the gonadal primordia become apparent between days 2 and 3 of incubation (Hamilton and Hamburger 16–18). At this stage, the gonads are morphologically identical in both sexes. Histological differentiation into testes or ovaries commences at day 6.5 (stage 29–30; Fig. 31), when Sertoli cells differentiate and begin organizing into seminiferous cords in males, while the outer cortical layer of the gonad begins to thicken in females (Smith and Sinclair, 2004). It has been reported that some sexual dimorphisms exist prior to gonadal sex differentiation. Germ cell numbers, for example, are apparently more numerous and asymmetrically distributed at an earlier stage in females (Zaccanti et al., 1990). In the chicken embryo, only the left gonad differentiates into a functional ovary, the right gonad regresses.

Genes thought to be involved in gonad formation and sexual differentiation in the chicken embryo are shown semi-schematically in Fig. 31. In mammals, some key genes have been identified that are required for the formation of the undifferentiated (or "bipotential") gonads. These genes are conserved in chicken and their embryonic expression profiles imply that they play the same role in birds. Genes encoding the transcription factors steroidogenic factor-1 *(SF1)* and Wilms tumor 1 *(WT1)*, for example, are both expressed in early forming gonads (Fig. 31). Meanwhile, the onset of testicular differentiation is characterized by the male-specific expression of the transcription factor *SOX9* (Kent et al., 1996) and Anti-Müllerian hormone *(AMH)*, as in mammals. Unlike mammals, ovarian differentiation is marked by the expression of rate-limiting enzymes required for estrogen synthesis, namely, aromatase and 17-β hydroxysteroid dehydrogenase (17β-HSD). Several lines of evidence indicate that estradiol is required for ovarian differentiation in birds (as in lower vertebrates but in contrast to eutherian mammals) (Smith and Sinclair, 2004). The winged helix transcription factor, *FOXL2*, has a female-specific expression pattern that is coincident with that of aromatase, suggesting potential interaction.

In birds, as in mammals, sexual differentiation of the gonads must be controlled by genes on the sex chromosomes. Chickens and other birds have a ZZ male/ZW female sex chromosome system, but the sex determining gene(s) remain unknown. Under the Z dosage hypothesis, males inherit two copies of a gene required for testis development, and females

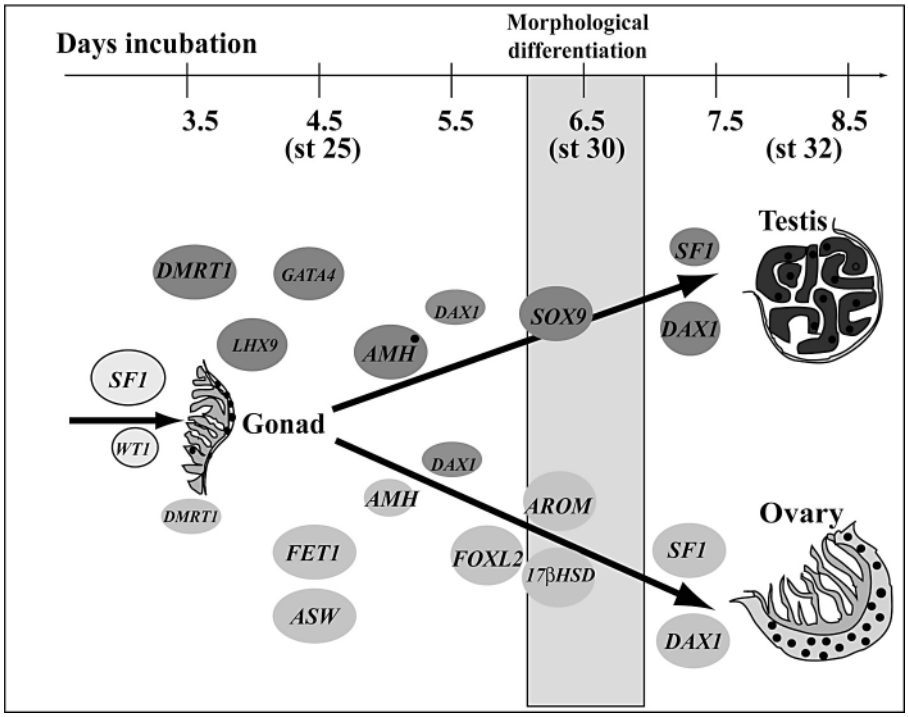


Fig. 31. Profile of genes expressed during gonadal development and sexual differentiation in the chicken embryo. Genes shown in open circles are expressed at similar levels in both sexes. Genes shown in dark gray are more highly expressed in males, genes shown in light gray are more highly expressed in females. The onset of morphological differentiation of the gonads is highlighted (day 6.5/stage 29–30). Small black circles represent germ cells. Modified from Smith and Sinclair (2004).

inherit one, resulting in ovary differentiation. The Z-linked *DMRT1* gene is conserved, and more highly expressed in male embryonic gonads prior to and during sexual differentiation (Smith et al., 1999). This gene is the strongest candidate sex determinant under the Z dosage hypothesis. The W chromosome is analogous to the mammalian Y because it is largely heterochromatic and carries few bona fide genes. Evidence from ZZW triploid chickens (Thorne and Sheldon, 1993) and from a female warbler with an apparent ZZW phenotype (Arit et al., 2004) support the view that the W chromosome carries an ovary-determining gene in birds. (The triploid chicken data, however, suggest that the effect of the W can be overridden by two Z chromosomes, as the birds are initially feminized but later become masculinized.) The W-linked gene, *ASW* (also called *WPCKI* or *HINTW*) is a strong candidate ovary determinant. It is present in multiple copies on the W, is widely expressed only in female embryos, and is conserved in non-ratite birds (Hori et al., 2000; O'Neill et al., 2000). It encodes a divergent form of the histidine triad nucleotide hydrolase enzyme, HINT, while the bona fide *HINT* gene is present on the Z. It has been suggested that the derived W protein, ASW/WPKC, may interfere with the Z copy to elicit ovary development (Hori et al., 2000). There may be other interactions between Z and W, involving methylation of the Z, with a W-linked factor transcribed in females repressing male genes carried on the Z (Teranishi et al., 2001). These observations suggest that both the W and Z sex chromosomes are involved in avian sex determination. Another candidate female-determining gene is *FET1* (female-expressed transcript #1), which is also W-linked and expressed in female urogenital systems (Reed and Sinclair, 2002). *FET1* encodes an avian retroviral element. If it plays a role in gonadal sex differentiation, it would represent an intriguing case of viral co-option by the embryo for a developmental process.

Definitive proof that any of these genes are involved in avian sex determination will require transgenic over-expression or deletion. There are a number of features of avian reproductive biology that make the production of transgenic birds more difficult than in laboratory mammals, although progress has been made (Mozdziak et al., 2003; Sang, 2004). More rapid alternative approaches include gene transfer using avian-specific retroviral vectors and/or electroporation in the developing embryo, and direct assessment of the embryos (See the special issues devoted to chicken embryology in *Developmental Dynamics*, vol 229, 2004, and *Mechanisms of Development*, vol 121, 2004). Using this approach, embryos could then be examined for phenotypic effects of over-expression (such as gonadal sex reversal). Electroporation has proven very useful for delivering plasmid-based expression constructs into tissues such as the neural tube or limb (Krull, 2004; Nakamura et al., 2004). However, gene expression is transient and the method is not well suited to targeting mesenchymal tissue or tissues containing extensive basement membrane, such as the gonads. Avian retroviral vectors do provide longer lasting expression of transgenes (Ishii et al., 2004). Vectors such as RCAS are replication competent and can spread from cell to cell following initial transfection into early embryos. Attempts are currently being made to over-express candidate male *(DMRT1)* or female *(ASW)* genes cloned into RCAS vectors and injected into chicken embryos prior to gonadal sex differentiation. The aim of these experiments is to induce complete or partial sex reversal (Smith and Sinclair, 2004).

Alternatively, candidate gene expression could theoretically be down-regulated, using RNA antisense technology in ovo (Kos et al., 2003) or in isolated gonads grown in vitro. RNA interference is now being widely used for specific gene knockdown in vertebrate cells and tissues, and in whole embryos (Pekarik et al., 2003). In the chicken embryo, however, this approach has focused primarily upon accessible tissues such as the brain, neural tube or limb. Recently, researchers have combined the superior delivery provided by replication-competent avian retroviruses with antisense RNA to development vectors capable of down-regulating target genes, at least in vitro (Bromberg-White et al., 2004). For all of these approaches, the challenge will be to ensure sufficient delivery of over-expression or knockdown constructs such that an effect on gonadal differentiation is observed.

The ribosomal DNAs of the chicken genome

(Prepared by M.E. Delany)

The translation of mRNA into protein in eukaryotes occurs within the cytoplasmic ribosomes which number in the tens of millions per cell. An appropriate capacity for ribosome biogenesis is intimately associated with cell growth, differentiation and homeostasis. The mature 80S ribosome consists of two subunits, large (60S) and small (40S), and contains over 80 ribosomal (r) proteins and four different species of rRNA. The 28S, 5.8S and 5S rRNAs are incorporated into the large subunit along with ~50 r-proteins (L1–L50); the 18S rRNA is incorporated into the small subunit along with ~33 r-proteins (S1–S33). Pre-ribosome subunit assembly occurs within the nucleolus. The nucleolus, first described in 1781, is located within the nucleus and is a prominent cytological feature of interphase cells, disappearing during mitosis and re-forming in daughter cells (Hadjiolov, 1985). Decades ago McClintock developed the cytogenetic concept for the "nucleolus organizer region" (NOR), i.e., the chromosomal region which controls the formation of the nucleolus (McClintock, 1934); years later it was discovered that the NOR encodes the high copy number, tandemly repeated 18S-5.8S-28S rRNA genes (Brown and Gurdon, 1964; Ritossa and Spiegelman, 1965). Within the nucleolus the pre-ribosomal subunits are assembled utilizing the processed local transcripts (18S, 5.8S, 28S), imported 5S rRNA synthesized at an unlinked locus (in most eukaryotes, excepting yeast), and the r-proteins imported following their message translation in the cytoplasm (most r-protein genes are single-copy and unlinked, found scattered around the genome). The 18S-5.8S-28S genes are transcribed by Pol I, the r-protein genes by Pol II, and the 5S rRNA by Pol III. The coordination of the transcription of numerous genes, protein translation and molecular shuttling necessary to accomplish ribosome manufacture is remarkable. In addition to the components necessary for rDNA processing and ribosome biogenesis, other important cellular molecules, e.g., the telomerase components TR and TERT, localize to the nucleolus (Etheridge et al., 2002). Interestingly, abnormal NOR and nucleolar architecture is a hallmark feature of both metastatic and aging cells (see Hadjiolov, 1985 and references therein; Delany et al., 1998).

18S-5.8S-28S rDNA (NOR)

In higher vertebrates, the 18S, 5.8S and 28S rRNA genes are arranged as a single transcription unit (18S-5.8S-28S) which is tandemly repeated at one or more loci (e.g., five NOR loci in human and mouse); the repeat units are present in high copy number (100's to 1000's of genes/haploid genome). The single NOR locus in chicken was initially mapped to microchromosome 16 (q arm) and linked to the MHC by trisomy mapping utilizing the aneuploid "Trisomic" line which segregates individuals with extra copies of the NOR chromosome (Bloom and Bacon, 1985). The map location was further confirmed by both cytogenetic analysis (Fillon et al., 1998) and sequencing of cosmid clusters (Guillemot et al., 1988). Gene copy number was established by studies utilizing the NOR-aneuploid "Trisomic" line (Muscarella et al., 1985), and other research and commercial stocks (Su and Delany, 1998; Delany and Krupkin, 1999; Delany, 2000) with diploid values ranging from ~300 to ~500 copies (~150 to ~250 repeats/chromosome). NOR-deficient chromosomes from genetic populations selected on the basis of reduced nucleolar size were found to have 65 genes ("PNU" line) and as few as 40 genes ("mPNU" line) (Delany et al., 1994a, b, 1995). The NOR-deficient lines were used to establish the minimum number of 18S-5.8S-28S genes necessary for embryonic viability (~160/diploid) and the stage at which embryonic failure occurs in severely rDNA-deficient individuals (gastrulation) (Delany et al., 1991, 1994a, b, 1995). Studies of gene expression in NOR-aneuploid and NOR-deficient genetic lines indicate regulated expression of the 18S-5.8S-28S genes accommodating variations in gene copy number and developmental stage (Muscarella et al., 1985; Delany et al., 1994b, 1995). Regulation appears to be mediated through chromatin architecture (Muscarella et al., 1987). The degree of copy number variation and the incidence of nucleolar size polymorphisms within diploid populations (Delany et al., 1994a, b; Su and Delany, 1998) suggest that alteration in gene copy number is a frequent event. The underlying genetic mechanism for shifts in gene copy number may be unequal recombination promoted by out-of-register pairing (see Delany et al., 1991 for discussion); it has been proposed that the NOR is located between the MHC and the "MHC-like" Rfp-Y locus, and that a high recombination rate within the NOR is the genetic mechanism responsible for the lack of genetic linkage between the MHC and Rfp-Y (Miller et al., 1996).

Molecular features of the 18S-5.8S-28S rRNA genes

Each gene repeat unit consists of an intergenic spacer (IGS) 5′ of the gene, the 18S-coding region, an internal transcribed spacer (ITS1), the 5.8S gene, an ITS2, and the 28S-coding region. The primary rRNA transcript (~10 kb) includes both 5′ and 3′ external transcribed spacer sequences (ETS) plus the 18S, ITS1, 5.8S, ITS2 and 28S sequences; the primary transcript is processed via a series of cleavage reactions into the individual 18S, 5.8S and 28S rRNAs assuring production of equimolar amounts of these rRNAs (Hadjiolov, 1985). The order of the genes within the repeat unit, their sequences, and some aspects of the processing steps are highly conserved among eukaryotes and even prokaryotes. In contrast, the adjacent IGS regions vary significantly for size and sequence and this genetic

variation has been studied in a number of organisms (see Delany and Krupkin, 1999 and references therein). In chicken, the size of the repeat unit varies according to the size of the adjacent IGS, and enormous heterogeneity exists with repeat sizes from 11 to 50 kb. IGS region variation is important because of the *cis*-control regions 5′ and 3′ of the repeat unit (promoter, enhancer and termination sites) among other important sequences, e.g., replication origins, recombination hotspot motifs, matrix attachment regions (MARs), etc. (see Gonzalez and Sylvester, 1995). Repeat size variation exists at several levels including both within the NOR locus, between NOR chromosomes within a population and between populations (Delany and Krupkin, 1999; Delany, 2000). The average size of the repeat in UCD 001 (Red Jungle Fowl, RJF) was 7 kb smaller than that of UCD 003 (Single Comb White Leghorn, SCWL). UCD 001 exhibited two predominant repeat size classes (29.5 and 30.5 kb, with minor repeats ranging from 15 to 50 kb) whereas UCD 003 exhibited one main repeat class (37 kb, with minor repeats ranging from 19 kb to over 50 kb). The average size of the entire rDNA array (based on copy number and repeat size) was 5 Mb in UCD 001 and 7 Mb in UCD 003 (Delany and Krupkin, 1999). NOR variation was also examined in broiler and layer chickens (Delany, 2000). The average gene repeat size in commercial white-egg populations was 36 kb whereas in brown egg layers and broilers the average gene repeat sizes were 32.5 and 33.9 kb, respectively. NOR array size was similar among the three types of chicken populations, 6 Mb; populations with larger repeat unit size generally possessed a lower number of gene repeats. The diversity patterns of NOR-gene repeat variation within and between populations suggested that breed origin as well as selection scheme may have influenced the degree of variation seen within and between NORs; repeat unit size variation was greatest in the broiler populations.

Molecular and cytogenetic features of 5S rDNA

The 5S rRNA genes in higher eukaryotes are arranged in arrays of tandem repeats. The repeat unit contains a non-transcribed IGS region (spacer) adjacent to the highly conserved, small coding region (~ 120 bp). Most vertebrate genomes contain only one or two loci, *Xenopus* being an unusual exception with repeats located at the telomeres of most chromosomes (Pardue et al., 1973). The function of the 5S rRNA is unknown. Studies of prokaryote ribosomes indicate that it crowns the large ribosomal subunit having extensive interactions with proteins and may serve to stabilize the r-proteins (Ban et al., 2000). Unlike the 18S-5.8S-28S genes, transcript levels appear unaffected by changes in growth conditions (de la Serna et al., 2000).

The molecular characteristics of the chicken 5S rRNA genes were recently described (see Daniels, 2001; Daniels and Delany, 2003). A predominant 2.2-kb gene (α) repeat (coding plus IGS) was found in both research and commercial chicken stocks and a minor 0.6-kb (β) repeat (having a significantly smaller IGS) was found in a subset of the populations. FISH mapping indicated a single locus and placed the chicken 5S rRNA gene array on chromosome 9 near the centromere on the q arm. Positioning of the 5S array orients the markers of the linkage group (E36, GGA9) and suggests there are no markers yet for the p arm. Linkage analysis conducted using *Alu*I PCR-RFLP and the E. Lansing reference population indicated a high double recombination rate (25%) involving 5S, ROS0152 and MSU0346 suggesting that the 5S array might be a recombination hotspot. The arrangement of the α and β genes (interspersed or separate clusters) within the array is unknown; in human separate clusters of 5S genes map to chromosome 1 (Kost et al., 1995). The size of the 5S rDNA array in UCD 001 (RJF) (possessing only the α gene repeat) was found to be 83 kb and can be used to provide an estimated gene copy number of 76 genes per diploid genome; the human genome estimates being more than double, ~180 genes/diploid genome (Little and Braaten, 1989). Sequence analysis was conducted on both 5Sα (UCD 001 and UCD 003) and 5Sβ (UCD 003) genes (AF419700, AF419701) and features characteristic of Pol III promoter systems were identified in both gene classes (A-, C- and D-box, termination sequences) along with homopolymeric G/C stretches, and a matrix attachment region (MAR) (Daniels and Delany, 2003).

Chicken telomere biology: telomeres and telomerase
(Prepared by M.E. Delany)

The telomere is the specialized nucleoprotein structure which "caps" the ends of linear chromosomes and telomerase is the specialized enzyme responsible for maintaining the length of the telomere (Greider and Blackburn, 1985, 1989). Telomerase catalyzes the addition of the telomere repeat (TTAGGG) to the 3′ end of parental DNA strand(s) thus preventing diminution of the telomere resulting from the 5′ end replication problem (Olovnikov, 1973). In human somatic cells, the developmental regulation of telomerase activity plays an important role in genome stability and cellular lifespan. Lack of telomerase activity in aging somatic cells (in vitro or in vivo) correlates with telomere shortening and once telomeres reach a critical threshold size, ensuing cytogenetic abnormalities involving the chromosome ends eventually trigger apoptosis and/or cellular senescence (Counter et al., 1992; Karlseder et al., 1999). This telomere-initiated genetic mechanism provides an important limitation to cellular lifespan and thus prevents uncontrolled cell proliferation of aging and abnormal cell populations (i.e., if other control mechanisms fail to remove aged cells from the cell cycle, eventually the telomere mechanism will be invoked). Subversion of this mechanism occurs in human cancers with the resumption of telomerase activity and stabilization of telomere length (Shay and Bacchetti, 1997). Notably, the telomere biology of the laboratory mouse model differs from that of human, i.e., telomerase activity is constitutive in somatic cells and telomere shortening is not normally a feature of aging cells or a primary genetic mechanism involved in murine cell cycle control (reviewed in Forsyth et al., 2002). Recent experiments have focused on the molecular details of telomere array organization and telomerase activity in chicken to establish the key features of telomere biology in this important food animal and model system (Delany et al., 2000, 2003; Taylor and Delany, 2000; Delany and Daniels, 2003; Swanberg and Delany, 2003).

Array organization

Chicken telomere arrays can be divided into three main categories based on size, chromosomal location, and stability. Class I arrays are interstitial, range from 0.5 to 10 kb in size, and show no evidence of telomere shortening (Delany et al., 2000). Class II arrays are terminal, range from 10 to 40 kb, and show evidence of age-related telomere shortening in vivo and in vitro (Delany et al., 2000; Taylor and Delany, 2000; Swanberg and Delany, 2003). Class III arrays are terminal, range from 40 kb to 1–2 Mb, and do not exhibit telomere shortening (possibly a resolution issue given their large size) although the telomeres in this category show evidence of hypervariability (Delany et al., 2003; Rodrigue and Delany, in preparation). Nanda et al. (2002b) studied the distribution of the $(TTAGGG)_n$ repeat sequences by FISH analysis of chromosomes from 16 bird species and report the enrichment of telomeric DNA on the microchromosomes as compared to the macrochromosomes and the variability among genomes of interstitial telomere sequence content; similarly, not all bird species possess the extremely large telomere tracts (Delany et al., 2000; Nanda et al., 2002b).

Telomerase activity and telomere shortening

Telomerase activity is high during the early differentiation stages of chicken embryogenesis (pre-blastula Stage X to neurula Stage 8) and organogenesis (E5 to E10). In the late stage embryo and post-natally, down-regulation of activity occurs in a tissue-specific manner (Taylor and Delany, 2000) except in germline and highly proliferative tissues (e.g., intestine) where high telomerase activity is found even in older adults. Estimates for telomere shortening in vivo are 160 bp/cell division based on germline vs erythrocyte comparisons of adults from 2 to 9 years of age (Delany et al., 2000) and ~600 bp/year based on adult organ vs germline (gonad) comparisons of 5-year-old adult males (Taylor and Delany, 2000).

Telomerase activity is not routinely detected in primary cultures of chicken embryo fibroblasts (CEFs), although activity can be detected occasionally (and transiently) in flasks at different passages. Telomere profiles of CEF cultures studied over time suggest a dynamic shift in cell populations over the course of a culture lifespan, although ultimately a significant loss of telomeric DNA occurs concomitant with senescence (Swanberg and Delany, 2003). Overall, losses in Class II arrays ranged from 40 to 85% (of original levels) and mean array lengths were reduced by 621 to 2,191 bp (data from five different cultures established from single E11 embryos, UCD 003 line). Telomere shortening values from the cultures exhibiting reductions in telomere length ranged from –28 to –88 bp per population doubling (average value of –61 bp).

In contrast, transformed avian (chicken, turkey, quail) cells in vitro (and in vivo tumor samples) exhibited telomerase activity (cell lines examined: RP-19, DT40, RP-9, MSB-1, MQ-NCSU, QT6, QT35, LMH, LMH/2A) (Swanberg and Delany, 2003). Interestingly, the telomeric DNA profiles of the transformed cells were similar to those of the senescent CEFs, exhibiting very little DNA in the Class II array category, perhaps indicating stabilization of telomere-deficient genomes (although this remains to be tested experimentally). In other studies of cells in vitro, telomerase activity was detected in short-term turkey muscle satellite (stem) cells but did not correlate with proliferation potential of large and small-cell colonies (Mozdziak et al., 2000). Quail embryo myoblasts and chicken neuroretina cells in culture were telomerase negative but became telomerase positive upon v-myc transformation (Falchetti et al., 1999).

