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First Report on Chicken Genes and Chromosomes 2000

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Galleto (young cock). Bronze sculpture by Giambologna (1529–1608). Museo Nazionale del Bargello, Florence

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Chicken genomics matures for the 21st century

(Prepared by D.W. Burt)

Chicken genetics has a rich history spanning almost 100 years, since Spillman (1908) showed that barring was sex-linked. The first genetic linkage map was published by Hutt (1936) followed by many revisions, with the latest published by Bitgood and Somes (1993). These early "classical" maps were based on feather colour, morphological, immunological and physiological genetic markers. As with most other livestock species, these classical maps have progressed slowly and ultimately 44 loci were mapped onto eight linkage groups. When international collaborative efforts to produce a molecular map of the chicken genome were established (Burt et al., 1995a; Burt and Cheng, 1998), progress and interest in the chicken as a model genome have accelerated. These collaborations started at the 23rd Conference of the International Society for Animal

Genetics (ISAG) in Interlaken, Switzerland in 1992. Two preliminary maps were published soon after (Bumstead and Palyga, 1992; Levin et al., 1994), culminating in a consensus map of almost 2,000 loci by Groenen et al. (2000). These collaborations have been aided by support from both national and international research programmes, in particular the ChickMap and Avianome projects within the EC Framework 4 Biotechnology programme.

The aim of this report was to summarise the progress made in the last eight years. Through the efforts of 34 authors in 21 departments, we have produced a report reviewing the current status of genomics in the chicken and other birds. This review summarises some successes that have highlighted to the wider scientific community the value of the chicken as a model species. Progress on the genetic and physical mapping of the chicken has come a long way; even the problem of identification of microchromosomes has been overcome by physical mapping methods. New tools open the way to examine the evolution, not only of avian genomes but also of other vertebrates. Analysis of the comparative map between chicken, mouse and human built on the gene mapping efforts of the chicken mapping community, provided new insights into the evolution of the vertebrate genome. The accumulation of over 20,000 chicken ESTs marks a new phase in the exploration of the avian genome and its function. We hope this review will be of interest and direct benefit not only to those in the poultry industry, but also to those in other fields, such as, medicine and developmental biology.

Chicken classical genetic maps

(Prepared by M. Tixier-Boichard)

The first "classical" genetic map of the chicken was established in 1936 by F.B. Hutt from the compilation of segregation analyses and chromosome studies. It was extensively reviewed and completed by Etches and Hawes (1973). The latest update included 119 loci identified by morphological mutations, biochemical polymorphisms or chromosome breakpoints (Bitgood and Somes, 1993). Map position was only available for 44 loci, grouped onto seven autosomal linkage groups and chromosome Z (Table 1). In addition, 38 loci were assigned to one of the groups but not mapped precisely, 12 loci were assigned to microchromosomes, and 16 loci were linked two by two but not assigned to any linkage group (Bitgood and Somes, 1990). Accuracy of map position was variable due to differences in sample size and linkage was sometimes expressed in crossover percentage rather than map unit (cM). The recent development of the genetic map based upon molecular markers makes it possible to establish linkage between the so-called "classical" mutations and anonymous markers (mainly microsatellites). This integrates both the former "classical" map and the molecular map, and opens the way to the molecular identification of mutations with major phenotypic effects. Molecular mapping of a mutant involves the production of experimental families that are segregating for one or several mutations. When there is no prior knowledge of the chromosome position of a mutant, mapping has to be done by screening the whole genome with molecular markers. The cost and effort of this approach can be efficiently reduced by using the strategy of bulked segregant analysis (Michelmore et al., 1991), where typings are done on pooled DNA samples prepared according to the phenotype.

Table 1. Linkage groups of the chicken classical map (Bitgood and Somes, 1990)

Linkage group	I	II	III	IV	V	VI	VII	VIII	X
Chromosome	2[a]	2[a]	1	2[a]	Z	W	4[b]	7	16
Number of mapped loci	3	4	12	3	17	-	-	1	4
Number of assigned loci	2	-	11	-	20	-	4	-	1

[a] LG I, II, IV were suggested to correspond to chromosomes 2, 3 and 4 (Bitgood and Somes, 1993), but the exact correspondence was not known.
[b] LG VII, previously assigned to chromosome 6, actually corresponds to chromosome 4, since one of the LG VII loci, ALB, maps to chromosome 4.