The telomerase RNA gene

Telomerase consists of two components, a small RNA (TR) having template binding and copying functions and a specialized reverse transcriptase (TERT) responsible for nucleotide addition. Chicken TR (chTR) is a single copy gene which maps to chromosome 9 (GGA9q terminal). Thus, of seven coding loci on GGA9, four map to human chromosome 3 (*TRFC*, *EIF4A2*, *SKIL*, TR), two map to HSA2 *(NCL*, *PAX3)* and one maps to HSA1 (5S rDNA) (Delany and Daniels, 2003).

The TR-coding region is 465 bp and both the primary sequence and proposed secondary structure share many features in common with other vertebrate TRs (Chen et al., 2000). A unique feature of chTR is an expanded GC-rich region (termed the "co-axial stacking" region) between two conserved domains. The coding region along with 600 bp 5′ and 2,700 bp 3′ was sequenced in UCD 001 (3.7 kb total, AY312571) for analysis of local and distal characteristics (Delany and Daniels, 2003) including regulatory element/transcription factor binding motifs, GC content and identification of regions homologous to the TR sequences identified in oncogenic strains of Marek's disease virus (see also Fragnet et al., 2003).

The GC content of the chTR gene and associated sequences is high, the 5′ sequence (–604 to –1) is 67.1% GC, the coding region (+1 to +465) is 77% GC, and the 3′ region (+466 to +3159) is 52.2% GC. A CpG island is indicated for positions –556 to +1436. Five blocks of sequences (from 5′, coding, and 3′ regions) have homology with MDV (GA, Md5, RB1B) sequences (sequence identities ranging from 82 to 95%, see Fig. 1 in Delany and Daniels, 2003). Interestingly, the GC-rich coaxial stacking region is the only region of the coding sequence missing from the MDV chicken TR sequence. Chen et al. (2000) hypothesize that that region imparts binding stability. The biological significance of the presence of TR sequences in the MDV genome in regard to transformation remains to be elucidated; Fragnet et al. (2003) report expression of the MDV TR in peripheral blood of infected birds. In human cancer cells, there exists evidence for upregulation ("dysregulation") of TR via aneuploid and amplification mechanisms (Avilion et al., 1996; Soder et al., 1997, 1998).

More than 30 regulatory element motifs exist 5′ of the chTR coding sequence, including many Sp1 sites, oncogene transcription factor motifs (c-Myb), steroid receptor binding sites. Many of these motifs are also found 5′ of the human TR gene, e.g., Sp1, GR, c-Myb, ER, CCAAT and in positions of the same relative proximity to the coding region. Additionally, numerous motifs are found 3′ of the gene and many of these (more than 50) correspond to elements also known to be involved in TERT regulation as defined by studies in model organisms (see Delany and Daniels, 2003). In human cells, TR is constitutively expressed whereas TERT exhibits regulated expression (Mergny et al., 2002).

In summary, key aspects of telomere biology established for the chicken are similar to that described for human and include (1) age-associated telomere shortening in somatic cells both in vivo and in vitro, (2) down-regulation of telomerase activity in non-transformed non stem-cell types in vivo and in vitro, (3) telomerase activity in transformed cell types (tumors and cell lines), (4) TR gene organization including a single locus (GGA9) with a number of important regulatory element motifs (e.g., oncogene transcription factor binding sites) both 5′ and 3′ of the gene.

Tropomodulin genes in *Gallus domesticus* and in mammals: gene structure, protein homologies and tissue distribution

(Prepared by C.A. Conley and V.M. Fowler)

The tropomodulins (Tmods) are a relatively small family of actin-binding proteins that are present in both vertebrates and invertebrates as an essential component of the actin cytoskeleton. Biochemically, Tmods bind to tropomyosin and bind or cap the slow-growing (pointed) ends of actin filaments (Fowler, 1996, 1997; Fowler and Conley, 1999). In vivo, Tmod proteins are localized to the pointed ends of a variety of actin filaments, including the short filaments that form the vertices of the spectrin-actin membrane skeleton (Ursitti and Fowler, 1994), the contractile actin filaments of striated muscle sarcomeres (Fowler et al., 1993; Gregorio and Fowler, 1995; Almenar-Queralt et al., 1999), and the dynamic actin filaments in the dendritic network in lamellipodia of migrating endothelial cells (Fischer et al., 2003). In cultured chick cardiomyocytes, disruption of Tmod function using microinjected antibodies leads to elongation of sarcomeric thin filaments from their pointed ends and inhibition of cell beating (Gregorio et al., 1995). Transgenic mice overexpressing the Tmod1 isoform under the control of a cardiac promoter develop dilated cardiomyopathy (Sussman et al., 1998), and the human gene for Tmod1 maps very close to a human familial cardiomyopathy (Krajinovic et al., 1995). Tmod3 is a negative regulator of cell migration in endothelial cells (Fischer et al., 2003) and Tmod2 may play a role in synaptic plasticity in neurons, based on behavioral deficits and enhanced long term potentiation in a Tmod2 knockout mouse (Cox et al., 2003). Understanding the comparative biology of the Tmods should lead to improvements in our understanding of basic biological processes, as well as provide the potential for novel therapeutic procedures.

Tmod gene family

Although Tmod function was first studied in the chicken, the Tmod gene family is best understood in mammals. Two subclasses of Tmods are known, an ~40-kDa subclass also called Tmods and a larger ~65-kDa subclass that is referred to as leiomodins (Lmods). The entire Tmod family is characterized by two conserved regions present in all isoforms, with variable sequences present elsewhere that distinguish particular isoforms (Fig. 32A). The first conserved region is located at the amino-terminus of all family members, and has been demonstrated to bind tropomyosin (Babcock and Fowler, 1994; Kostyukova et al., 2000; Greenfield and Fowler, 2002). The second conserved region is located in the carboxy-terminal half of the ~40-kDa Tmods and in the middle of the ~65-kDa Lmods, and has been shown to contain the actin-capping region (Fowler et al., 2003). In the Lmods, the sequences intervening between the conserved regions are of variable length, and the Lmods also carry a carboxy-terminal extension following the second conserved region (Conley et al., 2001 and manuscript in preparation).

In the nearly complete genomes of mice, rats, and humans, there are four ~40-kDa Tmod genes and three ~65-kDa Lmod genes (Cox and Zoghbi, 2000; Conley et al., 2001, and manuscript in preparation). Each of the mammalian genes displays distinct but overlapping patterns of expression, with some isoforms being found widely while others are restricted to a small subset of tissue types. In chickens, two Tmod genes have been reported previously, that are most similar to the mammalian *TMOD1* and *TMOD4*. Chicken *TMOD1*, otherwise known as erythrocyte Tmod or *E-Tmod*, was the first chicken Tmod isoform cloned (Babcock and Fowler, 1994), and has been the subject of considerable study. In chickens, TMOD1 is associated with the pointed ends of the thin filaments in the sarcomeres of cardiac and slow skeletal muscle and with the costameres on the sarcolemma of fast skeletal muscle (Almenar-Queralt et al., 1999; Greenfield and Fowler, 2002). A crystal structure for the carboxy-terminal half of chicken TMOD1 has recently become available, and shows that this portion of the molecule is composed of a series of five leucine-rich repeats (Krieger et al., 2002). TMOD4, also known as skeletal-muscle Tmod or *Sk-Tmod*, was also originally studied in chickens and is found at the pointed ends of thin filaments in chicken fast skeletal muscle fibers (Almenar-Queralt et al., 1999) and in mammalian skeletal muscle (Conley et al., 2001). Curiously, *TMOD4* is expressed in the fiber cells of the eye lens and in mature erythrocytes in chickens, whereas in mammals *TMOD1* is expressed in these cell types (Almenar-Queralt et al., 1999; Cox and Zoghbi, 2000; Fischer et al., 2000). This suggests that some Tmod isoforms have exchanged tissue-specific functions during evolution.

Considerably less is known concerning Lmod function and expression. In humans, the two published Lmods have been shown to display distinct patterns of expression (Conley et al., 2001). *LMOD1* is expressed widely and predominantly in tissues containing smooth muscle, and LMOD1 is localized to smooth muscle cells in tissues containing multiple cell types (Conley, 2001). *LMOD2* is expressed in adult skeletal muscle as well as fetal and adult cardiac muscle, a pattern resembling *TMOD1* expression in muscle tissue (Conley et al., 2001). The expression of Lmod3 has not yet been reported in the literature (Conley et al., manuscript in preparation), however based on the abundance of EST sequences from fetal tissues *LMOD3* is likely to be highly expressed in embryos, both in mammals and in chickens (Table 9).

Avian-mammalian conservation

For this review, we have performed searches of the chicken EST database and have identified chicken sequences encoding the two remaining Tmod isoforms *(TMOD2* and *TMOD3)* as well as the three Lmod isoforms (Table 9). All Tmod sequences

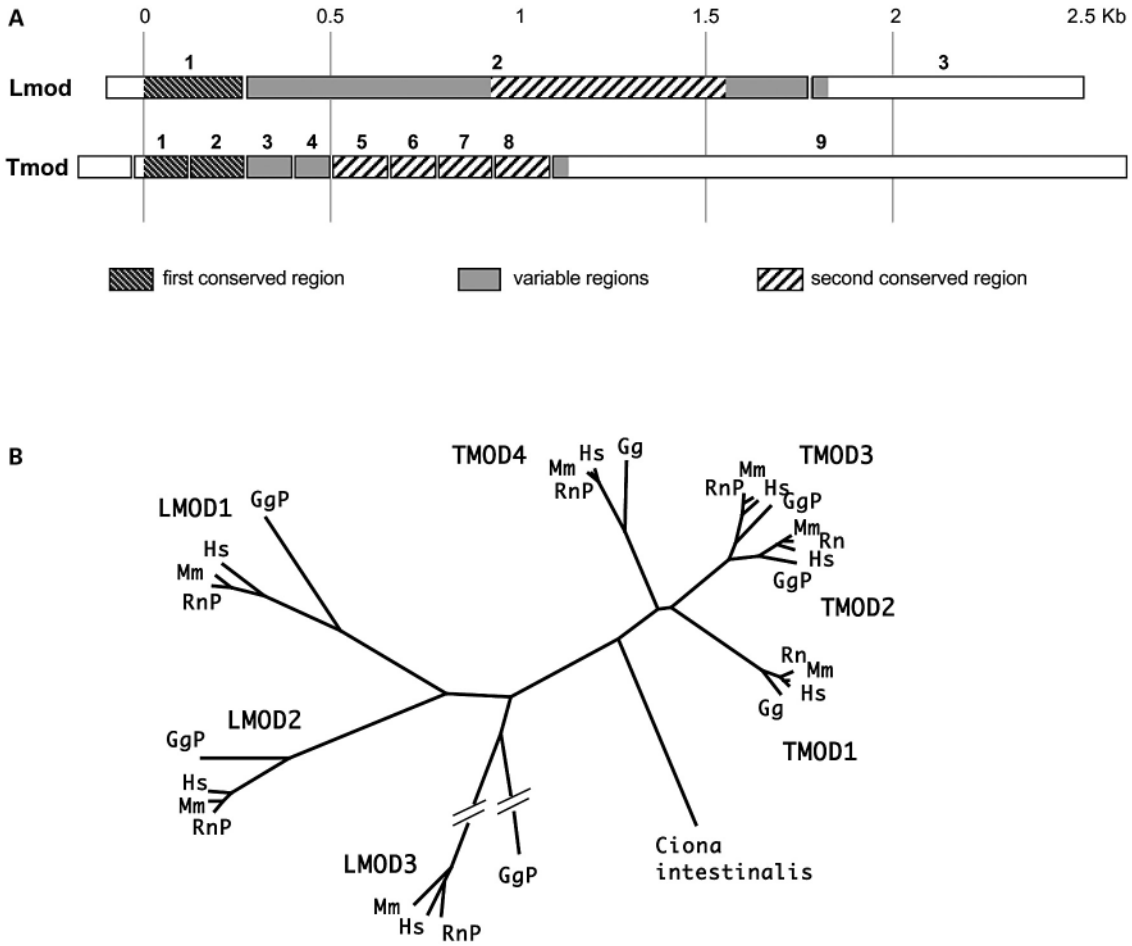


Fig. 32. Conservation of chicken and mammalian Tmod sequences. (**A**) Exon structure of mammalian Tmods and Lmods. Different hatching indicates the conserved and variable regions within the two subclasses. Regions were considered variable if the amino acid sequences were less than 60% similar over 20 amino acids. (**B**) Phylogenetic tree of mammalian and chicken Tmod proteins. Each of the mammalian and chicken sequences for Tmods1–4 and Lmods1–3 are grouped together. The longer branch lengths in the Lmod portion of the tree are probably a result of incomplete or inaccurate sequences that are currently in GenBank. The tree was constructed from sequences available in GenBank, with ClustalW to perform the multiple alignment and the protdist, kitch, and drawtree programs of the Phylip package to analyze phylogeny, using the single *Ciona intestinalis* Tmod as outgroup and default settings. Gg: chicken; Hs: human; Mm: mouse; Rn: rat; RnP/GgP: partial sequences.

found in chicken showed high similarity to known mammalian genes (Figs. 32B, 33A, B). A complete ORF has been constructed for chicken *TMOD3*, and partial cDNA sequences containing incomplete ORFs have been constructed for chicken *TMOD2* and chicken *LMODs 1, 2,* and *3*. Each of the chicken TMOD1–4 proteins shows greater than 85% similarity to the corresponding mammalian Tmod and lower similarity to the others (Fig. 33A), suggesting orthology, so we have named them appropriately. Chicken TMOD3 displays a relatively uniform distribution of amino acid sequence divergence from human TMOD3 throughout the entire ORF (Fig. 33C). The chicken Lmod sequences are also most similar to the correspondingly named mammalian proteins (Fig. 33B). These results demonstrate that all duplication events producing the Tmod genes in both mammals and chickens had occurred prior to the mammalian-avian divergence, which occurred 310 million years ago (Kumar and Hedges, 1998).

Genomic structure

No sequences corresponding to the chicken Tmod genes are present in the chicken genomic sequence available as of April 2003; thus we have not been able to deduce the intron-exon structure of the chicken Tmod genes. However, with three available mammalian genomes, the gene structure of the mammalian Tmods is relatively well characterized (Chu et al., 2000; Conley et al., 2001; Cox et al., 2001). In mammals, the gene structure of the four genes encoding TMODs1–4 is conserved, with eight introns in an ~1.1-kb open reading frame (ORF). The mammalian Lmod gene structures are also conserved but are different from that of the *TMODs1–4*, having only two introns in a ~1.7-kb ORF (Fig. 32A). The exon boundaries for the Lmods are located within the regions conserved between Lmods and Tmods, and fall very near to exon boundaries in *TMODs1–4* (Conley et al., 2001). No evidence for alternative exon splicing within the coding region has been reported for

mammalian *TMODs1–4*, although an additional gene has been reported that is interspersed within the introns of the tandemly repeated *TMODs 2* and *3* (Cox et al., 2001). No evidence for alternative splicing was found during our examination of chicken Tmod ESTs.

The conservation of Tmod vs. Lmod gene structure suggests that an ancient gene duplication event created the genes encoding the ~65-kDa Lmods and genes encoding the ~40-kDa Tmods, and that subsequent duplication events created multiple isoforms in the different subclasses that retained the ancestral gene structure. Thus it is reasonable to predict that the chicken gene structure for both *TMODs1–4* and the Lmods will be similar to that of mammals.

Using the reported synteny between chicken and mammalian chromosomes (Schmid et al., 2000), we can also predict the location of unmapped chicken Tmod genes. The genetic location of the two previously reported chicken Tmod genes, *TMOD1* and *TMOD4*, has been determined on the physical map of the chicken genome. Chicken *TMOD1* maps to one tip of the Z chromosome at Zq21 (Nanda et al., 2000), which is syntenic to the portion of the human chromosome 9 and mouse chromosome 4 that carry the respective human *TMOD1* (9q22.3) and mouse *Tmod1* (4 at 21.5 cM) genes (White et al., 1995; Sung et al., 1996). *TMOD4* maps to 1q12 in humans and to chromosome 3 at 52.0 cM in mice (Cox and Zoghbi, 2000). One named microchromosome, E26C13, shares synteny with both human chromosome 1 and mouse chromosome 3, and may be the location of chicken *TMOD4*. Based on synteny, we can also predict that chicken *TMOD2* and *TMOD3* will be located in tandem on chicken chromosome 10 between *CRABP1* and *TPM1*, since in human they are located in tandem on chromosome 15q21.1-2 and in mouse on chromosome 9 at 38.0–52.0 cM (http://www.ncbi.nlm.nih.gov/LocusLink/).

The Lmods are somewhat less well characterized in mammals, with *LMOD1* located on human chromosome 1q32/mouse chromosome 1 at cytoband F and *LMOD2* located on human chromosome 7q31/mouse chromosome 6 at cytoband A3 (LocusLink). The locations of chicken *LMOD1* and *LMOD2* can be predicted to be chromosome 8 and chromosome 1 respectively. Human *LMOD3* is mapped to 3p14.1, which does not show synteny to any chicken chromosomes in the 2000 map (Schmid et al., 2000), thus this gene may be located on one of the smaller microchromosomes. These predicted locations could be useful for directing the screening of chicken BAC libraries for genomic Tmod sequences, as well as perhaps focusing efforts in mapping the unmapped chicken Tmod genes.

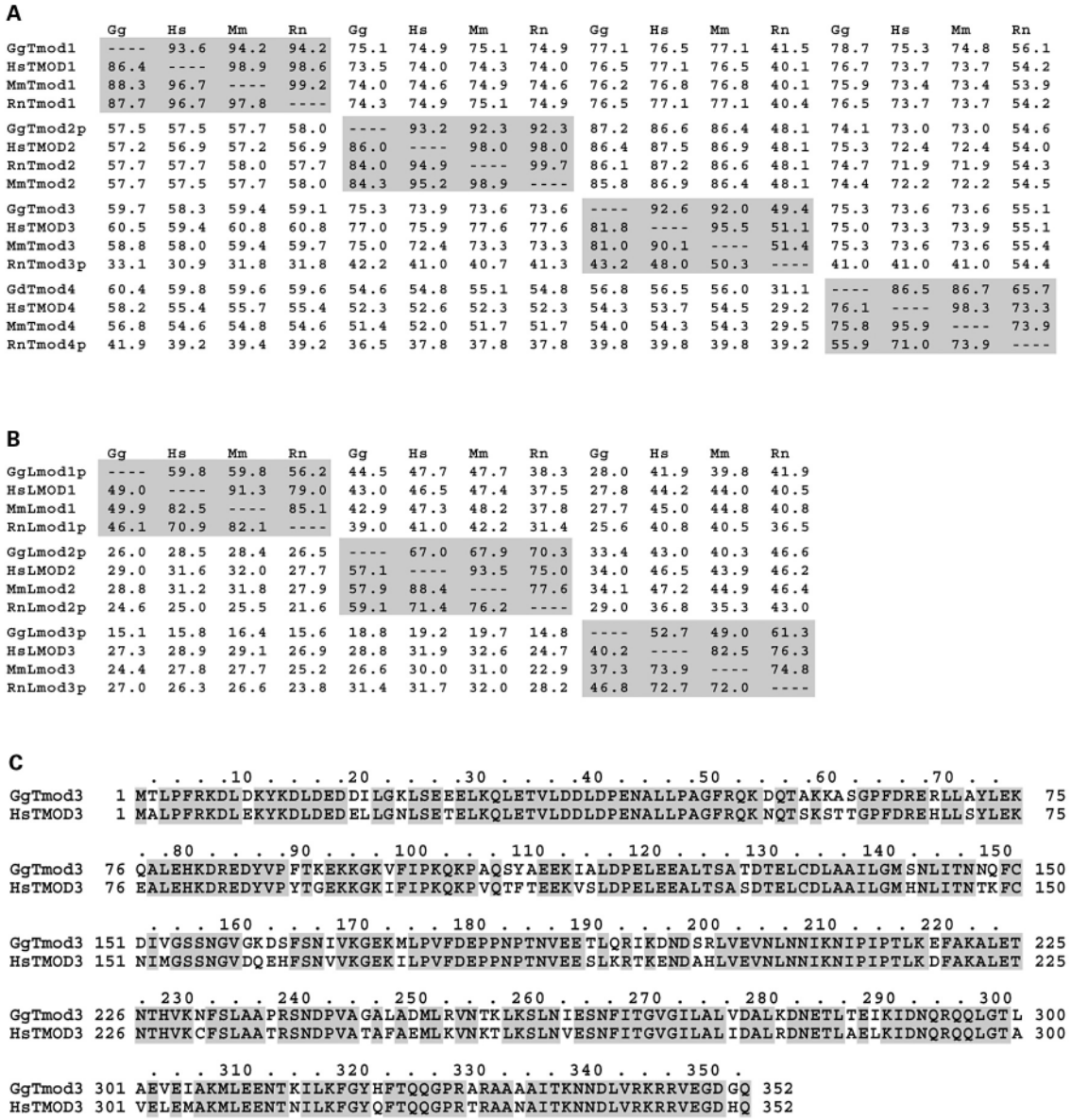
Table 9. Chicken ESTs encoding novel Tmod sequences. ESTs encoding Tmod protein sequences were collected as of April 2003. The corresponding mammalian protein and the mammalian tissue-level localization are given as headings, and the GenBank accession number and source tissue for that particular cDNA are listed below. The tissue sources for the chicken ESTs correspond quite well with the reported mammalian expression patterns. The mammalian expression for F-Lmod is only predicted from available ESTs.

	Accession #	Tissue source
Tmod1 (erythrocytes, striated muscle)	BU131801	stage 36 trunks
	BU250803	stage 36 trunks
	BU261195	stage 36 limbs
	BU320389	stage 22 heads
	BU411792	adult muscle
	BU411879	adult muscle
Tmod2 (brain)	BU353735	adult cerebellum
	BU353797	adult cerebellum
Tmod3 (ubiquitous)	AJ395122	bursal lymphocyte
	AJ445489	bursal lymphocyte
	AJ447852	bursal lymphocyte
	AJ449020	bursal lymphocyte
	AJ449021	bursal lymphocyte
	AJ450032	bursal lymphocyte
	AJ451371	bursal lymphocyte
	AJ453003	bursal lymphocyte
	AJ453905	bursal lymphocyte
	AJ454353	bursal lymphocyte
	AJ455036	bursal lymphocyte
	AJ456003	bursal lymphocyte
	AJ456819	bursal lymphocyte
	AL588234	brain
	BU142366	stage 20 – 21 embryo
	BU203073	stage 20 – 21 embryo
	BU104108	stage 20 – 21 embryo
	BU245532	adult liver
	BU286851	adult kidney + adrenal
	BU291377	adult kidney + adrenal
	BU309324	adult heart
	BU317272	stage 36 heads
	BU382716	stage 36 trunks
	BU403773	stage 36 limbs
Tmod4 (skeletal muscle)	BM486166	breast muscle
	BM486873	breast muscle
	BM488992	breast muscle
	BU407410	stage 36 limbs
	BX267380	multiple tissues
	BX267381	multiple tissues
Lmod1 (smooth muscle)	BI066925	fat
	BU295945	adult small intestine
	BU29755	adult small intestine
	BU455951	adult ovary
Lmod2 (striated muscle)	BU214157	stage 20-21 embryo
	BU402089	stage 36 limbs
	BU411942	adult muscle
Lmod3 (predicted embryos and muscle)	BU127797	stage 36 limbs
	BU236847	stage 22 heads
	BU411890	adult muscle
	BU475151	adult chondrocytes

DEAD box genes in chicken

(Prepared by R. Godbout and S. Katyal)

DEAD box proteins are members of an extensive family of putative RNA helicases that share nine conserved motifs (Q, I, Ia, Ib, II–VI) (Linder et al., 1989; Tanner et al., 2003). Motif II consists of D(asp)-E(glu)-A(ala)-D(asp), part of the more extended sequence V/I-L-D-E-A-D-X-(M/L)-L-X-X-G that represents the signature motif of this protein family. The conserved motifs in DEAD box proteins are required for ATP binding/hydrolysis and RNA binding (reviewed in Caruthers and McKay, 2002). In addition to RNA-dependent ATPase activity, some DEAD box proteins have been shown to have RNA unwinding or RNA destabilizing activity (Hirling et al., 1989; Rozen et al., 1990; Gururajan et al., 1994). DEAD box proteins



A

	Gg	Hs	Mm	Rn	Gg	Hs	Mm	Rn	Gg	Hs	Mm	Rn	Gg	Hs	Mm	Rn
GgTmod1	----	93.6	94.2	94.2	75.1	74.9	75.1	74.9	77.1	76.5	77.1	41.5	78.7	75.3	74.8	56.1
HsTMOD1	86.4	----	98.9	98.6	73.5	74.0	74.3	74.0	76.5	77.1	76.5	40.1	76.7	73.7	73.7	54.2
MmTmod1	88.3	96.7	----	99.2	74.0	74.6	74.9	74.6	76.2	76.8	76.8	40.1	75.9	73.4	73.4	53.9
RnTmod1	87.7	96.7	97.8	----	74.3	74.9	75.1	74.9	76.5	77.1	77.1	40.4	76.5	73.7	73.7	54.2
GgTmod2p	57.5	57.5	57.7	58.0	----	93.2	92.3	92.3	87.2	86.6	86.4	48.1	74.1	73.0	73.0	54.6
HsTMOD2	57.2	56.9	57.2	56.9	86.0	----	98.0	98.0	86.4	87.5	86.9	48.1	75.3	72.4	72.4	54.0
RnTmod2	57.7	57.7	58.0	57.7	84.0	94.9	----	99.7	86.1	87.2	86.6	48.1	74.7	71.9	71.9	54.3
MmTmod2	57.7	57.5	57.7	58.0	84.3	95.2	98.9	----	85.8	86.9	86.4	48.1	74.4	72.2	72.2	54.5
GgTmod3	59.7	58.3	59.4	59.1	75.3	73.9	73.6	73.6	----	92.6	92.0	49.4	75.3	73.6	73.6	55.1
HsTMOD3	60.5	59.4	60.8	60.8	77.0	75.9	77.6	77.6	81.8	----	95.5	51.1	75.0	73.3	73.9	55.1
MmTmod3	58.8	58.0	59.4	59.7	75.0	72.4	73.3	73.3	81.0	90.1	----	51.4	75.3	73.6	73.6	55.4
RnTmod3p	33.1	30.9	31.8	31.8	42.2	41.0	40.7	41.3	43.2	48.0	50.3	----	41.0	41.0	41.0	54.4
GdTmod4	60.4	59.8	59.6	59.6	54.6	54.8	55.1	54.8	56.8	56.5	56.0	31.1	----	86.5	86.7	65.7
HsTMOD4	58.2	55.4	55.7	55.4	52.3	52.6	52.3	52.3	54.3	53.7	54.5	29.2	76.1	----	98.3	73.3
MmTmod4	56.8	54.6	54.8	54.6	51.4	52.0	51.7	51.7	54.0	54.3	54.3	29.5	75.8	95.9	----	73.9
RnTmod4p	41.9	39.2	39.4	39.2	36.5	37.8	37.8	37.8	39.8	39.8	39.8	39.2	55.9	71.0	73.9	----

B

	Gg	Hs	Mm	Rn	Gg	Hs	Mm	Rn	Gg	Hs	Mm	Rn
GgLmod1p	----	59.8	59.8	56.2	44.5	47.7	47.7	38.3	28.0	41.9	39.8	41.9
HsLMOD1	49.0	----	91.3	79.0	43.0	46.5	47.4	37.5	27.8	44.2	44.0	40.5
MmLmod1	49.9	82.5	----	85.1	42.9	47.3	48.2	37.8	27.7	45.0	44.8	40.8
RnLmod1p	46.1	70.9	82.1	----	39.0	41.0	42.2	31.4	25.6	40.8	40.5	36.5
GgLmod2p	26.0	28.5	28.4	26.5	----	67.0	67.9	70.3	33.4	43.0	40.3	46.6
HsLMOD2	29.0	31.6	32.0	27.7	57.1	----	93.5	75.0	34.0	46.5	43.9	46.2
MmLmod2	28.8	31.2	31.8	27.9	57.9	88.4	----	77.6	34.1	47.2	44.9	46.4
RnLmod2p	24.6	25.0	25.5	21.6	59.1	71.4	76.2	----	29.0	36.8	35.3	43.0
GgLmod3p	15.1	15.8	16.4	15.6	18.8	19.2	19.7	14.8	----	52.7	49.0	61.3
HsLMOD3	27.3	28.9	29.1	26.9	28.8	31.9	32.6	24.7	40.2	----	82.5	76.3
MmLmod3	24.4	27.8	27.7	25.2	26.6	30.0	31.0	22.9	37.3	73.9	----	74.8
RnLmod3p	27.0	26.3	26.6	23.8	31.4	31.7	32.0	28.2	46.8	72.7	72.0	----

C

```
          . . . .10 . . . .20 . . . .30 . . . .40 . . . .50 . . . .60 . . . .70 . .
GgTmod3    1 MTLPFRKDLDKYKDLDEDDILGKLSEEELKQLETVLDDLDPENALLPAGFRQKDQTAKKASGPFDRERLLAYLEK  75
HsTMOD3    1 MALPFRKDLEKYKDLDEDELLGNLSETELKQLETVLDDLDPENALLPAGFRQKNQTSKSTTGPFDREHLLSYLEK  75

          . .80 . . . .90 . . . 100 . . . 110 . . . 120 . . . 130 . . . 140 . . . 150
GgTmod3   76 QALEHKDREDYVPFTKEKKGKVFIPKQKPAQSYAEEKIALDPELEEALTSATDTELCDLAAILGMSNLITNNQFC 150
HsTMOD3   76 EALEHKDREDYVPYTGEKKGKIFIPKQKPVQTFTEEKVSLDPELEEALTSASDTELCDLAAILGMHNLITNTKFC 150

          . . . 160 . . . 170 . . . 180 . . . 190 . . . 200 . . . 210 . . . 220 . .
GgTmod3  151 DIVGSSNGVGKDSFSNIVKGEKMLPVFDEPPNPTNVEETLQRIKDNDSRLVEVNLNNIKNIPIPTLKEFAKALET 225
HsTMOD3  151 NIMGSSNGVDQEHFSNVVKGEKILPVFDEPPNPTNVEESLKRTKENDAHLVEVNLNNIKNIPIPTLKDFAKALET 225

          . 230 . . . 240 . . . 250 . . . 260 . . . 270 . . . 280 . . . 290 . . . 300
GgTmod3  226 NTHVKNFSLAAPRSNDPVAGALADMLRVNTKLKSLNIESNFITGVGILALVDALKDNETLTEIKIDNQRQQLGTL 300
HsTMOD3  226 NTHVKCFSLAATRSNDPVATAFAEMLKVNKTLKSLNVESNFITGVGILALIDALRDNETLAELKIDNQRQQLGTA 300

          . . . 310 . . . 320 . . . 330 . . . 340 . . . 350 .
GgTmod3  301 AEVEIAKMLEENTKILKFGYHFTQQGPRARAAAAITKNNDLVRKRRVEGDGQ 352
HsTMOD3  301 VELEMAKMLEENTNILKFGYQFTQQGPRTRAANAITKNNDLVRKRRVEGDHQ 352
```

Fig. 33. Sequence similarity of chicken and mammalian Tmod genes. (**A**) Matrix of identity (below diagonal) and similarity (above diagonal) between chicken and mammalian Tmod1–4 proteins. (**B**) Matrix of identity (below diagonal) and similarity (above diagonal) between chicken and mammalian Lmod1–3 proteins. The shaded boxes indicate sequences that are taken to be orthologous, as they show much higher similarity within the box than to other sequences. Among the Lmods, many of these sequences are incomplete, thus the degree of similarity is reduced. The matrices were produced from the same ClustalW alignment used to generate the tree displayed in Fig. 32. (**C**) Alignment of chicken and human Tmod3 protein sequences, generated using ClustalW. The shading indicates identical amino acids. Gg: chicken; Hs: human; Mm: mouse; Rn: rat.

have been implicated in virtually every aspect of RNA metabolism, including transcription, splicing, stability, ribosome biogenesis and translation initiation (reviewed in Abdelhaleem et al., 2003). With few exceptions (e.g. translation initiation factor eIF-4A), the function of most DEAD box proteins expressed in higher eukaryotes is either not known or only partially understood.

At least twenty-nine mammalian DEAD box proteins have been reported, in either the literature or in databases (Table 10). With the exception of the conserved domains which tend to be similarly spaced, members of the DEAD box protein family show little sequence similarity to each other. However, a significant level of similarity has been observed for the same DEAD box protein in different species. For example, *Drosophila melanogaster* DDX1 is 58.9% identical (424 identities over 720 amino acids) to human DDX1 (Rafti et al., 1996). It is therefore possible to distinguish one DEAD box protein from another in widely divergent species, even though all DEAD box proteins share a core of conserved sequences.

Table 10. Percent identity and similarity between human and chicken DEAD box proteins

DEAD Box	Size of human and chicken proteins (aa)	Identity (%)	Similarity (%)	Comments[a]
DDX1	740/740	93	97	Godbout et al. (2002)
DDX2B	407/407	98	99.5	Accession number AF515726
DDX4	691/663	54	67	Tsunekawa et al. (2000)
DDX5	614/595	90	94	Jost et al. (1999)
DDX2A	406/402	96.2	99	Missing first 4 aa*
DDX3	662/402	92	93.5	Missing first 258 aa, contains a 9 aa duplication
DDX6	472/472	99	99	Full-length sequence
DDX10	875/526	57.4	71.9	Missing first 349 aa
DDX17	651/526	92.4	94.7	Missing first 17 aa and last 105 aa
DDX18	670/311	91.6	95.8	Missing first 163 aa and last 196 aa
DDX19	479/267	95.5	98.9	Missing first 212 aa
DDX20	No ESTs			
DDX23	820/420	99.5	100	Missing first 241 aa and last 159 aa
DDX24	859/538	50.7	61.3	Missing last 340 aa
DDX25	483/236	68.4	85	Missing first 104 aa
DDX27	769/717	80.9	89.3	Missing first 53 aa
DDX28	540/260	56.9	73.5	Missing first 281 aa
DDX31	851/458	67.7	80.6	Missing first 351 aa, area of similarity ends abruptly at position 805 in human DDX31
DDX41	623/369	98.1	99.7	Missing first 252 aa
DDX42	No ESTs			
DDX43	No ESTs			
DDX46	1032/543	93.4	96.1	Missing first 496 aa
DDX47	455/440	91.4	95.5	No similarity in first 15 aa
DDX48	411/411	97.6	99.3	Full-length sequence
DDX49	483/392	77.8	85.7	Missing first 91 aa
DDX51	666/488	65.6	80.5	Missing first 181 aa
DDX54	865/247	63.6	78.5	Missing first 620 aa
DDX55	600/497	85.3	93.8	Missing first 77 aa, gap in open reading frame
DDX56	No ESTs			

[a] *: Compiled chicken ESTs lack sequences corresponding to the first 4 amino acids of the human protein.

To date, full-length cDNA sequences corresponding to four chicken DEAD box proteins have been reported, including DDX1, DDX2B (eIF-4AII), DDX4 (Vasa) and DDX5 (p68). Both the chicken and human DDX1 have a predicted size of 740 amino acids, with 93% (689/740) identical residues and 97% similar residues (Godbout et al., 2002) (Table 10, top section). Chicken DDX2B (Accession No. AF515726) is 98% identical (398/407) and 99.5% similar to human DDX2B. The level of identity between chicken and human DDX4 is considerably lower, with 54% identical residues (363/663) and 67% similar residues over the entire sequence (Tsunekawa et al., 2000). The predicted sizes of chicken and human DDX5 are 595 and 614 amino acids, respectively, with most of the extra residues in human DDX5 located at the N-terminus (Jost et al., 1999). Chicken DDX5 is 90% identical (535/595) and 94% similar to human DDX5.

With the increasing number of chicken ESTs available through DNA databases, we reasoned that it should be possible to identify the majority of DEAD box transcripts expressed in chicken, based on similarity to their mammalian counterparts. To carry out this analysis, we used the TBLASTN program to identify chicken ESTs corresponding to each of the 25 mammalian DEAD box genes listed in Table 10 (bottom section). Chicken ESTs representing the same transcript were linked together using the BBSRC EST assembly program (www.chick.umist.ac.uk) (Boardman et al., 2002). Each compiled cDNA sequence was then used to carry out BLASTX searches of NCBI protein databases. With this approach, we were able to conclusively identify the chicken counterparts for 21 of the 25 DEAD box transcripts. No ESTs were identified for *DDX20*, *DDX42*, *DDX43* and *DDX56*. Inability to identify the chicken orthologues of these four genes may indicate that: (i) these transcripts are expressed only at low levels in chicken tissues and/or ESTs have not yet been identified, (ii) these genes are absent in the chicken genome, or (iii) the level of similarity between the chicken and human transcripts is too low to allow identification of the chicken orthologue.

As shown in Table 10, full-length (or close to full-length) sequences were obtained for five chicken DEAD box proteins: DDX2A (also called eIF-4AI), DDX6 (p54, RCK), DDX48 (eIF-4A-like), DDX27 and DDX47. The first three showed exceptional identity (>96%) to their human counterparts, while DDX27 and DDX47 were 80.9 and 91.4% identical, respectively. In the remaining 16 cases, the compiled cDNAs represented only partial protein sequences, usually lacking part of the N-terminal region. The number of amino acids missing from the compiled chicken sequence in relation to the human protein sequence is indicated in Table 10. By combining all

data (published, full-length sequences and partial sequences), 14 out of 25 DEAD box proteins were found to be highly conserved (>90% identity) between human and chicken. Substantially lower levels of sequence conservation were observed for four DEAD box proteins: DDX4 (54% identical; 67% similar), DDX10 (57.4% identical; 71.9% similar over 526 aa), DDX24 (50.7% identical; 61.3% similar over 538 aa) and DDX28 (56.9% identical; 73.5% similar over 260 aa).

In summary, there are at least 25 DEAD box genes in the chicken genome, in comparison to at least 29 in the human genome. Failure to identify four DEAD box genes in chicken may reflect low transcript and/or EST abundance in the libraries that have been screened to date, rather than their absence in the chicken genome. The high level of sequence similarity observed for the majority of DEAD box proteins expressed in human and chicken suggest that the functions of these proteins in birds and mammals are strictly conserved. The less evolutionarily conserved DEAD box proteins may have functions that allow more sequence flexibility or, alternatively, may have adapted to fulfill roles specific to the individual species.

Tissue distribution of DEAD box transcripts in chicken

(Prepared by R. Godbout and S. Katyal)

D(Asp)-E(glu)-A(ala)-D(Asp) box genes encode putative RNA helicases that have been implicated in a wide variety of RNA metabolic processes. There are at least 29 DEAD box genes in the human genome. Using chicken expressed sequence tag (EST) databases, we have identified cDNAs corresponding to 25 of the 29 human DEAD box genes. Here, we carry out in silico analysis of ESTs in order to study the tissue expression patterns of these 25 chicken DEAD box genes.

DEAD box proteins are putative RNA helicases characterized by eight to nine conserved motifs including the D(asp)-E(glu)-A(ala)-D(asp) box motif (Linder et al., 1989; Tanner et al., 2003). These motifs have been shown to be involved in ATP binding/hydrolysis, RNA binding and RNA unwinding (Pause and Sonenberg, 1992; Pause et al., 1993; Caruthers and McKay, 2002). Related to the DEAD box proteins are the DEAH and the DExD/H family of proteins, all three of which are members of the superfamily 2 (SF2) of helicases (Silverman et al., 2003). DEAD box proteins have been found in a wide variety of organisms, from complex multicellular organisms to bacteria and viruses. DEAD box proteins have been implicated in virtually every aspect of RNA metabolism, including translation initiation, RNA splicing, RNA stability, RNA export and ribosome biogenesis (Abdelhaleem et al., 2003). While some DEAD box proteins are well characterized (e.g. translation initiation factor eIF-4A), the role of the majority of DEAD box proteins expressed in higher eukaryotes remains poorly defined.

Only a few chicken DEAD box proteins have been described in the literature, including DDX1, DDX4 (Vasa), DDX5 (p68) and DDX17 (p72) (Table 11). DDX1 is an 82-kDa protein primarily found in the nucleus which has been shown to be associated with the RNA processing factor CstF-64 (Bléoo et al., 2001). DDX1 is widely expressed in the developing chick, with highest levels in tissues of neural origin such as retina and brain (Godbout et al., 2002). DDX4, first shown to be expressed in *Drosophila* germ cells, plays a role in germline determination (Hay et al., 1988; Lasko and Ashburner, 1990). In chicken, DDX4 (CVH) is found in granulofilamentous structures in the cytoplasm of germ cells and is detected as early as the first cleavage of fertilized eggs (Tsunekawa et al., 2000). The expression pattern of DDX5 in chicken suggests a role in neural, and to a lesser extent, mesodermal, development (Seufert et al., 2000). Chicken DDX5 has been shown to be tightly associated with 5-methylcytosine (5MeC)-DNA glycosylase, involved in DNA demethylation (Jost et al., 1999). DDX17 is widely expressed in the developing chick embryo, with highest levels in brain and testis. Brain, muscle and liver maturation is accompanied by down-regulation of DDX17, suggesting a role in early development (Ip et al., 2000).

The last few years have seen a great expansion in the number of chicken ESTs available through various databases making it possible to carry out in silico analysis of tissue expression. We have used these EST databases to provide a more comprehensive view of the expression patterns of DEAD box genes in chicken. For the purpose of this study, DEAD box genes are defined as those encoding proteins that have the D-E-A-D motif as well as the additional seven or eight motifs (depending on how the motifs are defined) characteristic of this protein family. At the time of analysis, >50 human DDX (DEAD Box) genes *(DDX1* to *DDX56)* had been reported either in the literature or in databases, of which 31 had DEAD box motifs (Table 11). Two of these 31 genes have been excluded from this analysis: *DDX26 (DICE1)*, which has a DEAD box motif but is missing some of the other motifs characteristic of DEAD box proteins, and *DDX7*, originally classified as a human DEAD box gene but subsequently found to be of bacterial origin. The remaining *DDX* genes either have DEAH or DExD/H motifs or could not be linked to any database entry.

Chicken ESTs corresponding to the majority of the 29 DEAD box genes (25/29) were identified using the TBLASTN program, based on sequence similarity to the previously characterized human DEAD box proteins. The chicken ESTs were assembled into contiguous cDNA sequences using the BBSRC EST assembly program (www.chick.umist.ac.uk) (Boardman et al., 2002). The resulting cDNA sequences were then used to carry out BLASTN searches of the BBSRC ChickEST database (www.chick.umist.ac.uk) which contains 339,314 ESTs from 21 embryonic and adult tissues. Additional ESTs from normal tissue cDNA libraries were identified by BLASTN searches of the NCBI chicken EST database (www.ncbi.nlm.nih.gov). As results obtained from these searches are not quantitative, this approach was used to identify trends in the relative abundance and tissue-specific distribution of the various DDX transcripts.

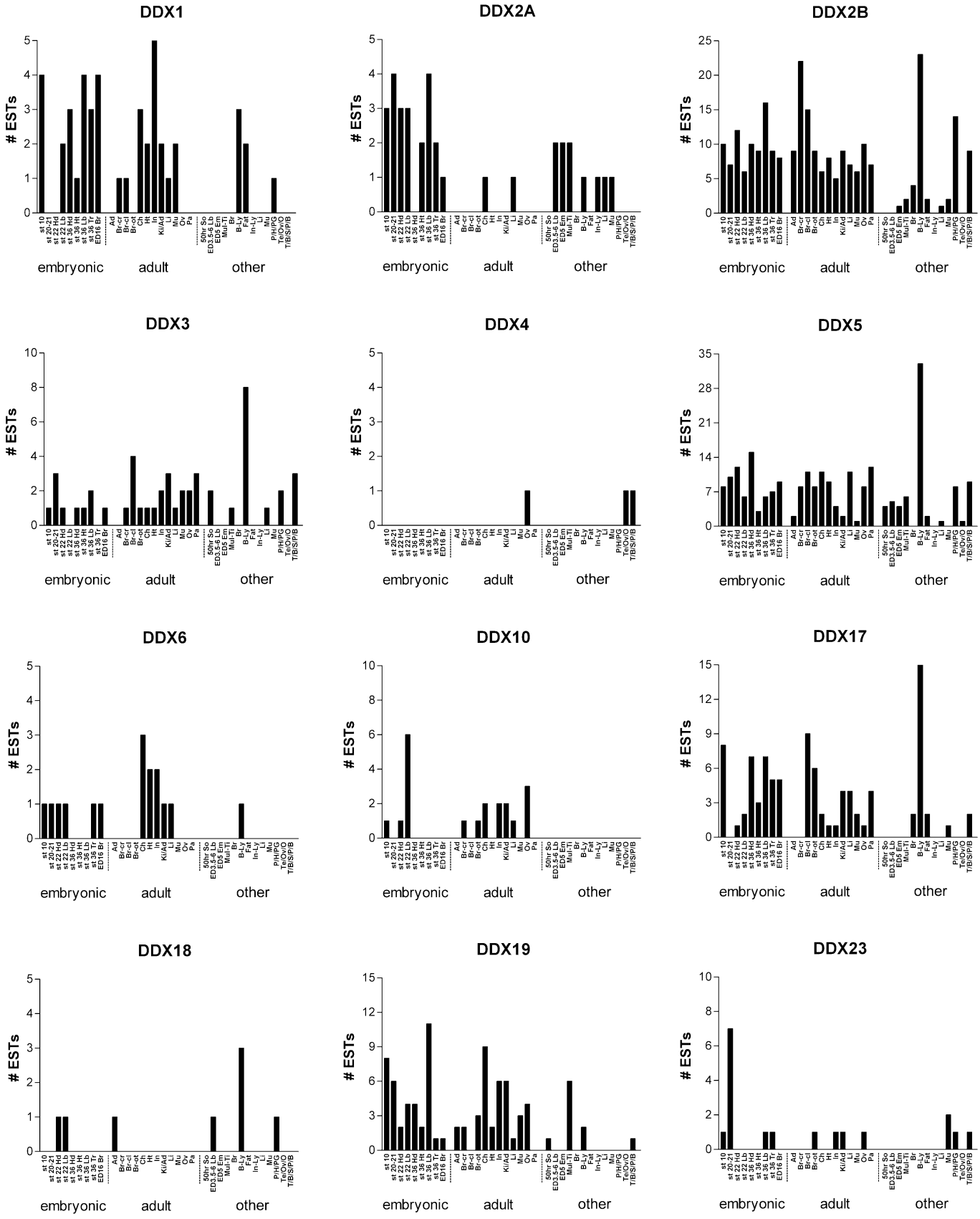
Results for 25 of the 29 DEAD box genes are shown in Fig. 34. No ESTs were identified for *DDX20*, *DDX42*, *DDX43* and *DDX56*, perhaps due to the absence of the corresponding genes in the chicken genome or because of a relatively low level of similarity between the human and chicken proteins. Alternatively, the corresponding transcripts may be in low abundance in chicken tissues and/or corresponding ESTs have not yet been

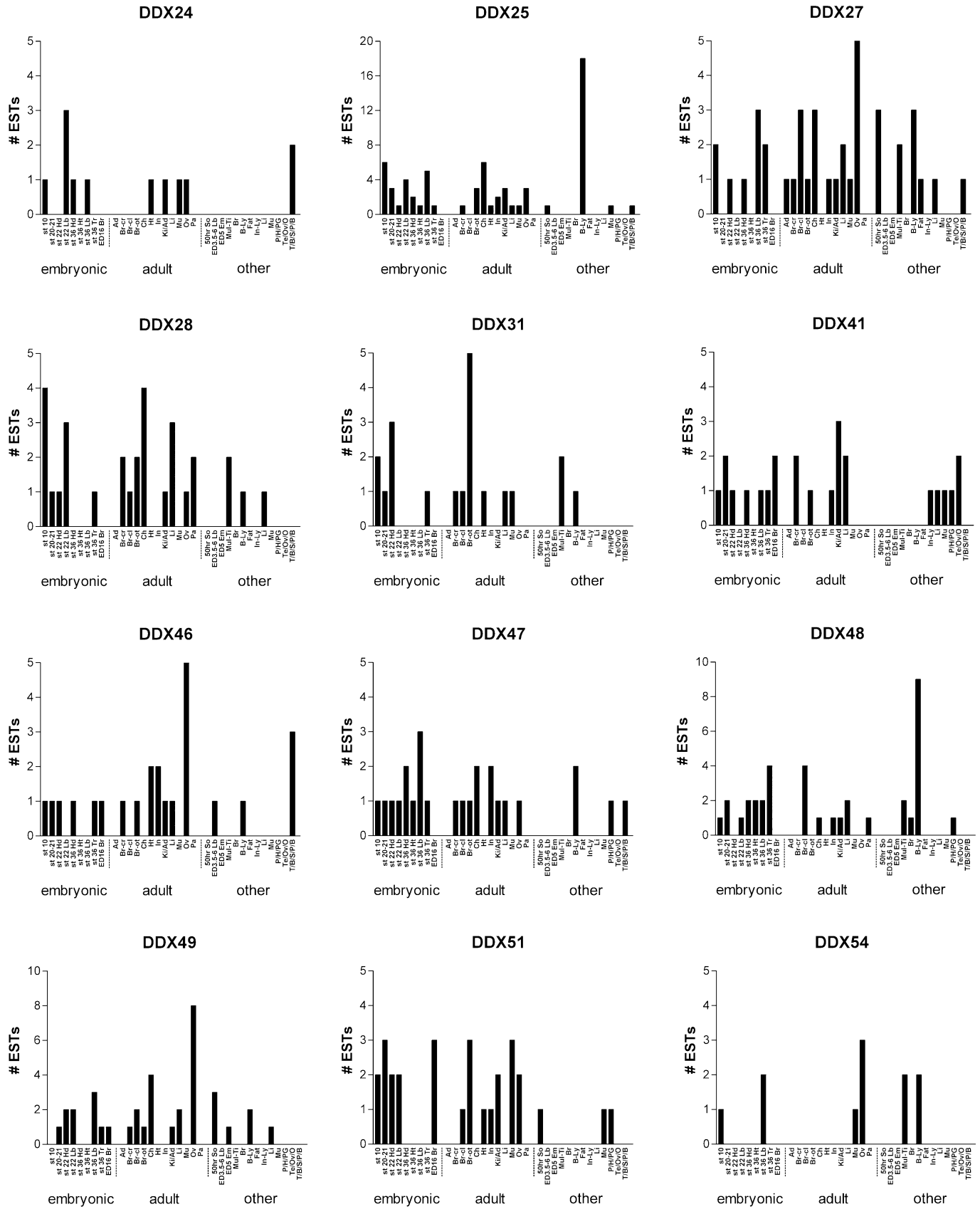
Table 11. List of DEAD, DEAH, DexD/H genes with DDX designation. DEAD box proteins are highlighted in gray.

Name	Other Names	Motif	cDNAs published	ESTs	Notes
DDX1	HuDBP-RB	DEAD	AY057383	yes	Godbout et al. (2002)
DDX2A	eIF-4AI	DEAD	no	yes	
DDX2B	eIF-4AII	DEAD	AF515726	yes	also called DDX14
DDX3	PL10, An3, DBX, DBY	DEAD	no	yes	
DDX4	VASA	DEAD	AB004836	yes	Tsunekawa et al. (2000)
DDX5	p68, HLR1	DEAD	AF158370	yes	Jost et al. (1999)
DDX6	p54, HLR2, RCK	DEAD	no	yes	
DDX7	NP-52	DEAD			bacterial DEAD box
DDX8	HRH1, PRP22	DEAH			
DDX9	RNA Helicase A, NDHII	DEIH			
DDX10		DEAD	no	yes	
DDX11	CLHR1, KRG2	DEAH			
DDX12	CHLR2	DEAH			
DDX13	SKI2W, HLH	DEVH			
DDX15	DBP1, PRP43, HRH2	DEAH			
DDX16	DBP2, PRP8	DEAH			
DDX17	p72	DEAD	no	yes	
DDX18	MrDb	DEAD	no	yes	
DDX19	DBP5	DEAD	no	yes	
DDX20	DP103, Gemin3	DEAD	no	no	
DDX21	RNA Helicase II/Gu	DEVD			
DDX22	-	-	-		not identified
DDX23	PRP28	DEAD	no	yes	
DDX24		DEAD	no	yes	
DDX25	GRTH	DEAD	no	yes	
DDX26	DICE1	DEAD			other motifs highly divergent
DDX27		DEAD	no	yes	
DDX28	MDDX28	DEAD	no	yes	mitochondrial (m) DDX28
DDX29		DEVH			
DDX30		DEVH			
DDX31		DEAD	no	yes	
DDX32		DCVD			
DDX33		DEAH			
DDX34		DEVH			
DDX35		DEAH			
DDX36		DEIH			
DDX37		DEAH			
DDX38	PRP16	DEAH			
DDX39		DECD			
DDX40		DEAH			
DDX41	abstrakt	DEAD		yes	
DDX42	SF3b125	DEAD		no	
DDX43		DEAD		no	
DDX44	-	-	-		not identified
DDX45	-	-	-		not identified
DDX46	PRP5, U5-200 kDa, HEL117	DEAD	no	yes	
DDX47		DEAD	no	yes	
DDX48	eIF-4A-like NUK-34	DEAD	no	yes	
DDX49		DEAD	no	yes	
DDX50	RH-II/Gubeta (GU2)	DEVD			
DDX51		DEAD	no	yes	
DDX52	ROK1	DESD			
DDX53	-	-	-		not identified
DDX54	APR-5, DP97	DEAD	no	yes	only described in mouse
DDX55		DEAD	no	yes	
DDX56	NOH61	DEAD	no	no	

isolated. Based on the number of hits obtained from the BLAST searches, the most abundant DEAD box transcripts are *DDX2B* (eIF-4AII) and *DDX5* (summarized in Table 12). Both transcripts appear to be widely expressed in embryonic and adult tissues. In contrast to *DDX2B*, *DDX2A* (eIF-4AI) ESTs were primarily found in the embryonic libraries. Interestingly, this pattern appears to be reversed in mouse, with eIF-4AI being ubiquitously expressed and eIF-4AII showing a more variable tissue distribution (Nielsen and Trachsel, 1988).

DDX4 and *DDX18* generated the least number of hits, with a total of three ESTs and eight ESTs, respectively. Two of the three *DDX4* ESTs were found in the ovary and in the testis/ovary/oviduct libraries. Other DDXs demonstrating significant developmental stage- or tissue-specific patterns include *DDX10* (6/20 ESTs from stage 22 limb), *DDX23* (7/18 ESTs from stage 20–21 embryo), *DDX24* (3/12 ESTs from stage 22 limb), *DDX31* (5/20 ESTs from brain, parts other than cerebrum and cerebellum), *DDX46* (5/24 ESTs from ovary), *DDX49* (8/36 ESTs from ovary) and *DDX54* (3/11 ESTs from ovary) (Table 12). Some DDXs appeared especially abundant in 2–3 week bursal lymphocytes, including *DDX3* (8/48 ESTs), *DDX5* (33/261), *DDX17* (15/94), *DDX25* (18/64) and *DDX48* (9/37).


DDX1
DDX2A
DDX2B
DDX3
DDX4
DDX5
DDX6
DDX10
DDX17
DDX18
DDX19
DDX23
ESTs
embryonic
adult
other



(For Fig. 34 continued on page 466.)

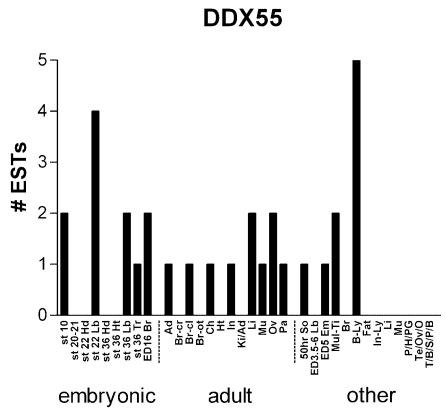


Fig. 34. Graphs of the number of DDX ESTs obtained for each of the tissues listed. Data for embryonic and adult tissues were obtained by BLAST searches of the BBSRC ChickEST database. Data for other tissues were obtained by BLAST searches of the NCBI chicken EST database. The nine BBSRC embryo/embryonic tissues are (with total number of ESTs obtained from the cDNA libraries): stage 10 whole embryo (~ 12,000 ESTs); stage 20–21 whole embryo (~ 26,000); stage 22 head (~ 18,000); stage 22 limb (~ 15,000); stage 36 head (~ 15,000); stage 36 heart (~ 8000); stage 36 limb (~ 19,000); stage 36 trunk (~ 13,000); embryonic day 16 brain (~ 16,000). The twelve BBSRC adult tissues are: adipose (~ 2,500); brain–cerebrum (~ 14,000); brain–cerebellum (~ 14,000); brain–other parts (~ 15,000); chondrocytes (~ 32,000); heart (~ 9,000); small intestine (~ 17,000); kidney and adrenal (~ 19,000); liver (~ 13,000); muscle (~ 9,000); ovary (~ 29,000); pancreas (~ 8,000). The thirteen NCBI libraries included in this analysis are: 50 hour somites associated with neural tube; embryonic day 3.5 to 6 limb bud; embryonic day 5 embryo; multiple tissues from embryonic to adult stages; brain–unknown stage; 2–3 weeks old bursal lymphocytes; fat; intestine-lymphocytes; liver; muscle–breast, leg, epiphyseal growth plate; pituitary/hypothalamus/pineal gland; testis/ovary/oviduct; thymus/bursa/spleen/peripheral blood lymphocytes/bone marrow (lymphoid). Abbreviations used are underlined.

Table 12. Number of ESTs in BBSRC embryonic libraries, BBSRC adult tissue libraries and other (NCBI) libraries

DEAD Box	# ESTs embryonic	# ESTs adult	# ESTs other	Comments
DDX1	21	17	6	
DDX2A	22	2	10	mainly embryonic
DDX2B	87	113	58	abundant, widely expressed
DDX3	10	21	17	
DDX4	0	1	2	reproductive organs - 2/3
DDX5	76	87	73	abundant, widely expressed
DDX6	6	9	1	
DDX10	8	12	0	st. 22 limb - 6/20
DDX17	38	34	22	bursal lymphocytes - 15/94
DDX18	2	1	5	bursal lymphocytes - 3/8
DDX19	39	38	10	
DDX23	10	4	4	st. 20-21 embryo - 7/18
DDX24	6	4	2	st. 22 limb - 3/12
DDX25	23	21	21	bursal lymphocytes - 18/64
DDX27	9	19	11	
DDX28	10	16	4	
DDX31	7	10	3	brain - 5/20
DDX41	9	9	6	
DDX46	6	13	5	ovary - 5/24
DDX47	11	10	4	
DDX48	14	10	13	bursal lymphocytes - 9/37
DDX49	10	19	7	ovary - 8/36
DDX51	12	13	3	
DDX54	3	4	4	ovary - 3/11
DDX55	11	10	9	

Although this preponderance of DDX ESTs in bursal lymphocytes may reflect to some extent the large number of ESTs isolated from this library (Abdrakhmanov et al., 2000), not all DDXs are well-represented in this library, suggesting enrichment of some DDXs in bursal lymphocytes compared to others.

Most tissues expressed many DEAD box genes (Fig. 34). For example, ESTs representing 13 different DDX genes were observed in embryonic day 16 brain. This number increased to 19 DDX genes in adult brain. In stage 36 heart, ESTs corresponding to 10/25 DDX transcripts were observed, with the number increasing to 12 in adult heart. There was concordance for 17/25 DDX genes in embryonic and adult heart, with seven genes represented in both tissues and ten genes represented in neither. The adult chondrocyte library had ESTs from 16 of the DDX genes, while the ovarian library had 18 different DDX ESTs. Even though only ~3,000 ESTs have been sequenced from the adult adipocyte library, ESTs representing six DDX genes were identified in this tissue: *DDX2B*, *DDX5*, *DDX18*, *DDX19*, *DDX27*, *DDX55*. Of note, the only *DDX18* EST found in the 13 BBSRC adult libraries was derived from adipocyte tissue.

From this analysis, one can conclude that: (i) very few DEAD box transcripts are tissue specific; in fact, with the possible exception of DDX4, it is not clear that any DEAD box transcripts are tissue specific, (ii) a number of DEAD box transcripts are enriched in specific tissues, (iii) the majority of DEAD box transcripts appear equally abundant in embryonic and adult tissues, (iv) most tissues express a variety of DEAD box transcripts. These observations are consistent with the multiple roles in RNA metabolism proposed for DEAD box proteins and would suggest that the need for an extended DEAD box protein family is not based so much on tissue-specific functions as on a requirement for these proteins to carry out a variety of functions in the cell. Except for their conserved motifs, most DEAD box proteins show little similarity to each other. Specificity of function for the different DEAD box proteins may be governed through specific protein-protein and/or protein-RNA interactions.

DT40 in the genomic age

(Prepared by J.-M. Buerstedde, H. Arakawa and R.B. Caldwell)

The chicken B cell line DT40 has been extensively used to study the function of genes by disruption due to its high homologous recombination activity. Topics of fruitful research include among others B cell-specific receptor signaling and immunoglobulin repertoire development as well as ubiquitous cellular processes like DNA repair and mRNA processing.

The entire chicken genome has recently been released with profound implications for researchers using the DT40 genetic

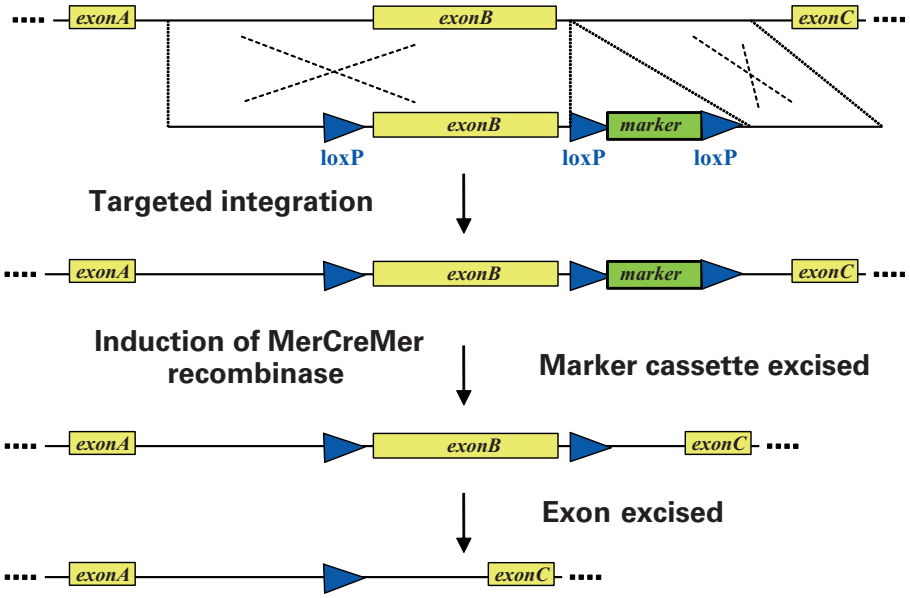


Fig. 35. Conditional gene knockouts using Cre-mediated exon deletion.

model. Here, we shall review how the genome sequence will enable better identification of genes and sequence motives, facilitate technical aspects of gene targeting and lead to advances in mutant phenotype analysis.

Identification of evolutionarily conserved genes and sequence motives

Studies in DT40 are generally undertaken to discover aspects of gene function which are conserved during vertebrate evolution and relevant for our understanding of mammalian biology (Kurosaki, 2002; Arakawa and Buerstedde, 2004; Hochegger et al., 2004; Shin et al., 2004). Due to the lack of forward mutant screens, almost all genes disrupted in DT40 have counterparts previously described for other organisms. In the early days, the chicken homologs had to be cloned by cross-hybridization or reverse PCR using degenerate PCR primers corresponding to conserved sequences. Later, with the release of EST databases from bursal B cells (Abdrakhmanov et al., 2000) and other chicken tissue (Boardman et al., 2002), partial cDNA sequences of knock-out candidate genes could often be retrieved by BLAST searches using mammalian cDNAs as query sequences. This approach suffered however from the disadvantage that the available ESTs did not cover the conserved coding regions of all genes. In the absence of a reliable chicken gene catalog, it was also difficult to ascertain that the discovered cDNA represented indeed the homolog of the mammalian gene and not a related paralog. The release of the chicken genome sequence (International Chicken Genome Sequencing Consortium, 2004) solves most of these problems, since it is now possible to search for the most closely related gene by genome BLAST (http://genome.ucsc.edu/cgi-bin/hgBlat). However, one needs to keep in mind that the current genome assembly contains gaps which by various estimates encompass about 5–10% of all transcripts, so that a homolog missed by genomic BLAST searches might still exist.

The chicken genome can now also be searched for conserved non-coding sequences with roles in gene expression, mRNA processing or translation. These sequences might be worthwhile targets for disruption and substitution analysis in DT40, if the associated gene is active in DT40 and its altered expression can be measured in mutant clones. Other intergenic sequences, which are often strongly conserved between the human, murine and chicken genomes, represent more challenging mutation targets, as it is difficult to predict the mutant phenotypes.

Design of targeting constructs

The genome sequence greatly facilitates the design and the construction of the targeting vectors. Conventional DT40 knock-out vectors require genomic fragments upstream and downstream of the dominant drug resistance marker for alignment and crossing-over at the target locus (Arakawa et al., 2001). In addition, at least partial knowledge of the exon-intron structure of the target gene locus is needed to predict the effect of gene targeting. These prerequisites often led to the unattractive task to clone and characterize an unknown chicken locus. One of the possible shortcuts was to amplify genomic DNA by long-range PCR using primers from the cDNA sequence (Bezzubova et al., 1997). In most cases a partial exon-intron structure and a crude restriction map of the locus could be deduced from the amplified fragments. Suitable parts were then amplified by appropriately modified primers and cloned into the target vector. To the delight of DT40 veterans, this somehow unpredictable procedure is now obsolete, because a BLAST search of a cDNA sequence against the genome reveals the precise exon-intron structure and a complete restriction map of the locus. Since isogenic vector target arms are expected to increase the targeting efficiency, one would simply amplify suitable fragments by PCR from genomic DNA of DT40 after confirming that the restriction sites relevant for the cloning do not differ between the wild-jungle fowl genome sequence and DT40.

The availability of sequences from entire loci not only eases conventional targeting vector design, but also encourages more precise and subtle mutagenesis. Whereas most DT40 knock-outs in the past have combined truncations and deletions of conserved coding regions, entire gene deletions are now possible, if the target locus is not larger than 10–20 kb. In addition, single amino acid substitutions or added in-frame coding regions can be incorporated into the vector target arms and then recombined into the locus. As the co-inserted drug resistance marker might interfere with gene expression, mutant loxP flanked marker should be used which can be removed by transient Cre recombinase expression (Arakawa et al., 2001). Such strategies should be considered, if physiological gene expression control is needed and the complementation of a gene knock-out by the expression of the cDNA is challenging.

The function of genes essential for cell survival has been traditionally studied in DT40 by gene disruption under the protection of conditional cDNA expression (Wang et al., 1996). With the precise exon-intron structure of the target genes at hand, an alternative approach is the conditional gene inactivation by Cre-mediated deletion of loxP-flanked exons (Kwan, 2002). After targeted integration of vectors designed for this purpose (Fig. 35), transfectants with crossover distal for an intragenic loxP site are identified. In the next step, the loxP resistance marker would be removed by brief Cre recombinase leaving a single loxP site which can recombine with the distal intragenic loxP site. Vectors are currently developed for use in DT40 with convenient restriction sites to insert genomic fragments at the right position with regard to the loxP site and the drug resistance marker (Arakawa et al., unpublished). Again this strategy is most advantageous, if physiologic transcription control of the knock-out target gene is required.

Gene expression profiling

Changes in the transcription of multiple genes accompany many of the processes studied in DT40, for example, B cell signaling or DNA damage responses. In addition, various groups have started to disrupt transcription factor genes involved in B cell differentiation and immunoglobulin gene diversification. To the resulting phenotypes, the transcription profiles of wild-type and mutant cells need to be analyzed. Micro-array analysis is able to measure the transcription levels of thousands of genes and first results using filter- or chip-based micro-arrays have been reported (Neiman et al., 2001; Koskela et al., 2003). Nevertheless, the cDNA inserts used for the micro-arrays were not completely sequenced thus complicating the assignment of signals to defined transcripts. Another problem is that the results of different laboratories are difficult to compare due to differences in micro-array production and hybridization conditions. Efforts are now underway to use the chicken gene catalog based on automatic gene predictions and the genomic mapping of ESTs and cDNAs to generate a normalized chicken unigene chip (Affymetrix Inc, Santa Clara, USA, personal communication) which should improve and standardize the analysis of transcription profiles of DT40 mutants.

Another way to investigate the transcription profile of a given cell sample is Serial Analysis of Gene Expression (SAGE) based on large scale sequencing of short cDNA tags derived from the bulk mRNA (Velculescu et al., 1995). Whereas tag mapping to cDNA and genome sequences indicates the type of expressed genes, the prevalence of individual tags within the library reflects their relative expression levels. SAGE analysis is most profitable, if a large percentage of tags can be mapped unambiguously to transcripts. This represented a problem for the first chicken SAGE analysis performed recently on bursal cells and DT40 (Wahl et al., 2004) as only one third of all tags with matches could be mapped to annotated transcripts. Nevertheless, another third of the tags mapped into the neighborhood of transcripts and these can now be used to improve the transcript annotations. Accumulation of additional data will further facilitate the interpretation of results and thus establish the technique as a useful tool for the analysis of DT40 mutants.

Full-length cDNA databases

Although gene finder programs can define evolutionarily conserved exons with reasonable accuracy, they often fail to correctly predict 5′ and 3′ transcript boundaries and poorly conserved exons. This was confirmed when the cDNA sequences of more than 2,000 new bursal genes were sequenced and compared with the chicken transcript build of the Ensembl team (Caldwell et al., 2005). If the bursal full-length cDNAs are combined with previously known chicken transcript sequences and cDNA recently sequenced from other tissues (Hubbard et al., 2005), this collection will encompass a large fraction of all transcripts expressed in DT40. The full-length cDNAs not only help to define the transcript borders, but also facilitate the complementation of mutant DT40 phenotypes by genetic complementation. It is a convenience that many of the cDNAs are available as fully sequenced plasmid inserts ready to be cloned into cDNA expression vectors.

Studies of protein interactions

Recent advances in protein tagging and the improved accuracy and sensitivity of partial peptide sequencing assist the analysis of protein complexes. Although some techniques still need to be optimized for DT40, there are clear benefits to combine the study of protein interactions with gene targeting. For example, it is possible to express a tagged protein either as a modified cDNA in a deletion background or by adding the tag to the genomic open reading frame. In addition, all modified proteins can be easily checked for their functionality by transfecting a disruption mutant and looking for complementation. The assignment of peptide sequences to proteins is helped by the bursal cDNA collection (http://pheasant.gsf.de/DEPARTMENT/DT40/dt40Transcript.html) and the Ensembl chicken gene catalog (International Chicken Genome Sequencing Consortium, 2004).

Turning RNAi into an advantage

RNAi now allows studying the function of many genes in mammalian cell culture models by gene knock-down and thus has created alternatives to knock-out experiments in DT40. However, the possibility to easily modify the genome of DT40 remains a distinct advantage enabling clean knock-outs of genes and regulatory sequences, genetic complementation by

mutants and multiple gene disruptions. Still, RNAi has the potential to significantly enhance the DT40 genetic model. Libraries of RNAi vectors corresponding to all genes expressed in DT40 should allow genome-wide screening for recessive mutants similar to what was recently reported for mammalian cell lines (Berns et al., 2004; Paddison et al., 2004). As transfection by electroporation is difficult to scale up and to adapt to microtiter plate format, replication-incompetent derivatives of RCAS vectors (http://home.ncifcrf.gov/hivdrp/RCAS/plasmid.html) appear like a reasonable choice to express the RNAi sequences. Any results obtained by RNAi could subsequently be confirmed by gene disruption. In addition, by using a mutant DT40 clone as a recipient of the RNAi library it may be possible that suppressor (van Haaften et al., 2004) or synthetic lethal mutants could be identified.

We would like to thank all members of the lab for helpful discussions.

An overview of avian evolution

(Prepared by S.B. Hedges)

Present and past diversity

The living birds form a large and diverse group of vertebrates, with nearly 10,000 species in approximately 2,000 genera, 200 families and 29 orders (Sibley and Monroe, 1990; IUCN, 2004). New species are discovered each year, but at a low rate indicating that our knowledge of avian diversity is quite good. The major uncertainty in the total number of species concerns disagreement over the definition of particular species, such as the recognition of subspecies as full species, rather than discovery of new species. In contrast, other groups of tetrapods (e.g., mammals, lizards, snakes, amphibians) have fewer species and a higher rate of species discovery (Uetz, 2004).

The fossil record of birds is relatively poor, probably because the avian skeleton is fragile (hollow bones), and most species are small and occur in environments (humid forests) where decomposition is rapid. However, the early history of birds has become much better known in the last decade with the discovery of exceptionally preserved fossils of birds and dinosaurs from the Cretaceous (142–65 million years ago, Mya), especially in China (Chiappe and Dyke, 2002). The earliest bird is *Archaeopteryx* from the late Jurassic (~ 150 Mya) of Germany, and the consensus view is that birds evolved from carnivorous (theropod) dinosaurs called coelurosaurs (e.g., *Tyrannosaurus rex*), and specifically from a group of relatively small and agile species called dromaeosaurids (e.g., *Velociraptor*). This was suggested in the 19th century when *Archaeopteryx* was first classified as a small dinosaur, before the impressions of feathers were noted, and has since been supported by detailed comparisons of anatomy and (recently) egg morphology (Varricchio et al., 1997). To recognize this derivation from dinosaurs, paleontologists sometimes refer to birds as "living dinosaurs" and refer to classical dinosaurs (e.g., *Stegosaurus*) as "non-avian dinosaurs". The carnivorous (theropod) ancestry of birds is also illustrated by the fact that *Archaeopteryx* and many of the other Mesozoic (251–65 Mya) species possessed sharp teeth and raptorial claws.

Discoveries of feathered dinosaurs in the last decade have also bolstered the link between birds and dinosaurs. In the earliest-branching species of coelurosaurs, the feathers were filamentous and probably functioned more like the down of some living birds, providing insulation (Chiappe and Dyke, 2002). However, the maniraptoran coelurosaurs (including dromaeosaurids), which were the closest relatives of birds, possessed vaned feathers that more closely resembled avian flight feathers. There is no evidence that these dinosaurs could undergo the sustained flapping flight of modern birds, but extensive development of feathers and their configuration indicate that at least some of these dinosaurs were gliders (Xu et al., 2003). These fossils have helped to blur the distinction between dinosaurs and birds. Nonetheless, the full suite of adaptations for sustained, flapping flight, such as asymmetric feathers, alulas (wing structures for improving the airfoil function), and other aerodynamic structures, are found only in birds. Even *Archaeopteryx* probably was able to initiate flight directly from the ground (Chiappe and Dyke, 2002).

Debate continues as to how the Cretaceous birds are classified. One arrangement divides them into two large groups (Sauriurae and Ornithurae) whereas another places them in a ladder-like tree of lineages leading to modern birds (Chiappe and Dyke, 2002). In either case, the closest relative of modern birds is believed to be *Ichthyornis*, a small, toothed, tern-like marine bird of the late Cretaceous. Some Cretaceous fossils have been postulated to be representatives of modern orders such as Galliformes (e.g., fowl), Anseriformes (e.g., ducks), Psittaciformes (e.g., parrots), Charadriiformes (e.g., plovers), Procellariformes (e.g., petrels, albatrosses), Gaviiformes (e.g., loons), Gruiformes (e.g., cranes), and Pelecaniformes (e.g., pelicans). However, all of these fossils are considered to be problematic in some way (Chiappe and Dyke, 2002). With those aside, the fossil record of birds shows a major dichotomy at the Cretaceous-Tertiary boundary (65 Mya), when a large asteroid collided with Earth causing the extinction of the dinosaurs and other life. Almost no unambiguous fossils of modern (neornithine) birds are known before that event and no fossils of non-neornithine birds are known after that event. One important exception is *Presbyornis*, an aquatic neornithine bird apparently related to ducks (Anseriformes) which is known from the late Cretaceous and the early Tertiary (Kurochkin et al., 2002). Fossils of most orders of modern birds appear in the early part of the Cenozoic (65–0 Mya).

Phylogenetic relationships

In general, the phylogeny of modern birds is poorly known despite decades of attention from morphologists and molecular phylogeneticists. Although molecular studies (Sibley and Ahlquist, 1990) have identified some obvious cases of convergence in previous morphological classifications and have helped to better organize the higher-level classification of birds, it is unclear why those studies and the numerous DNA sequence studies collected in recent years have yet to resolve the avian tree, even at the ordinal level (Cracraft et al., 2004). Probably the best explanation is that the large number of taxa (e.g., species, genera, and families) requires even more characters than have been applied thus far (i.e., it is data limited). An addition-

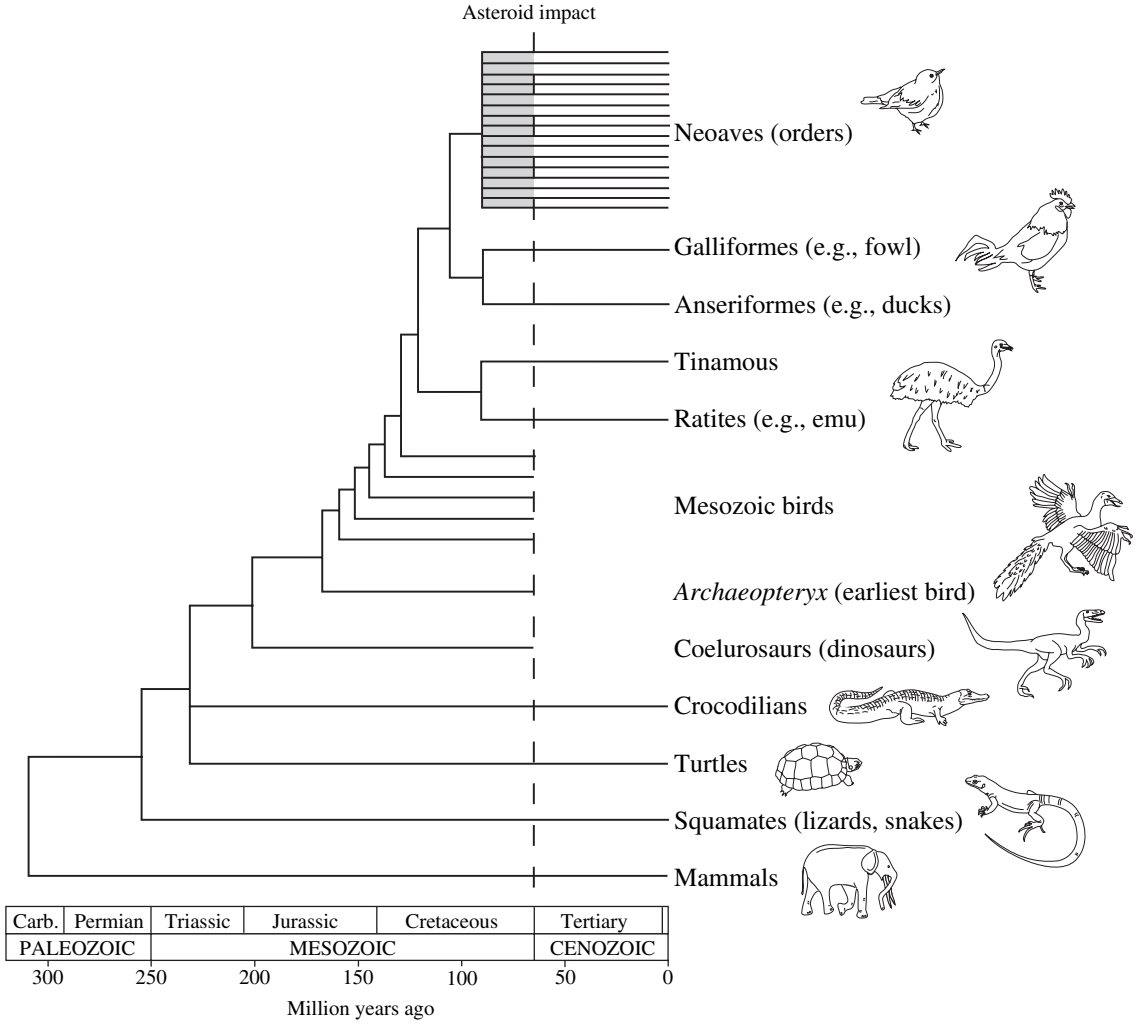


Fig. 36. Relationships and divergence times of birds and other amniote vertebrates (see text). Shaded area in Neoaves represents a time when most ordinal and superordinal lineages diverged based on molecular clocks. The lineages of Mesozoic birds and *Archaeopteryx* are shown ending arbitrarily at the Cretaceous/Tertiary boundary, although fossil data are sparse and some lineages may have disappeared earlier.

al compounding problem is that the tree of avian orders has unusually short internodes, suggesting that there was a relatively rapid radiation which will require even more characters to resolve (Cracraft et al., 2004).

While a detailed tree of birds remains a future task, some of the higher level clades are now firmly established (Fig. 36). For example, there is wide agreement that modern birds (Neornithes) form three major clades: Paleognathae (tinamous and ratites), Galloanserae (e.g., ducks, fowl), and Neoaves (all other birds). In addition, DNA sequence analyses, primarily of nuclear genes, indicate that Galloanserae and Neoaves are closest relatives (Groth and Barrowclough, 1999; van Tuinen et al., 2000). Although earlier DNA hybridization studies had correctly identified the three groups (Sibley and Ahlquist, 1990), those data were ambiguous regarding their relationships. Initially, even data from complete mitochondrial genomes (~ 16 kb) provided a conflicting signal, indicating that Passeriformes was the most basal clade of modern birds and that the paleognaths and galloanserines were close relatives (Harlid and Arnason, 1999; Mindell et al., 1999). Later mitochondrial analyses with additional taxa found support for the paleognath-basal tree (Paton et al., 2002; Garcia-Moreno et al., 2003).

The living ratites (flightless paleognaths) include the ostrich of Africa, rheas of South America, the emu of Australia, the cassowaries of Australia and New Guinea, and the kiwis of New Zealand. Extinct species occurred on Madagascar (elephant bird), New Zealand (moas), and elsewhere. The phylogeny of these birds has garnered considerable interest, in part because of their primarily southern distribution and the expectation that the breakup of Gondwana influenced their biogeographic history. Although the moas are now extinct, complete mitochondrial genome sequences have been obtained from fossil remains, and phylogenetic trees have been constructed with large data sets of DNA sequences. Nonetheless, the relation-

ships have proven to be difficult to resolve. Three of the Australasian species (kiwi, emu, and cassowary) form a group in most analyses, but the moa usually branches basally when present in the tree (Lee et al., 1997; van Tuinen et al., 1998; Cooper et al., 2001; Haddrath and Baker, 2001; Paton et al., 2002). In contrast morphological trees place the kiwi as a basal lineage, separate from the other Australasian species (Lee et al., 1997). Firm biogeographic conclusions must await additional data and analyses.

The Galloanserae includes two orders of primarily robust-bodied birds. The Galliformes consists of mostly non-aquatic, ground-dwelling birds such as game fowl, megapodes, guans, and chachalacas. The chicken *(Gallus gallus*; common name, Domestic Fowl) belongs to this order. Its wild counterpart (also *Gallus gallus)* goes by the common name Red Jungle Fowl and is native to the lowlands (< 2,000 m) of southern Asia and the Malay archipelago (Sibley and Monroe, 1990). It is placed in the family Phasianidae along with other widely domesticated species such as the common quail *(Coturnix coturnix)*, turkey *(Meleagris gallopavo)*, and pheasant *(Phasianus colchicus)*. Other members of the order Galliformes include the New World quails (Odontophoridae) and guinea fowl (Numididae). The other order within Galloanserae, the Anseriformes (ducks, geese, swans, screamers), is primarily aquatic and comprises about 450 species in ~125 genera (Sibley and Monroe, 1990).

Neoaves is the third major clade of living birds and accounts for 95% of the species. Although some of the 20 or so neoavian orders are well defined, others are not (Cracraft et al., 2004). For example, the diving birds, wading birds, and marine birds are placed by most morphologists into about 8–9 different orders, including Charadriiformes (e.g., gulls, plovers), Ciconiiformes (e.g., storks), Gaviiformes (loons), Gruiformes (e.g., cranes), Pelecaniformes (e.g., pelicans, boobies, tropicbirds), Phoenicopteriformes (flamingos), Podicipediformes (e.g., grebes), Procellariiformes (albatrosses, shearwaters), and Sphenisciformes (penguins). Most of these orders are recognized today in avian field guides and other reference sources. However, DNA hybridization data (Sibley and Ahlquist, 1990) and DNA sequences (Hedges and Sibley, 1994; van Tuinen et al., 2001; Cracraft et al., 2004) have revealed relationships that place into question the recognition of those orders. For example, the pelicans are most closely related to the shoebill stork and hammerkop, and not to the boobies or tropicbird, and the grebes are most closely related to the flamingos, not to the loons (Hedges and Sibley, 1994; van Tuinen et al., 2001; Cracraft et al., 2004). Those surprising findings have been obtained with multiple genes, in different laboratories, and they have strong statistical support. Other controversial findings among the water birds, such as a clustering of New World vultures with storks (Sibley and Ahlquist, 1990), have not yet been corroborated.

Besides the water birds, progress has been made with DNA sequence analyses in understanding relationships within other neoavian groups, including the Passeriformes (Johansson et al., 2001, 2002; Barker et al., 2002; Yuri and Mindell, 2002; Cracraft et al., 2004). One particularly problematic bird has been the hoatzin *(Opisthocomus hoazin)* of South America, sometimes placed in its own order (Opisthocomiformes). It is the only bird that uses microbial foregut fermentation to convert cellulose into simple sugars, as in some mammals (e.g., ruminants). It feeds on young leaves and twigs of marsh plants and has a large muscular crop for fermentation. Most phylogenetic analyses have placed the hoatzin in the Galloanserae, but DNA hybridization studies and DNA sequence studies have agreed that it is a neoavian species (Sibley and Ahlquist, 1990; Hedges et al., 1995; Hughes and Baker, 1999; Sorenson et al., 2003). However, its particular relationship within Neoaves has not yet been resolved. In fact, the relationships of nearly all neoavian orders remain essentially unresolved and are best viewed at present as a "comb" (Cracraft et al., 2004) (Fig. 36).

Molecular clocks and biogeography

A literal reading of the fossil record of birds is that the modern orders evolved and radiated in the early Cenozoic, after the Cretaceous-Tertiary extinctions (Feduccia, 1995, 2003). Even the problematic Cretaceous fossils of Neornithine birds noted above are mostly from the final stage of the Cretaceous (Maastrichian; 71–65 Mya). However, molecular clocks have instead indicated that the orders of modern birds branched relatively deeply in the Cretaceous (120–75 Mya) (Hedges et al., 1996; Cooper and Penny, 1997; Kumar and Hedges, 1998; Haddrath and Baker, 2001; van Tuinen and Hedges, 2001; Paton et al., 2002). If these molecular clocks are correct, why is there such a large gap in the avian fossil record? It is possible that some error in time estimates can be explained by rate variation on lineages and calibrations made with problematic avian fossils. However, local clock methods (e.g., Bayesian) have been used in addition to global clocks, and some studies (Hedges et al., 1996; Kumar and Hedges, 1998; van Tuinen and Hedges, 2001) have calibrated from outside of the avian fossil record to avoid potential calibration biases. Despite the use of these different methods, the resulting time estimates have been fairly consistent in supporting relatively deep divergences among modern birds (Fig. 36).

The divergence of the chicken (Galliformes) and duck (Anseriformes) has proven to be an important anchor point for avian molecular clocks. Anseriformes is about the only order of modern birds that can be confidently placed in the Cretaceous, with the earliest fossils from the Campanian (84–71 Mya) (Chiappe and Dyke, 2002). Calibrating outside of birds and using 12 constant-rate nuclear genes, this divergence was estimated to be 90 ± 7 Mya (van Tuinen and Hedges, 2001). A similar date of 85 ± 17 Mya was obtained with different data (mitochondrial DNA), calibrations (internal avian calibrations), and clock methods ("rate smoothing" local clock) (Haddrath and Baker, 2001). The advantage of establishing an anchor point within birds is to provide a robust calibration for obtaining other time estimates among birds (van Tuinen and Hedges, 2004). Using that chicken-duck calibration point, several data sets were analyzed (mitochondrial DNA, DNA-DNA hybridization, and transferrin immunological data), resulting in the following mean divergence times for major nodes: 119 ± 5 Mya (paleognath-neognath), 104 ± 3 (Galloanserae-Neoaves), and 89 ± 9.6 Mya (base of neoavian radiation). Dates among paleognaths ranged from 50 Mya (kiwi-emu) to 83 (tinamous-ratites), between orders 76–80 Mya, and within

orders 39 Mya (chicken-quail) to 80 Mya (kingfisher-hornbill). However, sequence data were limited and most neoavian orders were not represented.

In another study (Paton et al., 2002), divergence time estimates were obtained using mitochondrial sequence data, local clock methods, and calibrations of 85 Mya (chicken-duck) and 35 Mya (emu-cassowary). The resulting times were similar to the previous study in finding deep splits (Cretaceous) among neoavian orders and a deep divergence between paleognaths and neognaths (123 Mya; 156–108 Mya). However, their dates for divergences among paleognaths were slightly older: 105 Mya (tinamous versus ratites), 89 Mya (rhea versus ostrich), and 81 Mya (kiwi versus emu). As more sequences are obtained, especially from nuclear genes, time estimates from molecular clocks should become more stable until the point where most or all error is from the fossil record and calibration points (Hedges and Kumar, 2003, 2004).

With this current timescale and phylogeny of avian evolution (Fig. 36), and recognizing its limitations, it is possible to draw some general inferences concerning the niche and morphology (habitus) of birds during their early history. For example, the heavy-bodied habitus and primarily ground dwelling, non-marine habits of the Galloanserae and Paleognathae, and their dinosaurian ancestors, suggest that these features were common among the stem neornithine birds of the Cretaceous (van Tuinen et al., 2000). Also, considering that shallow, marine environments are favorable for fossilization, this (the predominance of non-marine species) may in part explain the sparseness of the early fossil record of modern birds. Nonetheless, fossils indicate that Cretaceous birds also occupied other niches (e.g., perching, diving, etc.).

The early (Cretaceous) branching of ordinal lineages of modern birds found with molecular clocks "is compatible with the extensive speciation that occurred within orders following the sudden availability of niches in the early Tertiary period" (Hedges et al., 1996). Those niches were vacated by dinosaurs, pterosaurs, and other vertebrate groups that became extinct following the Cretaceous-Tertiary asteroid impact. The near simultaneous appearance of so many major lineages of neoavian birds, adapted to different environments, in the early Tertiary supports the niche-filling hypothesis.

One explanation for the early branching of ordinal and superordinal lineages of birds is that they reflect the splitting of landmasses in the Cretaceous, the Continental Breakup hypothesis (Hedges et al., 1996). The same hypothesis was proposed for the orders of placental mammals (Hedges et al., 1996), based on molecular clock evidence, and subsequent phylogenetic analyses have provided support (Springer et al., 1997; Stanhope et al., 1998). In birds, it has proven more difficult to associate particular orders with geographic regions (e.g., continents) because of their wider distributions and ease of dispersal. As noted above, ratites have been singled out as having an evolutionary history associated with continental breakup (Cracraft, 1973, 2001; van Tuinen et al., 1998), but biogeographic details will remain sketchy until their relationships are better resolved. A good case has been made that modern (neornithine) birds in general are Gondwana in origin, based on a review of fossils, phylogeny, and molecular divergence times (Cracraft, 2001). However, the extent that plate tectonics and the divergence of continents have had on avian evolution remains unanswered.

Completion of the chicken genome will be a major benefit for evolutionary studies. For example, orthology determination, which is necessary for assembling phylogenetic data sets of nuclear genes, will be greatly facilitated by having an avian genome for comparison. At a higher level, genome comparisons will now be able to include a representative bird (reptile) and therefore help fill the current genomic gap between mammals and fishes. Finally, detailed studies within birds, involving molecular clocks and phylogenies, will have the benefit of an avian genome for primer design, clock calibration, and better genetic comparisons in general.

I thank Jennifer Hines for drawings of animals.

References

Abdelhaleem M, Maltais L, Wain H: The human DDX and DHX gene families of putative RNA helicases. Genomics 81:618–622 (2003).

Abdrakhmanov I, Lodygin D, Geroth P, Arakawa H, Law A, Plachy J, Korn B, Buerstedde JM: A large database of chicken bursal ESTs as a resource for the analysis of vertebrate gene function. Genome Res 10:2062–2069 (2000).

Aerts J, Crooijmans RPMA, Cornelissen S, Hemmatian K, Veenendaal T, Jaadar A, et al: Integration of chicken genomic resources to enable whole-genome sequencing. Cytogenet Genome Res 102: 297–303 (2003).

Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, Schanen C, Palotie A, Arnold AP: Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. Proc Natl Acad Sci USA 100: 4873–4878 (2003).

Akey JM, Zhang K, Xiong M, Jin L: The effect of single nucleotide polymorphism identification strategies on estimates of linkage disequilibrium. Mol Biol Evol 20:232–342 (2003).

Almenar-Queralt A, Lee A, Conley CC, Ribas de Pouplana Ll, Fowler VM: Identification of a novel tropomodulin isoform, Skeletal-tropomodulin, that caps actin filament pointed ends in fast skeletal muscle. J Biol Chem 274:28466–28475 (1999).

Arakawa H, Buerstedde JM: Immunoglobulin gene conversion: insights from bursal B cells and the DT40 cell line. Dev Dyn 229:458–464 (2004).

Arakawa H, Lodygin D, Buerstedde JM: Mutant loxP vectors for selectable marker recycle and conditional knock-outs. BMC Biotechnol 1:7 (2001).

Arit D, Bensch S, Hansson B, Hasselquist D, Westerdahl H: Observation of a ZZW female in a natural population: implications for avian sex determination. Proc R Soc Lond B Biol Sci 271:S249–251 (2004).

Auer H, Mayr B, Lambrou M, Schleger W: An extended chicken karyotype, including the NOR chromosome. Cytogenet Cell Genet 45:218–221 (1987).

Avilion AA, Piatyszek MA, Gupta J, Shay JW, Bacchetti S, Greider CW: Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res 56:645–650 (1996).

Babcock GG, Fowler VM: Isoform specific interaction of tropomodulin with skeletal muscle and erythrocyte tropomyosins. J Biol Chem 269:27510–27518 (1994).

Ban N, Nissen P, Hansen J, Moore P, Steitz T: The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science 289:905–919 (2000).

Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, et al: The syntenic relationship of the zebrafish and human genomes. Genome Res 10:1351–1358 (2000).

Barker FK, Barrowclough GF, Groth JG: A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proc R Soc Lond B Bio 269:295–308 (2002).

Baverstock PR, Adams M, Polkinghorne RW, Gelder M: A sex-linked enzyme in birds – Z-chromosome conservation but no dosage compensation. Nature 296:763–766 (1982).

Beaumont C, Protais J, Pitel F, Leveque G, Malo D, Lantier F, et al: Effect of two candidate genes on the *Salmonella* carrier state in fowl. Poult Sci 82:721–726 (2003).
Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Heimerikx M, et al: A large-scale RNAi screen in human cells identifies new components of the p53 pathway. Nature 428:431–437 (2004).
Bezzubova O, Silbergleit A, Yamaguchi-Iwai Y, Takeda S, Buerstedde JM: Reduced X-ray resistance and homologous recombination frequencies in a RAD54 –/– mutant of the chicken DT40 cell line. Cell 89:185–193 (1997).
Bieganowski P, Garrison PN, Hodawadekar SC, Faye G, Barnes LD, Brenner C: Adenosine monophosphoramidase activity of Hint and Hnt1 supports function of Kin28, Ccl1, and Tfb3. J Biol Chem 277:10852–10860 (2002).
Bléoo S, Sun X, Hendzel MJ, Rowe JM, Packer M, Godbout R: Association of human DDX1 with a cleavage stimulation factor involved in 3′-end processing of pre-mRNA. Mol Biol Cell 12:2046–3059 (2001).
Bloom SE, Bacon LD: Linkage of the major histocompatibility (B) complex and the nucleolar organizer in the chicken: assignment to a microchromosome. J Hered 76:146–154 (1985).
Boardman PE, Sanz-Ezquerro J, Overton IM, Burt DW, Bosch E, Fong WT, et al: A comprehensive collection of chicken cDNAs. Curr Biol 12:1965–1969 (2002).
Boehnke MK, Lange K, Cox DR: Statistical methods for multipoint radiation hybrid mapping. Am J Hum Genet 49:1174–1188 (1991).
Bonnen PE, Wang PJ, Kimmel M, Chakraborty R, Nelson DL: Haplotype and linkage disequilibrium architecture for human cancer-associated genes. Genome Res 12:1846–1853 (2002).
Botstein D, Risch N: Discovering genotypes underlying human phenotypes: past successes for Mendelian disease, future approaches for complex disease. Nat Genet 33:228–237 (2003).
Boyd Y, Goodchild M, Morroll S, Bumstead N: Mapping of the chicken and mouse genes for toll-like receptor 2 (TLR2) to an evolutionarily conserved chromosomal segment. Immunogenetics 52:294–298 (2001).
Brenner C: Hint, Fhit, and GalT: function, structure, evolution, and mechanism of three branches of the histidine triad superfamily of nucleotide hydrolases and transferases. Biochemistry 41:9003–9014 (2002).
Brenner C, Garrison P, Gilmour J, Peisach D, Ringe D, Petsko GA, Lowenstein JM: Crystal structures of HINT demonstrate that histidine triad proteins are GalT-related nucleotide-binding proteins. Nat Struct Biol 4:231–238 (1997).
Bromberg-White JL, Webb CP, Patacsil VS, Miranti CK, Williams BO, Holmen SL: Delivery of short hairpin RNA sequences by using a replication-competent avian retroviral vector. J Virol 78: 4914–4916 (2004).
Brown DD, Gurdon JB: Absence of ribosomal RNA synthesis in the anucleolate mutant of *Xenopus laevis*. Proc Natl Acad Sci USA 51:139–146 (1964).
Buerstedde JM, Arakawa H, Watahiki A, Carninci PP, Hayashizaki YY, Korn B, Plachy J: The DT40 web site: sampling and connecting the genes of a B cell line. Nucleic Acids Res 30:230–231 (2002).
Buitenhuis AJ, Crooijmans RPMA, Bruijnesteijn van Coppenraet ES, Veenendaal A, Groenen MAM, van der Poel JJ: Improvement of the comparative map of chicken chromosome 13. Anim Genet 33:249–254 (2002).
Bumstead N, Palyga J: A preliminary linkage map of the chicken genome. Genomics 13:690–697 (1992).
Burt D: Origin and evolution of avian microchromosomes. Cytogenet Genome Res 96:97–112 (2002).
Burt DW: The chicken genome and the developmental biologist. Mech Dev 121:1129–1135 (2004a).
Burt DW: Chicken genomics charts a path to the genome sequence. Brief Funct Genomic Proteomic 3:60–67 (2004b).
Caldwell R, Kierzek A, Arakawa H, Bezzubov Y, Zaim J, Fiedler P, et al: Full-length cDNAs from chicken bursal lymphocytes to facilitate gene function analysis. Genome Biol 6:R6 (2005).
Callan HG: Lampbrush Chromosomes (Springer-Verlag, Berlin 1986).
Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, et al: Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 22:231–238 (1999).
Carre W, Diot C, Fillon V, Crooijmans RP, Lagarrigue S, Morisson M, et al: Development of 112 unique expressed sequence tags from chicken liver using an arbitrarily primed reverse transcriptase-polymerase chain reaction and single strand conformation gel purification method. Anim Genet 32:289–297 (2001).
Caruthers JM, McKay DB: Helicase structure and mechanism. Curr Opin Struct Biol 12:123–133 (2002).
Chelysheva LA, Solovei IV, Rodionov AV, Yakovlev AF, Gaginskaya ER: Lampbrush chromosomes of the chicken: the cytological map of the macrobivalents. Cytology (Russ.) 32:303–316 (1990).
Chen J-L, Blasco MA, Greider CW: Secondary structure of vertebrate telomerase RNA. Cell 100:503–514 (2000).
Chen L, Couchman JR, Smith J, Woods A: Molecular characterization of chicken syndecan-2 proteoglycan. Biochem J 366:481–490 (2002).
Chiappe LM, Dyke GJ: The Mesozoic radiation of birds. Annu Rev Ecol Syst 33:91–124 (2002).
Chu X, Thompson D, Yee LJ, Sung LA: Genomic organization of mouse and human erythrocyte tropomodulin genes encoding the pointed end capping protein for the actin filaments. Gene 256:271–281 (2000).
Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, et al: Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA 99:13675–13680 (2002).
Collignon J, Sockanathan S, Hacker A, Cohen-Tannoudji M, Norris D, Rastan S, et al: A comparison of the properties of *Sox-3* with *Sry* and two related genes, *Sox-1* and *Sox-2*. Development 122:509–520 (1996).
Collins FS, Guyer MS, Chakravarti A: Variations on a theme: cataloging human DNA sequence variation. Science 278:1580–1581 (1997).
Colombo P, Yon J, Garson K, Fried M: Conservation of the organization of five tightly clustered genes over 600 million years of divergent evolution. Proc Natl Acad Sci USA 89:6358–6362 (1992).
Conley CA: Leiomodin and tropomodulin in smooth muscle. Am J Physiol Cell Physiol 280: C1645–C1656 (2001).
Conley CA, Fritz-Six KL, Almenar-Queralt A, Fowler VM: Leiomodins: Larger members of the tropomodulin (Tmod) gene family. Genomics 73:127–139 (2001).
Constancia M, Pickard B, Kelsey G, Reik W: Imprinting mechanisms. Genome Res 8:881–900 (1998).
Cooper A, Penny D: Mass survival of birds across the Cretaceous-Tertiary boundary: molecular evidence. Science 275:1109–1113 (1997).
Cooper A, Lalueza-Fox C, Anderson S, Rambaut A, Austin J, Ward R: Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. Nature 409:704–707 (2001).
Coullin P, Crooijmans RP, Groenen MA, Heilig R, Mollicone R, Oriol R, Candelier JJ: Assignment of *FUT8* to chicken chromosome band 5q1.4 and to human chromosome 14q23.2→q24.1 by in situ hybridization. Conserved and compared synteny between human and chicken. Cytogenet Genome Res 97:234–238 (2002).
Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S: Telomere shortening associated with chromosome stability is arrested in immortal cells which express telomerase activity. EMBO J 11:1921–1929 (1992).
Cox PR, Zoghbi HY: Sequencing, expression analysis, and mapping of three unique human tropomodulin genes and their mouse orthologs. Genomics 63:97–107 (2000).
Cox PR, Siddique T, Zoghbi HY: Genomic organization of *Tropomodulins 1* and *4* and unusual intergenic and intraexonic splicing of *YL-1* and *Tropomodulin 4*. BMC Genomics 2:7–16 (2001).
Cox PR, Fowler VM, Xu B, Sweatt JD, Paylor R, Zoghbi HY: Mice lacking tropomodulin-2 (Tmod2) show enhanced long-term potentiation, hyperactivity and deficits in learning and memory. Mol Cell Neurosci 23:1–12 (2003).
Cracraft J: Continental drift, paleoclimatology, and the evolution and biogeography of birds. J Zool 169: 455–545 (1973).
Cracraft J: Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. Proc R Soc Lond B Bio 268:459–469 (2001).
Cracraft JC, Barker FK, Braun M, Harshman J, Dyke GJ, Feinstein J, et al: Phylogenetic relationships among modern birds (Neornithes), in Cracraft JC, Donoghue MJ (eds): Assembling the Tree of Life (Oxford University Press, Oxford 2004).
Crittenden LB, Provencher L, Santangelo L, Levin I, Abplanalp H, Briles RW, Briles WE, Dodgson JB: Characterisation of a Red Jungle Fowl by White Leghorn backcross reference population for molecular mapping of the chicken genome. Poultry Sci 72:334–348 (1993).
Crooijmans RPMA, Vrebalov J, Dijkhof RJ, van der Poel JJ, Groenen MA: Two-dimensional screening of the Wageningen chicken BAC library. Mamm Genome 11:360–363 (2000).
Crooijmans RPMA, Dijkhof RJ, Veenendaal T, van der Poel JJ, Nicholls RD, Bovenhuis H, Groenen MA: The gene orders on human chromosome 15 and chicken chromosome 10 reveal multiple inter- and intrachromosomal rearrangements. Mol Biol Evol 18:2102–2109 (2001).
Daniels LM: Molecular analysis of the 5S rDNA array in chicken *(Gallus gallus domesticus)*. M.S. Thesis, University of California, Davis (2001).
Daniels LM, Delany ME: Molecular and cytogenetic organization of the 5S ribosomal DNA in chicken *(Gallus gallus)*. Chromosome Res 11:305–317 (2003).
de Kloet RS, de Kloet SR: Evolution of the spindlin gene in birds: independent cessation of the recombination of sex chromosomes at the spindlin locus in neognathous birds and tinamous, a palaeognathous avian family. Genetica 119:333–342 (2003).
de la Serna I, Cujec T, Shi Y, Tyler B: Non-coordinate regulation of 5S rRNA genes and the gene encoding the 5S rRNA-binding ribosomal protein homolog in *Neurospora crassa*. Mol Gen Genet 263:987–994 (2000).
Deeb N, Lamont SJ: Use of a novel outbred by inbred F1 cross to detect genetic markers for growth. Anim Genet 34:205–212 (2003).
Delany ME: Patterns of ribosomal gene variation in elite commercial chicken pure line populations. Anim Genet 31:110–116 (2000).

Delany ME, Daniels LM: The chicken telomerase RNA gene: Conservation of sequence, regulatory elements and synteny among viral, avian and mammalian genomes. Cytogenet Genome Res 102:309–317 (2003).

Delany ME, Krupkin AB: Molecular characterization of ribosomal gene variation within and among NORs segregating in specialized populations of chicken. Genome 42:60–71 (1999).

Delany ME, Muscarella DE, Bloom SE: Formation of nucleolar polymorphisms in trisomic chickens and subsequent microevolution of rRNA gene clusters in diploids. J Hered 82:213–220 (1991).

Delany ME, Emsley A, Smiley M, Putnam JR, Bloom SE: Nucleolar size polymorphisms in commercial layer chickens: determination of incidence, inheritance, and nucleolar sizes. Poultry Sci 73:1211–1217 (1994a).

Delany ME, Muscarella DM, Bloom SE: Effects of rRNA gene copy number and nucleolar variation on early development: inhibition of gastrulation in rDNA-deficient chick embryos. J Hered 85:211–217 (1994b).

Delany ME, Taylor RL Jr, Bloom SE: Teratogenic development in chicken embryos associated with a major deletion in the rRNA gene cluster. Dev Growth Differ 37:403–412 (1995).

Delany ME, Gurel V, Taylor RL Jr: Ribosomal DNA genotypes and nucleolar phenotypes of v-src initiated tumors in 6.B congenic chickens. Poultry Sci 77(s1):5 (1998).

Delany ME, Krupkin AB, Miller MM: Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. Cytogenet Cell Genet 90:139–145 (2000).

Delany ME, Daniels LM, Swanberg SE, Taylor HA: Telomeres in the chicken: Chromosome ends and genome stability. Poultry Sci 82:917–926 (2003).

Denjean B, Ducos A, Darré A, Pinton A, Séguéla A, Berland H, et al: Caryotypes des canards commun *(Anas platyrhynchos)*, Barbarie *(Cairina moschata)* et de leur hybride. Revue Méd Vét 148:695–704 (1997).

Desjardins P, Morais R: Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. J Mol Biol 212: 599–663 (1990).

Dunnington EA, Stallard LC, Hillel J, Siegel PB: Genetic diversity among commercial chicken populations estimated from DNA fingerprints. Poultry Sci 73:1218–1225 (1994).

Emara MG, Kim H: Genetic markers and their application in poultry breeding. Poultry Sci 82:952–957 (2003).

Etheridge KT, Banik SSR, Ambruster BN, Zhu Y, Terns RM, Terns MP, Counter CM: The nucleolar localization domain of the catalytic subunit of human telomerase. J Biol Chem 277:24764–24770 (2002).

Ewing B, Green P: Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8:186–194 (1998).

Ewing B, Hillier L, Wendl MC, Green P: Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 8:175–185 (1998).

Falchetti ML, Falcone G, D'Ambrosio E, Verna R, Alema S, Levi A: Induction of telomerase activity in v-*myc*-transformed avian cells. Oncogene 18: 1515–1519 (1999).

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A: A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13:399–408 (1996).

Feduccia A: Explosive evolution in tertiary birds and mammals. Science 267:637–638 (1995).

Feduccia A: "Big bang" for tertiary birds? Trends Ecol Evol 18:172–176 (2003).

Fillon V, Morisson M, Zoorob R, Auffray C, Douaire M, Gellin J, Vignal A: Identification of 16 chicken microchromosomes by molecular markers using two-colour fluorescence in situ hybridization (FISH). Chromosome Res 6:307–313 (1998).

Fischer RS, Lee A, Fowler VM: Tropomodulin and tropomyosin mediate lens cell actin cytoskeleton reorganization in vitro. Invest Ophthalmol Vis Sci 41:166–174 (2000).

Fischer RS, Fritz-Six KL, Fowler VM: Actin pointed end capping by tropomodulin 3 negatively regulates cell motility in endothelial cells. J Cell Biol 161:1–12 (2003).

Forsyth NR, Wright WE, Shay JW: Telomerase and differentiation in multicellular organisms: Turn it off, turn it on, and turn it off again. Differentiation 69:188–197 (2002).

Fowler VM: Regulation of actin filament length in erythrocytes and striated muscle. Curr Opin Cell Biol 8:86–96 (1996).

Fowler VM: Capping actin filament growth: Tropomodulin in muscle and nonmuscle cells, in Cytoskeletal Regulation of Membrane Function, 50th Annual Meeting and Symposium of the Society of General Physiologists, Vol. 52, pp 79–89 (1997).

Fowler VM, Conley CC: Tropomodulin, in Kreis TE, Vale RD (eds): Guidebook to the Cytoskeletal and Motor Proteins, 2nd edition, pp 154–159 (Oxford University Press, Oxford 1999).

Fowler VM, Sussman MA, Miller PG, Flucher BE, Daniels MP: Tropomodulin is associated with the free (pointed) ends of the thin filaments in rat skeletal muscle. J Cell Biol 120:411–420 (1993).

Fowler VM, Greenfield NJ, Moyer J: Tropomodulin contains two actin filament pointed end-capping domains. J Biol Chem 278:40000–40009 (2003).

Fragnet L, Blasco MA, Klapper W, Rasschaert D: The RNA subunit of telomerase is encoded by Marek's Disease Virus. J Virol 77:5985–5996 (2003).

Fridolfsson AK, Cheng H, Copeland NG, Jenkins NA, Liu HC, Raudsepp T, et al: Evolution of the avian sex chromosomes from an ancestral pair of autosomes. Proc Natl Acad Sci USA 95:8147–8152 (1998).

Fritschi S, Stranzinger G: Fluorescent chromosome banding in inbred chicken: quinacrin bands, sequential chromomycin and DAPI bands. Theor Appl Genet 71:408–412 (1985).

Fukagawa T, Hayward N, Yang J, Azzalin C, Griffin D, Stewart AF, Brown W: The chicken *HPRT* gene: a counter selectable marker for the DT40 cell line. Nucleic Acids Res 27:1966–1969 (1999).

Fumihito A, Miyake T, Sumi S, Takada M, Ohno S, Kondo N: One subspecies of the red jungle fowl *(Gallus gallus gallus)* suffices as the matriarchic ancestor of all domestic breeds. Proc Natl Acad Sci USA 91:12505–12509 (1994).

Fumihito A, Miyake T, Takada M, Shingu R, Endo T, Gojobori T, Kondo N, Ohno S: Monophyletic origin and unique dispersal patterns of domestic fowls. Proc Natl Acad Sci USA 93:6792–6795 (1996).

Gaginskaya ER, Chin SH: Peculiarities of oogenesis in the chick II. Follicular period in the development of oocytes. Ontogenez (Russ.) 11:213–221 (1980).

Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, et al: BRCA1 supports XIST RNA concentration on the inactive X chromosome. Cell 111:393–405 (2002).

Garcia-Moreno J, Sorenson MD, Mindell DP: Congruent avian phylogenies inferred from mitochondrial and nuclear DNA sequences. J Mol Evol 57:27–37 (2003).

Godbout R, Packer M, Katyal S, Bléoo S: Cloning and expression analysis of the chicken DEAD box gene *DDX1*. Biochim Biophys Acta 1574:63–71 (2002).

Gonzalez IL, Sylvester JE: Complete sequence of the 43-kb human ribosomal DNA repeat: Analysis of the intergenic spacer. Genomics 27:320–328 (1995).

Goureau A, Garrigues A, Tosser-Klopp G, Lahbib-Mansais Y, Chardon P, Yerle M: Conserved synteny and gene order difference between human chromosome 12 and pig chromosome 5. Cytogenet Cell Genet 94:49–54 (2001).

Graves JAM: The rise and fall of *SRY*. Trends Genet 18:259–264 (2002).

Graves JAM: Sex and death in birds: a model of dosage compensation that predicts lethality of sex chromosome aneuploids. Cytogenet Genome Res 101: 278–282 (2003).

Greenfield NJ, Fowler VM: Tropomyosin requires an intact N-terminal coiled-coil to interact with tropomodulin. Biophys J 82:2580–2591 (2002).

Gregorio CC, Fowler VM: Mechanisms of thin filament assembly in embryonic chick cardiac myocytes: Tropomodulin requires tropomyosin for assembly. J Cell Biol 129:683–695 (1995).

Gregorio CC, Weber A, Bondad M, Pennise CR, Fowler VM: Requirement of pointed end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes. Nature 377: 83–86 (1995).

Greider CW, Blackburn EH: Identification of a specific telomere terminal transferase activity in *Tetrahymena* cell extracts. Cell 43:405–413 (1985).

Greider CW, Blackburn EH: A telomeric sequence in the RNA of *Tetrahymena* telomerase is required for telomere repeat synthesis. Nature 337:331–337 (1989).

Griffin DK, Habermann F, O'Brien PCM, Bagga M, Smith J, Burt D, Ferguson-Smith MA: Macro- and micro-chromosome paints generated by flow cytometry and chromosome microdissection: Tools for mapping the chicken genome. Cytogenet Cell Genet 87:278–281 (1999).

Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M, van der Poel JJ: A comprehensive microsatellite linkage map of the chicken genome. Genomics 49:265–274 (1998).

Groenen MAM, Cheng HH, Bumstead N, Benkel BF, Briles WE, Burke T, et al: A consensus linkage map of the chicken genome. Genome Res 10:137–147 (2000).

Groth JG, Barrowclough GF: Basal divergences in birds and the phylogenetic utility of the nuclear *RAG-1* gene. Mol Phyl Evol 12:115–123 (1999).

Grützner F, Rens W, Tsend-Ayush E, El-Mogharbel N, O'Brien PC, Jones RC, Ferguson-Smith MA, Graves JAM: In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. Nature 432:913–917 (2004).

Guillemot F, Billault A, Pourquie O, Behar G, Chausse AM, Zoorob R, Kreibick GC, Auffray C: A molecular map of the chicken major histocompatibility complex: the class II B genes are closely linked to the class I genes and the nucleolar organizer. EMBO J 7:2775–2785 (1988).

Guioli S, Schmitt K, Critcher R, Bouzyk M, Spurr NK, Ogata T, et al: Molecular analysis of 9p deletions associated with XY sex reversal: Refining the localization of a sex-determining gene to the tip of the chromosome. Am J Hum Genet 63:905–908 (1998).

Gururajan R, Mathews L, Longo FJ, Weeks DL: An3 mRNA encodes an RNA helicase that colocalizes with nucleoli in *Xenopus* oocytes in a stage-specific manner. Proc Natl Acad Sci USA 91:2056–2060 (1994).

Habermann FA, Cremer M, Walter J, Kreth G, von Hase J, Bauer K, et al: Arrangements of macro- and microchromosomes in chicken cells. Chromosome Res 9:569–584 (2001).

Hacia JG, Fan JB, Ryder O, Jin L, Edgemon K, Ghandour G, et al: Determination of ancestral alleles for human SNPs using high-density oligonucleotide arrays. Nat Genet 22:164–167 (1999).

Haddrath O, Baker AJ: Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. Proc R Soc Lond B Biol Sci 268:939–945 (2001).

Hadjiolov AA: The nucleolus and ribosome biogenesis. Cell Biology Monographs, Vol. 12 (Springer-Verlag, New York 1985).

Handley LJ, Ceplitis H, Ellegren H: Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. Genetics 167:367–376 (2004).

Harlid A, Arnason U: Analyses of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenous origin of ratite morphological characters. Proc R Soc Lond B Biol 266:305–309 (1999).

Hay B, Jan LY, Jan YN: A protein component of *Drosophila* polar granules is encoded by vasa and has extensive sequence similarity to ATP-dependent helicases. Cell 55:577–587 (1988).

Heard E, Rougeulle C, Arnaud D, Avner P, Allis CD, Spector DL: Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. Cell 107:727–738 (2001).

Hedges SB, Kumar S: Genomic clocks and evolutionary timescales. Trends Genet 19:200–206 (2003).

Hedges SB, Kumar S: Precision of molecular time estimates. Trends Genet 20:242–247 (2004).

Hedges SB, Sibley CG: Molecules vs. morphology in avian evolution: the case of the "pelecaniform" birds. Proc Natl Acad Sci USA 91:9861–9865 (1994).

Hedges SB, Simmons MD, VanDikj MA, Caspers G, DeJong WW, Sibley CG: Phylogenetic relationships of the Hoatzin, an enigmatic South American bird. Proc Natl Acad Sci USA 92:11662–11665 (1995).

Hedges SB, Parker PH, Sibley CG, Kumar S: Continental breakup and the ordinal diversification of birds and mammals. Nature 381:226–229 (1996).

Hillel J, Groenen MAM, Tixier-Boichard M, Korol A, David L, Ben-Ari G, et al: Genetic diversity of chickens, in 11th European Poultry Conference, August 6–10, Bremen, Germany (2002).

Hillel J, Groenen MAM, Tixier-Boichard M, Korol AB, David L, Kirzhner VM, et al: Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet Sel Evol 35:533–557 (2003).

Hirling H, Scheffner M, Restle T, Stahl H: RNA helicase activity associated with the human p68 protein. Nature 339:562–564 (1989).

Hochegger H, Sonoda E, Takeda S: Post-replication repair in DT40 cells: translesion polymerases versus recombinases. Bioessays 26:151–158 (2004).

Holl M: Über die Reifung der Eizelle des Huhnes. Sitzungsber Akad Wiss Wien 99:311–370 (1890).

Hori T, Asakawa S, Itoh Y, Shimizu N, Mizuno S: *Wpkci*, encoding an altered from of *PKCI*, is conserved widely on the avian W chromosome and expressed in early female embryos: Implication of its role in female sex determination. Mol Biol Cell 11:3645–3660 (2000).

Hubbard SJ, Grafham DV, Beattie KJ, Overton IM, McLaren SR, Croning MDR, et al: Transcriptome analysis for the chicken based on 19,626 finished cDNA sequences and 485,337 expressed sequence tags. Genome Res 15:174–183 (2005).

Hughes JM, Baker AJ: Phylogenetic relationships of the enigmatic hoatzin *(Opisthocomus hoazin)* resolved using mitochondrial and nuclear gene sequences. Mol Biol Evol 16:1300–1307 (1999).

Hutchison N: Lampbrush chromosomes of the chicken, *Gallus domesticus*. J Cell Biol 105:1493–1500 (1987).

International Chicken Genome Sequencing Consortium: Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695–716 (2004).

International Chicken Polymorphism Map Consortium: A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature 432:717–722 (2004).

Ip FCF, Chung SSK, Fu W-Y, Ip NY: Developmental and tissue-specific expression of the DEAD box protein p72. Mol Neurosci 11:457–462 (2000).

Ishii Y, Reece D, Mikawa T: Somatic transgenesis using retroviral vectors in the chicken embryo. Dev Dyn 229:630–642 (2004).

Itoh Y, Mizuno S: Molecular and cytological characterization of *Ssp*I-family repetitive sequence on the chicken W chromosome. Chromosome Res 10: 499–511 (2002).

Itoh Y, Hori T, Saitoh H, Mizuno S: Chicken spindlin genes on W and Z chromosomes: transcriptional expression of both genes and dynamic behavior of spindlin in interphase and mitotic cells. Chromosome Res 9:283–299 (2001).

IUCN: IUCN Redlist of Threatened Species. International Union for the Conservation of Nature, Cambridge, UK (2004).

Jacobsson L, Park H-B, Wahlberg P, Jiang S, Siegel PB, Andersson L: Assignment of fourteen microsatellite markers to the chicken linkage map. Poultry Sci 83:1825–1831 (2004).

Jennen DG, Crooijmans RP, Kamps B, Acar R, Veenendaal A, van der Poel JJ, Groenen MA: A comparative map of chicken chromosome 24 and human chromosome 11. Anim Genet 33:205–210 (2002).

Jennen DG, Crooijmans RP, Kamps B, Acar R, Veenendaal A, van der Poel JJ, Groenen MA: Comparative map between chicken chromosome 15 and human chromosomal region 12q24 and 22q11→q12. Mamm Genome 14:629–639 (2003).

Jennen DG, Crooijmans RP, Morisson M, Grootemaat AE, van der Poel JJ, Vignal A, Groenen MA: A radiation hybrid map of chicken chromosome 15. Anim Genet 35:63–65 (2004).

Jin L, Underhill PA, Oefner PJ, Cavalli-Sforza LL: Systematic search for polymorphisms in the human genome using denaturing high-performance liquid chromatography (DHPLC). Am J Hum Genet 57, Suppl:26 (1995).

Johansson US, Parsons TJ, Irestedt M, Ericson PGP: Clades within the "higher land birds", evaluated by nuclear DNA sequences. J Zool Syst Evol Res 39:37–51 (2001).

Johansson US, Irestedt M, Parsons TJ, Ericson PGP: Basal phylogeny of the tyrannoidea based on comparisons of cytochrome b and exons of nuclear c-myc and RAG-1 genes. Auk 119:984–995 (2002).

Jost J-P, Schwarz S, Hess D, Angliker H, Fuller-Pace FV, Stahl H, Thiry S, Siegmann M: A chicken embryo protein related to the mammalian DEAD box protein p68 is tightly associated with the highly purified protein-RNA complex of 5-MeC-DNA glycosylase. Nucleic Acids Res 27:3245–3252 (1999).

Judson R, Stephens JC: Notes from the SNP vs. haplotype front. Pharmacogenomics 2:7–10 (2001).

Kaelbling M, Fechheimer NS: Synaptonemal complexes and the chromosomes of the domestic fowl, *Gallus domesticus*. Cytogenet Cell Genet 35:87–92 (1983).

Karlseder JD, Broccoli Y, Dai Hardy S, de Lange T: p53 and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283:1321–1325 (1999).

Katoh K, Miyata T: A heuristic approach of maximum likelihood method for inferring phylogenetic tree and an application to the mammalian *SOX-3* origin of the testis-determining gene *SRY*. FEBS Lett 463:129–132 (1999).

Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P: A male-specific role for *SOX9* in vertebrate sex determination. Development 122:2813–2822 (1996).

Ko JH, Jin HK, Asano A, Takada A, Ninomiya A, Kida H, et al: Polymorphisms and the differential antiviral activity of the chicken *Mx* gene. Genome Res 12:595–601 (2002).

Koltzoff NK: The structure of the chromosomes and their participation in cell-metabolism. Biol Zh (Russ.) 7:44–46 (1938).

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R: Male development of chromosomally female mice transgenic for *Sry*. Nature 351:117–121 (1991).

Korsisaari N, Mäkelä TP: Interactions of Cdk7 and Kin28 with Hint/PKCI-1 and Hnt1 histidine triad proteins. J Biol Chem 275:34837–34840 (2000).

Kos R, Tucker RP, Hall R, Duong TD, Erickson CA: Methods for introducing morpholinos into the chicken embryo. Dev Dyn 226:470–477 (2003).

Koskela K, Kohonen P, Nieminen P, Buerstedde JM, Lassila O: Insight into lymphoid development by gene expression profiling of avian B cells. Immunogenetics 55:412–422 (2003).

Koskinen R, Salomonsen J, Goodchild M, Bumstead N, Boyd Y, Vainio O: Structure and chromosomal localization of chicken CD5. Scand J Immunol 54:141–145 (2001).

Kost M, Alimov A, Sarafanov A, Tikchomirova T, Bumeniuk R, Timofeeva M, Zelenin A: 5S rRNA gene hybridizes to human chromosome 1 at two sites (1q42.11→q42.13 and 1q43). Cytogenet Cell Genet 68:82–83 (1995).

Kostyukova A, Maeda K, Yamauchi E, Krieger I, Maeda Y: Domain structure of tropomodulin. Distinct properties of the N-terminal and C-terminal halves. Eur J Biochem 267:6470–6475 (2000).

Krajinovic M, Pinamonti B, Sinagra G, Vatta M, Severini GM, Milasin J, et al: Linkage of familial dilated cardiomyopathy to chromosome 9. Am J Hum Genet 57:846–852 (1995).

Krieger I, Kostyukova A, Yamashita A, Nitanai Y, Maeda Y: Crystal structure of the C-terminal half of tropomodulin and structural basis of actin filament pointed-end capping. Biophys J 83:2716–2725 (2002).

Kriglysh L, Nikerse DA: Variation is the spice of life. Nat Genet 27:234–236 (2001).

Kropotova EV, Gaginskaya ER: Lampbrush chromosomes from Japanese quail oocytes. Tsitologia 26:1008–1014 (1984).

Krull CE: A primer on using in ovo electroporation to analyse gene function. Dev Dyn 229:433–439 (2004).

Kumar S, Hedges SB: A molecular timescale for vertebrate evolution. Nature 392:917–920 (1998).

Kurochkin EN, Dyke GJ, Karhu AA: A new presbyornithid bird (Aves, Anseriformes) from the late cretaceous of Southern Mongolia. Am Museum Novitates 1–11 (2002).

Kuroda Y, Arai N, Arita M, Teranishi M, Hori T, Harata M, Mizuno S: Absence of Z-chromosome inactivation for five genes in male chickens. Chromosome Res 9:457–468 (2001).

Kuroiwa A, Yokomine T, Sasaki H, Tsudzuki M, Tanaka K, Namikawa T, Matsuda Y: Biallelic expression of Z-linked genes in male chickens. Cytogenet Genome Res 99:310–314 (2002).

Kurosaki T: Regulation of B-cell signal transduction by adaptor proteins. Nat Rev Immunol 2:354–363 (2002).

Kwan KM: Conditional alleles in mice: practical considerations for tissue-specific knockouts. Genesis 32:49–62 (2002).

Ladjali-Mohammedi K, Bitgood JJ, Tixier-Boichard M, Ponce de Leon FA: International system for standardized avian karyotypes (ISSAK): standardized banded karyotypes of the domestic fowl *(Gallus domesticus)*. Cytogenet Cell Genet 86:271–276 (1999).

Ladjali-Mohammedi K, Grapin-Botton A, Bonnin MA, Le Douarin NM: Distribution of HOX genes in the chicken genome reveals a new segment of conservation between human and chicken. Cytogenet Cell Genet 92:157–161 (2001).

Lahbib-Mansais Y, Tosser-Klopp G, Leroux S, Cabau C, Karsenty E, Milan D, et al: Contribution to high-resolution mapping in pigs with 101 type I markers and progress in comparative map between humans and pigs. Mamm Genome 14:275–288 (2003).

Lasko PF, Ashburner M: Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. Genes Dev 4:905–921 (1990).

Lee K, Feinstein J, Cracraft JC: The phylogeny of ratite birds: resolving conflicts between molecular and morphological data sets, in Mindell DP (ed): Avian Molecular Evolution and Systematics, pp 173–211 (Academic Press, San Diego 1997).

Lee MK, Ren C, Ling P, Cox B, Dodgson JB, Zhang HB: Development of a BAC-based physical map of chicken genome for high-throughput gene mapping and cloning. Plant & Animal Genome IX Conference, January 13–17, Town & Country Hotel, San Diego, CA (2001).

Lima CD, Klein MG, Weinstein IB, Hendrickson WA: Three-dimensional structure of human protein kinase C interacting protein I, a member of the HIT family of proteins. Proc Natl Acad Sci USA 93: 5357–5362 (1996).

Lindblad-Toh K, Winchester E, Daly MJ, Wang DG, Hirschhorn JN, Laviolette JP, et al: Large scale discovery and genotyping of single nucleotide polymorphisms in the mouse. Nat Genet 24:381–386 (2000).

Linder P, Lasko PF, Ashburner M, Leroy P, Nielsen PJ, Nishi K, Schnier J, Slonimski PP: Birth of the D-E-A-D box. Nature 337:121–122 (1989).

Little R, Braaten D: Genomic organization of human 5S rDNA and sequence of one tandem repeat. Genomics 4:376–383 (1989).

Liu W, Miller MM, Lamont SJ: Association of MHC class I and class II gene polymorphisms with vaccine or challenge response to *Salmonella enteritidis* in young chicks. Immunogenetics 54:582–590 (2002).

Liu W, Kaiser MG, Lamont SJ: Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with *Salmonella enteritidis* in young chicks. Poultry Sci 82:259–266 (2003).

Loyez M: Recherches sur le developpement ovarien des oeufs meroblastiques à vitellus nutritif abondant. Arch Anat Microsc Morphol Exp 8:69–397 (1906).

Lunetta KL, Boehnke M: Multipoint radiation hybrid mapping: comparison of methods, sample size requirements, and optimal study characteristics. Genomics 21:92–103 (1994).

Marth GT, Korf I, Yandell MD, Yeh RT, Gu Z, Zakeri H, et al: A general approach to single-nucleotide polymorphism discovery. Nat Genet 23:452–456 (1999).

Marth GT, Yeh RT, Minton M, Donaldson R, Li Q, Duan S, et al: Single-nucleotide polymorphisms in the public domain: how useful are they? Nat Genet 27:371–372 (2001).

Martins-Wess F, Milan D, Drögemüller C, Vobeta-Nemitz R, Brenig B, Robic A, Yerle M, Leeb T: A high resolution physical and RH map of pig chromosome 6q1.2 and comparative analysis with human chromosome 19q13.1. BMC Genomics 4:20 (2003).

Masabanda JS, Burt DW, O'Brien PC, Vignal A, Fillon V, Walsh PS, et al: Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. Genetics 166:1367–1373 (2004).

McClintock B: The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z Zellforsch Mikr Anat 21:294–328 (1934).

McKenzie SE, Mansfield E, Rappaport E, Surrey S, Fortina P: Parallel molecular genetic analysis. Eur J Hum Genet 6:417–429 (1998).

McQueen HA, McBride D, Miele G, Bird AP, Clinton M: Dosage compensation in birds. Curr Biol 11: 253–257 (2001).

Mergny JL, Riou JF, Mailliet P, Teulade-Fichous MP, Gilson E: Natural and pharmacological regulation of telomerase. Nucleic Acids Res 30:839–865 (2002).

Merriwether DA, Clark AG, Ballinger SW, Schurr TG, Soodyall H, Jenkins T, Sherry ST, Wallace DC: The structure of human mitochondrial DNA variation. J Mol Evol 33:543–555 (1991).

Miller MM, Goto RM, Taylor RL Jr., Zoorob R, Auffray C, Briles RW, Briles WE, Bloom SE: Assignment of Rfp-Y to the chicken major histocompatibility complex/NOR microchromosome and evidence for high-frequency recombination associated with the nucleolar organizer region. Proc Natl Acad Sci USA 93:3958–3962 (1996).

Mindell DP, Sorenson MD, Dimcheff DE, Hasegawa M, Ast JC, Yuri T: Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. Syst Biol 48:138–152 (1999).

Mizuno S, Macgregor H: The ZW lampbrush chromosomes of birds: a unique opportunity to look at the molecular cytogenetics of sex chromosomes. Cytogenet Cell Genet 80:149–157 (1998).

Mizuno S, Kunita R, Nakabayashi O, Kuroda Y, Arai N, Harata M, et al: Z and W chromosomes of chickens: studies on their gene functions in sex determination and sex differentiation. Cytogenet Genome Res 99:236–244 (2002).

Morisson M, Pitel F, Fillon V, Pouzadoux A, Berge R, Vit JP, et al: Integration of chicken cytogenetic and genetic maps: 18 new polymorphic markers isolated from BAC and PAC clones. Anim Genet 29: 348–355 (1998).

Morisson M, Lemiere A, Bosc S, Galan M, Plisson-Petit F, Pinton P, et al: ChickRH6: a chicken whole-genome radiation hybrid panel. Genet Sel Evol 34:521–533 (2002).

Morroll S, Goodchild M, Salmon N, Copeland NG, Gilbert DJ, Jenkins NA, Bumstead N, Boyd Y: The genes encoding E-selectin (SELE) and lymphotactin (SCYC1) lie on separate chicken chromosomes although they are closely linked in human and mouse. Immunogenetics 53:477–482 (2001).

Mozdziak PE, McFarland DC, Schultz E: Telomeric profiles and telomerase activity in turkey satellite cell clones with different in vitro growth characteristics. Biochim Biophys Acta 1492:362–368 (2000).

Mozdziak PE, Borwornpinyo S, McCoy DW, Petitte JN: Development of transgenic chickens expressing bacterial β-galactosidase. Dev Dyn 226:439–445 (2003).

Mullikin JC, Hunt SE, Cole CG, Mortimore BJ, Rice CM, Burton J, et al: An SNP map of human chromosome 22. Nature 407:516–520 (2000).

Muscarella DE, Vogt VM, Bloom SE: The ribosomal RNA gene cluster in aneuploid chickens: evidence for increased gene dosage and regulation of gene expression. J Cell Biol 101:749–756 (1985).

Muscarella DE, Vogt VM, Bloom SE: Characterization of ribosomal RNA synthesis in a gene dosage mutant: the relationship of topoisomerase I and chromatin structure to transcriptional activity. J Cell Biol 105:1501–1513 (1987).

Myakoshina YA, Rodionov AV: Meiotic lampbrush chromosomes in turkey, *Meleagris gallopavo* (Galliformes: Meleagrididae). Genetika (Russ.) 30: 577–583 (1994).

Nakamura H, Katahira T, Sato T, Watanabe Y, Funahashi J: Gain- and loss-of-function in chick embryos by electroporation. Mech Dev 121:1137–1143 (2004).

Nanda I, Shan Z, Schartl M, Burt DW, Koehler M, Nothwang H, et al: 300 million years of conserved synteny between chicken Z and human chromosome 9. Nat Genet 21:258–259 (1999).

Nanda I, Zend-Ajusch E, Shan Z, Grutzner F, Schartl M, Burt DW, et al: 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 (2000).

Nanda I, Haaf T, Schartl M, Schmid M, Burt DW: Comparative mapping of Z-orthologous genes in vertebrates: implications for the evolution of avian sex chromosomes. Cytogenet Genome Res 99:178–184 (2002a).

Nanda I, Schrama D, Feichtinger W, Haaf T, Schartl M, Schmid M: Distribution of telomeric $(TTAGGG)_n$ sequences in avian chromosomes. Chromosoma 111:215–227 (2002b).

Nei M: Molecular Evolutionary Genetics (Columbia University Press, New York 1987).

Neiman PE, Ruddell A, Jasoni C, Loring G, Thomas SJ, Brandvold KA, et al: Analysis of gene expression during myc oncogene-induced lymphomagenesis in the bursa of Fabricius. Proc Natl Acad Sci USA 98:6378–6383 (2001).

Nickerson DA, Tobe VO, Taylor SL: PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. Nucleic Acids Res 25:2745–2751 (1997).

Nielsen PJ, Trachsel H: The mouse protein synthesis initiation factor 4A gene family includes two related functional genes which are differentially expressed. EMBO J 7:2097–2105 (1988).

Nishida-Umehara C, Fujiwara A, Ogawa A, Mizuno S, Abe S, Yoshida MC: Differentiation of Z and W chromosomes revealed by replication banding and FISH mapping of sex-chromosome-linked DNA markers in the cassowary (Aves, Ratitae). Chromosome Res 7:635–640 (1999).

Niu D, Fu Y, Luo J, Ruan H, Yu XP, Chen G, Zhang YP: The origin and genetic diversity of Chinese native chicken breeds. Biochem Genet 40:163–174 (2002).

Noakes MA, Campbell MT, Van Hest BJ: The chicken *CLOCK* gene maps to chromosome 4. Anim Genet 31:333–334 (2000).

Noce T, Okamoto-Ito S, Tsunekawa N: Vasa homolog genes in mammalian germ cell development. Cell Struct Funct 26:131–136 (2001).

O'Brien SJ, Wienberg J, Lyons LA: Comparative genomics: lessons from cats. Trends Genet 13:393–399 (1997).

O'Donnell MJ, Little DP, Braun A: MassArray as an enabling technology for the industrial scale analysis of DNA. Genet Eng News 21:1–3 (1997).

Ogawa A, Murata K, Mizuno S: The location of Z- and W-linked marker genes and sequence on the homomorphic sex chromosomes of the ostrich and the emu. Proc Natl Acad Sci USA 95:4415–4418 (1998).

Ohno M, Fukagawa T, Lee JS, Ikemura T: Triplex-forming DNAs in the human interphase nucleus visualized in situ by polypurine/polypyrimidine DNA probes and antitriplex antibodies. Chromosoma 111:201–213 (2002).
Ohno S: Sex Chromosomes and Sex Linked Genes (Springer Verlag, Berlin 1967).
Olovnikov A: A theory of marginotomy: the incomplete copying of template margin in enzymatic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol 41:181–190 (1973).
O'Neill M, Binder M, Smith C, Andrews J, Reed K, Smith M, et al: *ASW*: a gene with conserved avian W-linkage and female specific expression in chick embryonic gonad. Dev Genes Evol 210:243–249 (2000).
Ottolenghi C, Veitia R, Quintana-Murci L, Torchard D, Scapoli L, Souleyreau-Therville N, et al: The region on 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. Genomics 64:170–178 (2000).
Ottolenghi C, Fellous M, Barbieri M, McElreavey K: Novel paralogy relations among human chromosomes support a link between the phylogeny of doublesex-related genes and the evolution of sex determination. Genomics 79:333–343 (2002).
Paddison PJ, Silva JM, Conklin DS, Schlabach M, Li M, Aruleba S, et al: A resource for large-scale RNA-interference-based screens in mammals. Nature 428:427–431 (2004).
Pardue M, Brown D, Birnstiel M: Location of the genes for 5S ribosomal RNA in *Xenopus laevis*. Chromosoma 42:191–203 (1973).
Paris Conference: Standardization in Human Cytogenetics: Birth Defects. Original Article Series 8:1–46 (1971).
Paton T, Haddrath O, Baker AJ: Complete mitochondrial DNA genome sequences show that modern birds are not descended from transitional shorebirds. Proc Roy Soc Lond B Bio 269:839–846 (2002).
Pause A, Sonenberg N: Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. EMBO J 11:2643–2654 (1992).
Pause A, Méthot N, Sonenberg N: The HRIGRXXR region of the DEAD box RNA helicase eukaryotic translation initiation factor 4A is required for RNA binding and ATP hydrolysis. Mol Cell Biol 13: 6789–6798 (1993).
Pekarik V, Bourikas D, Miglino N, Joset P, Preiswerk S, Stoeckli ET: Screening for gene function in chicken embryo using RNAi and electroporation. Nat Biotech 21:93–96 (2003).
Pitel F, Abasht B, Morisson M, Crooijmans RP, Vignoles F, Leroux S, et al: A high-resolution radiation hybrid map of chicken chromosome 5 and comparison with human chromosomes. BMC Genomics 5:66 (2004).
Ponce de Leon FA, Li Y, Weng Z: Early and late replicative chromosomal banding patterns of *Gallus domesticus*. J Hered 83:36–42 (1992).
Pukkila PJ: Identification of lampbrush chromosome loops which transcribe 5S ribosomal RNA by molecular hybridization. Dev Biol 63:197–212 (1975).
Rabie TKSM: Pulmonary hypertension syndrome in chicken: Peeking under QTL peaks. Chapter 4, Validation and fine-scale mapping of quantitative trait loci affecting pulmonary hypertension syndrome (PHS) in broilers using advanced intercross line. Thesis, pp 65–83 (ISBN: 90-8504-132-5) (2004).
Rafti F, Scarvelis D, Lasko PF: A *Drosophila melanogaster* homologue of the human DEAD-box gene *DDX1*. Gene 171:225–229 (1996).
Raudsepp T, Houck ML, O'Brien PC, Ferguson-Smith MA, Ryder OA, Chowdhary BP: Cytogenetic analysis of California condor *(Gymnogyps californianus)* chromosomes: comparison with chicken *(Gallus gallus)* macrochromosomes. Cytogenet Genome Res 98:54–60 (2002).
Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, Hodgkin J, Zarkower D: Evidence for evolutionary conservation of sex-determining genes. Nature 391:691–695 (1998).
Raymond CS, Kettlewell JR, Hirsch B, Bardwell VJ, Zarkower D: Expression of *Dmrt1* in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. Dev Biol 215: 208–220 (1999).
Razin E, Zhang ZC, Nechushtan H, Frenkel S, Lee Y-N, Arudchandran R, Rivera J: Suppression of microphthalmia transcriptional activity by its association with protein kinase C-interacting protein 1 in mast cells. J Biol Chem 274:34272–34276 (1999).
Reed KJ, Sinclair AH: *FET-1*: a novel W-linked, female specific gene up-regulated in the embryonic chicken ovary. Mech Dev 119, Suppl 1:S87–90 (2002).
Ren C, Lee MK, Yan B, Ding K, Cox B, Romanov MN, et al: A BAC-based physical map of the chicken genome. Genome Res 3:2754–2758 (2003).
Reumers J, Schymkowitz J, Ferkinghoff-Borg J, Stricher F, Serrano L, Rousseau F: SNPeffect: a database mapping molecular phenotypic effects of human non-synonymous coding SNPs. Nucleic Acids Res 33 (Database Issue):D527–D532 (2005).
Ritossa F, Spiegelman S: Localization of DNA complementary to rRNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc Natl Acad Sci USA 53:737–745 (1965).
Roberts L: Human genome research. SNP mappers confront reality and find it daunting. Science 287:1898–1899 (2000).
Rodionov AV, Chechik MS: Cytogenetic map of the Japanese quail lampbrush chromosomes. Genetika (Russ.) 38:1246–1251 (2002).
Rodionov AV, Myakoshina YA, Chelysheva LA, Solovei IV, Gaginskaya ER: Chiasmata in the lampbrush chromosomes of *Gallus gallus domesticus*: The cytogenetic study of recombination frequency and linkage map lengths. Genetika (Russ.) 28:53–63 (1992).
Rodionov AV, Galkina SA, Lukina NA, Solovei I, Saccone S: Crossing over in chicken oogenesis: cytological and chiasma-based genetic maps of the chicken lampbrush chromosome 1. J Hered 93: 125–129 (2002).
Romanov MN, Weigend S: Using RAPD markers for assessment of genetic diversity in chickens. Arch Geflügelk 65:1–4 (2001).
Ronaghi M, Uhlen M, Nyren P: A sequencing method based on real-time pyrophosphate. Science 281: 363–365 (1998).
Rosenberg NA, Burke T, Elo K, Feldman MW, Freidlin PJ, Groenen MA, et al: Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. Genetics 159:699–713 (2001).
Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC, Sonenberg N: Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. Mol Cell Biol 10:1134–1144 (1990).
Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al.: A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409: 928–933 (2001).
Saetre GP, Borge T, Lindroos K, Haavie J, Sheldon BC, Primmer C, Syvanen AC: Sex chromosome evolution and speciation in Ficedula flycatchers. Proc R Soc Lond B Biol Sci 270:53–59 (2003).
Saifi GM, Chandra HS: An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc R Soc Lond B Biol Sci 266:203–209 (1999).
Sang H: Prospects for transgenesis in the chick. Dev Dyn 121:1179–1186 (2004).
Schartl M: Sex chromosome evolution in non-mammalian vertebrates. Curr Opin Genet Dev 14:634–641 (2004).
Schmid M, Enderle E, Schindler D, Schempp W: Chromosome banding and DNA replication patterns in bird karyotypes. Cytogenet Cell Genet 52:139–146 (1989).
Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn H, Schartl M, et al: First report on chicken genes and chromosomes 2000. Cytogenet Cell Genet 90:169–218 (2000).
Seufert DW, Kos R, Erickson CA, Swalla BJ: p68, a DEAD-box RNA helicase, is expressed in chordate embryo neural and mesodermal tissues. J Exp Zool 28:193–204 (2000).
Shan Z, Nanda I, Wang Y, Schmid M, Vortkamp A, Haaf T: Sex-specific expression of an evolutionarily conserved male regulatory gene, *DMRT1*, in birds. Cytogenet Cell Genet 89:252–257 (2000).
Shastry BS: SNP alleles in human disease and evolution. J Hum Genet 47:561–566 (2002).
Shay JW, Bacchetti S: A survey of telomerase activity in human cancer. Eur J Cancer 33:787–191 (1997).
Shen XJ, Ito S, Mizutani M, Yamamoto Y: Phylogenetic analysis in chicken breeds inferred from complete cytochrome b gene information. Biochem Genet 40:129–141 (2002).
Shetty S, Griffin DK, Graves JA: Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Res 7:289–295 (1999).
Shetty S, Kirby P, Zarkower D, Graves JA: *DMRT1* in a ratite bird: evidence for a role in sex determination and discovery of a putative regulatory element. Cytogenet Genome Res 99:245–251 (2002).
Shibusawa M, Minai S, Nishida-Umehara C, Suzuki T, Mano T, Yamada K, Namikawa T, Matsuda Y: A comparative cytogenetic study of chromosome homology between chicken and Japanese quail. Cytogenet Cell Genet 95:103–109 (2001).
Shin C, Feng Y, Manley JL: Dephosphorylated SRp38 acts as a splicing repressor in response to heat shock. Nature 427:553–558 (2004).
Sibley CG, Ahlquist JE: Phylogeny and Classification of Birds (Yale University Press, New Haven 1990).
Sibley CG, Monroe BL Jr: Distribution and Taxonomy of Birds of the World (Yale University Press, New Haven 1990).
Silverman E, Edwalds-Gilbert G, Lin R-J: DExD/H-box proteins and their partners: helping RNA helicases unwind. Gene 312:1–16 (2003).
Sims SH, Macgregor HC, Pellatt PS, Horner HA: Chromosome 1 in crested and marbled newts *(Triturus)*: An extraordinary case of heteromorphism and independent chromosome evolution. Chromosoma 89:169–185 (1984).
Smith CA, Sinclair AH: Sex determination: Insights from the chicken. Bioessays 26:120–132 (2004).
Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH: Conservation of a sex-determining gene. Nature 402:601–602 (1999).
Smith CA, Katz M, Sinclair AH: *DMRT1* is upregulated in the gonads during female-to-male sex reversal in ZW chicken embryos. Biol Reprod 68: 560–570 (2003).
Smith EJ, Shi L, Drummond P, Rodriguez L, Hamilton R, Powell E, et al: Development and characterization of expressed sequence tags for the turkey *(Meleagris gallopavo)* genome and comparative sequence analysis with other birds. Anim Genet 31:62–67 (2000).

Smith EJ, Shi L, Drummond P, Rodriguez L, Hamilton R, Ramlal S, et al: Expressed sequence tags for the chicken genome from a normalized 10-day-old White Leghorn whole embryo cDNA library: 1. DNA sequence characterization and linkage analysis. J Hered 92:1–8 (2001).

Smith EJ, Shi L, Smith G: Expressed sequence tags for the chicken genome from a normalized 10-day-old white leghorn whole-embryo cDNA library. 3. DNA sequence analysis of genetic variation in commercial chicken populations. Genome 45:261–267 (2002).

Smith J, Burt DW: Parameters of the chicken genome *(Gallus gallus)*. Anim Genet 29:290–294 (1998).

Smith J, Paton IR, Murray F, Crooijmans RPMA, Groenen MAM, Burt DW: Comparative mapping of human chromosome 19 with the chicken shows conserved synteny and gives an insight into evolution. Mamm Genome 13:310–315 (2002).

Smith SF, Snell P, Gruetzner F, Bench AJ, Haaf T, Metcalfe JA, Green AR, Elgar G: Analyses of the extent of shared synteny and conserved gene orders between the genome of *Fugu rubripes* and human 20q. Genome Res 12:776–784 (2002).

Soder AI, Hoare SF, Muir S, Going JJ, Parksinson EK, Keith WN: Amplification, increased dosage and in situ expression of the telomerase RNA gene in human cancer. Oncogene 14:1013–1021 (1997).

Soder AI, Going JJ, Kaye SB, Keith WN: Tumor specific regulation of telomerase RNA gene expression visualized by in situ hybridization. Oncogene 16: 979–983 (1998).

Solovei I, Gaginskaya E, Allen T, Macgregor H: A novel structure associated with a lampbrush chromosome in the chicken, *Gallus domesticus.* J Cell Sci 101:759–772 (1992).

Solovei IV, Gaginskaya ER, Hutchison N, Macgregor H: Avian sex chromosomes in the lampbrush form: the ZW lampbrush bivalents from six species of bird. Chromosome Res 1:153–166 (1993).

Solovei IV, Ogawa A, Naito M, Mizuno S, Macgregor H: Specific chromomeres on the chicken W lampbrush chromosome contain specific repetitive DNA sequence families. Chromosome Res 6:323–327 (1998).

Sorenson MD, Oneal E, Garcia-Moreno J, Mindell DP: More taxa, more characters: the hoatzin problem is still unresolved. Mol Biol Evol 20:1484–1498 (2003).

Springer MS, Cleven GC, Madsen O, Jong WW, Waddell VG, Amrine HM, Stanhope MJ: Endemic African mammals shake the phylogenetic tree. Nature 388:61–63 (1997).

Stanhope MJ, Waddell VG, Madsen O, Jong W, Hedges SB, Cleven G, Kao D, Springer MS: Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. Proc Natl Acad Sci USA 95:9967–9972 (1998).

Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, et al: Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. Am J Hum Genet 72:83–87 (2003).

Steinmetz LM, Sinha H, Richards DR, Spiegelman JI, Oefner PJ, McCusker JH, Davis RW: Dissecting the architecture of a quantitative trait locus in yeast. Nature 416:326–330 (2002).

Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NST, Abeysinghe S, Krawczak M, Cooper DN: Human Gene Mutation Database (HGMD): 2003 Update. Hum Mutat 21:577–581 (2003).

Stock AD, Bunch TD: The evolutionary implications of chromosome banding pattern homologies in the bird order Galliformes. Cytogenet Cell Genet 34: 136–148 (1982).

Su M, Delany ME: Ribosomal RNA gene copy number variation in directionally selected enhanced-growth lines of chicken. Poultry Sci 77:1748–1754 (1998).

Suchyta SP, Cheng HH, Burnside J, Dodgson JB: Comparative mapping of chicken anchor loci orthologous to genes on human chromosomes 1, 4 and 9. Anim Genet 32:12–18 (2001).

Suka N, Shinohara Y, Saitoh Y, Saitoh H, Ohtomo K, Harata M, Shpigelman E, Mizuno S: W-heterochromatin of chicken; its unusual DNA components, late replication, and chromatin structure. Genetica 88:93–105 (1993).

Sumner AT, Evans HJ, Buchland RA: A new technique for distinguishing between human chromosomes. Nat New Biol 232:31–32 (1971).

Sun HS, Tuggle CK, Goureau A, Fitzsimmons CJ, Pinton P, Chardon P, Yerle M: Precise mapping of breakpoints in conserved synteny between human chromosome 1 and pig chromosomes 4, 6 and 9. Anim Genet 33:91–96 (2002).

Sung LA, Fan Y-S, Lin CC: Gene assignment, expression and homology of human tropomodulin. Genomics 34:92–96 (1996).

Sussman MA, Welch S, Cambon N, Klevitsky R, Hewett TE, Price RL, Witt SA, Kimball TR: Myofibril degeneration caused by tropomodulin overexpression leads to dilated cardiomyopathy in juvenile mice. J Clin Invest 101:51–61 (1998).

Suzuki T, Kansaku N, Kurosaki T, Shimada K, Zadworny D, Koide M, et al: Comparative FISH mapping on Z chromosomes of chicken and Japanese quail. Cytogenet Cell Genet 87:22–26 (1999).

Swanberg SE, Delany ME: Dynamics of telomere erosion in transformed and non-transformed avian cells in vitro. Cytogenet Genome Res 102:318–325 (2003).

Taillon-Miller P, Kwok PY: A high-density single-nucleotide polymorphism map of Xq25→q28. Genomics 65:195–202 (2000).

Tanaka SS, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M, Noce T: The mouse homolog of *Drosophila Vasa* is required for the development of male germ cells. Genes Dev 14:841–853 (2000).

Tanner NK, Cordin O, Banroques J, Doère M, Linder P: The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. Mol Cell 11:127–138 (2003).

Tarantul VZ, Rodionov AV, Makarova IV, Gol'tsov VA, Chelysheva LA, Khutinaeva MA, Gaginskaya ER, Gazaryan KG: Transcription of moderately repeated conservative sequences of the vertebrate genome: molecular and cytological analysis. Tsitologia 31:619–625 (1989).

Taylor HA, Delany ME: Ontogeny of telomerase in chicken: Impact of down-regulation on pre- and postnatal telomere length in vivo. Dev Growth Differ 42:613–621 (2000).

Tease C, Jones GH: Do chiasmata disappear? An examination of whether closely spaced chiasmata are liable to reduction or loss. Chromosome Res 3:162–168 (1995).

Teranishi M, Shimada Y, Hori T, Nakabayashi O, Kikuchi T, Macleod T, et al: Transcripts of the MHM region on the chicken Z chromosome accumulate as non-coding RNA in the nucleus of female cells adjacent to the *DMRT1* locus. Chromosome Res 9:147–165 (2001).

Thorne M, Sheldon B: Triploid intersex and chimeric chickens: useful models for studies of avian sex determination, in Reed KC, Graves JAM (eds): Sex Chromosomes and Sex Determining Genes, pp 201–208 (Harwood Academic, Chur 1993).

Tirunagaru VG, Sofer L, Cui J, Burnside J: An expressed sequence tag database of T-cell-enriched activated chicken splenocytes: sequence analysis of 5251 clones. Genomics 66:144–151 (2000).

Tsunekawa N, Naito M, Sakai Y, Nishida T, Noce T: Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells. Development 127:2741–2750 (2000).

Tsvetkov AG: Electronic microscope study of the spreaded lampbrush chromosomes from the chicken oocytes. PhD thesis, Leningrad (1987).

Twito T, Ben-Ari G, Blum S, David L, Fleischer H, Linial N, et al: Discovery and genotyping of SNPs in chicken for gene hunting and the study of biodiversity, in 11th European Poultry Conference, August 6–10 Bremen, Germany (2002).

Twyman RM, Primrose SB: Techniques patents for SNP genotyping. Pharmacogenomics 4:67–79 (2003).

Uetz P: The EMBL Reptile Database. Peter Uetz and the European Molecular Biology Laboratory, Heidelberg (2004).

Ursitti JA, Fowler VM: Immunolocalization of tropomodulin, tropomyosin and actin in spread human erythrocyte skeletons. J Cell Sci 107:1633–1639 (1994).

Vallejo RL, Bacon LD, Liu HC, Witter RL, Groenen MAM, Hillel J, Cheng HH: Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens. Genetics 148:349–360 (1997).

van Haaften G, Vastenhouw NL, Nollen EA, Plasterk RH, Tijsterman M: Gene interactions in the DNA damage-response pathway identified by genome-wide RNA-interference analysis of synthetic lethality. Proc Natl Acad Sci USA 101:12992–12996 (2004).

van Tuinen M, Hedges SB: Calibration of avian molecular clocks. Mol Biol Evol 18:206–213 (2001).

van Tuinen M, Hedges SB: The effect of external and internal fossil calibrations on the avian evolutionary timescale. J Paleontol 78:45–50 (2004).

van Tuinen M, Sibley CG, Hedges SB: Phylogeny and biogeography of ratite birds inferred from DNA sequences of the mitochondrial ribosomal genes. Mol Biol Evol 15:370–376 (1998).

van Tuinen M, Sibley CG, Hedges SB: The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. Mol Biol Evol 17:451–457 (2000).

van Tuinen M, Butvill DB, Kirsch JA, Hedges SB: Convergence and divergence in the evolution of aquatic birds. Proc R Soc Lond B Biol Sci 268:1345–1350 (2001).

Varricchio DJ, Jackson F, Borkowski JJ, Horner JR: Nest and egg clutches of the dinosaur *Troodon formosus* and the evolution of avian reproductive traits. Nature 385:247–250 (1997).

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW: Serial analysis of gene expression. Science 270: 484–487 (1995).

Vignal A, Milan D, SanCristobal M, Eggen A: A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol 34: 275–305 (2002).

Voorrips RE: MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93: 77–78 (2002).

Wahl MB, Caldwell RB, Kierzek AM, Arakawa H, Eyras E, Hubner N, et al: Evaluation of the chicken transcriptome by SAGE of B cells and the DT40 cell line. BMC Genomics 5:98 (2004).

Wallis JW, Aerts J, Groenen MA, Crooijmans RP, Layman D, Graves TA, et al: A physical map of the chicken genome. Nature 432:761–764 (2004).

Wang DG, Fan JB, Siao C, Berno A, Young P, Sapolsky R, et al: Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077–1082 (1998).

Wang J, Takagaki Y, Manley JL: Targeted disruption of an essential vertebrate gene: ASF/SF2 is required for cell viability. Genes Dev 10:2588–2599 (1996).

Warren M, Smith A, Partridge N, Masabanda J, Griffin D, Ashworth A: Structural analysis of the chicken *BRCA2* gene facilitates identification of functional domains and disease causing mutations. Hum Mol Genet 11:841–851 (2002).

Weber T, Schmidt E, Scheer U: Mapping of transcription units of *Xenopus laevis* lampbrush chromosomes by in situ hybridization with biotin-labeled cDNA probes. Eur J Cell Biol 50:144–153 (1984).

White RA, Dowler LL, Woo M, Adkison LR, Pal S, Gershon D, Fowler VM: The tropomodulin (Tmod) gene maps to chromosome 4, closely linked to Mup1. Mamm Genome 6:332–333 (1995).

Wienberg J, Stanyon R: Chromosome painting in mammals as an approach to comparative genomics. Curr Opin Genet Dev 5:792–797 (1995).

Xu X, Zhou ZH, Wang XL, Kuang XW, Zhang FC, Du XK: Four-winged dinosaurs from China. Nature 421:335–340 (2003).

Yonash N, Bacon LD, Witter RL, Cheng HH: High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Anim Genetics 30:126–135 (1999).

Yonash N, Cheng HH, Hillel J, Heller DE, Cahaner A: DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. Poultry Sci 80:22–28 (2001).

Yoshimura T, Suzuki Y, Makino E, Suzuki T, Kuroiwa A, Matsuda Y, Namikawa T, Ebihara S: Molecular analysis of avian circadian clock genes. Brain Res Mol Brain Res 78:207–215 (2000).

Yuri T, Mindell DP: Molecular phylogenetic analysis of Fringillidae, "New World nine-primaried oscines" (Aves: Passeriformes). Mol Phylogenet Evol 23: 229–243 (2002).

Zaccanti F, Vallisneri M, Quaglia A: Early aspects of sex differentiation in the gonads of chick embryos. Differentiation 43:71–80 (1990).

Zechner U, Wilda M, Kehrer-Sawatzki H, Vogel W, Fundele R, Hameister H: A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? Trends Genet 17:697–701 (2001).

Zhou H, Lamont SJ: Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. Anim Genet 30:256–264 (1999).

Zhou H, Lamont SJ: Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. Immunogenetics 55:133–140 (2003).

Zimmer R, King WA, Verrinder Gibbins AM: Generation of chicken Z-chromosome painting probes by microdissection for screening large-insert genomic libraries. Cytogenet Cell Genet 78:124–130 (1997).

Zoorob R, Billault A, Severac V, Fillon V, Vignal A, Auffray C: Two chicken genomic libraries in the PAC and BAC cloning systems: Organization and characterization. Anim Genet 27, Suppl 2:69 (1996).