At present, the map position of 12 classical mutants and one blood group has been established following linkage with molecular markers (Table 2). The mapping of polydactyly mutation on 2p makes it possible to propose markers for other mutants from the former linkage group IV, *M* "multiple spurs" and *D* "duplex comb". Among former linkage groups of the classical map, group I which contains five mutations (creeper, rose comb, uropygial gland, lavender plumage color, and ametapodia) is the last one not to be connected with the molecular map. The integration of molecular and classical maps would also benefit from mapping of the crest and frizzle mutations, which flank the dominant white mutation on linkage group E22. As shown with the Henny-feathering mutant, prior knowledge of the candidate gene for a mutation also offers a tool to map the gene, either by FISH or by the identification of a polymorphism within the gene. Thus, it is expected that the number of "classical" mutants mapped with molecular markers will increase in the future, provided that carriers of these mutants are still kept as part of chicken genetic resources.

Genetic map

(Prepared by M.A.M. Groenen and H.H. Cheng)

In chicken three different populations have been used for the construction of a genetic map. The East Lansing population (Crittenden et al., 1993) consists of 52 BC1 animals derived from a backcross between a partially inbred Jungle Fowl line and a highly inbred White Leghorn line. The Compton population (Bumstead and Palyga, 1992) consists of 56 BC1 animals derived from a backcross between two inbred White Leghorn lines that differed in their resistance to salmonella. Reference panel DNAs from these two mapping populations are available and have been distributed widely to the poultry community. The Wageningen population (Groenen et al., 1998) consists of 456 F2 animals from a cross between two broiler dam lines originating from the White Plymouth Rock breed. The number of informative meioses for the two backcross populations var-

Table 2. Mapping "classical" mutants with molecular markers in the chicken

'Classical mutant'	Former map position[a]	Candidate gene or closest molecular marker	Current map position, estimated in cM from pter	References on molecular studies
Extension of eumelanin	chr. 1	alpha-MSH receptor, (*MC1R*)	*MC1R* maps to a micro-chromosome	Takeuchi et al.,1996 Sazanov et al., 1998
Autosomal dwarfism	nd	*LEI*0146	1p (169 cM)	Ruyter-Spira et al., 1998b
Pea-Comb	1p	*ALVE*1	1p (209 cM)	Bartlett et al., 1996
Blue egg-shell	1p	*ALVE*1	1p (203 cM)	Bartlett et al., 1996
Polydactyly	IV	*MCW*0071	2p (45 cM)	Pitel et al., 2000
Naked neck	chr.1 ?	*ADL*0237	3q (278 cM)	Pitel et al., 2000
Blood group P, *CPPP*	chr.1 ?	*MSU*0084	3q (317 cM)	Crittenden et al., 1993
Dominant white	II	*MCW*0188	E22 (0 cM)	Ruyter-Spira et al., 1997
Henny feathering	nd	aromatase gene (*CYP19*)	10 (30 cM)	Dunn et al., 1999
Nanomelia	nd	aggrecan *AGC1*	10 (71 cM)	Li et al., 1993 Jones et al., 1997
Dermal melanin inhibitor	Z	*MSU*0035	Z (214 cM)	Levin et al., 1993
Sex-linked dwarfism	Zp	Growth hormone receptor, *GHR*	Zp (52 cM)	Burnside et al., 1991, Crittenden et al., 1993
Late-feathering *K*	Zp	*ALVE*21	Zp	Bacon et al., 1988 Levin and Smith, 1990

[a] nd: Not determined.

ies from 20 to 56 and the average mapping resolution therefore is only 5–7 cM. In the Wageningen population the number of informative meioses varies from 15 to 886 with an average mapping resolution of 1 cM.

Recently the chicken linkage maps based on these three mapping populations have been integrated into one consensus linkage map (Groenen et al., 2000). Since that time 58 additional loci have been added to the East Lansing map, 4 to the Compton map and 20 to the Wageningen map. These loci have now been integrated into the consensus linkage map (Fig. 1) bringing the total number of loci that have been placed on this map to 1965. Furthermore, the eight anonymous markers *ROS0054, ROS0119, ROS0149, ROS0153, ROS0249, ROS0250, ROS310* and *ROS0332* represent *ERBB3, CCNC*, the *KIAA0677* homologue, *SUV3* (*SUPV3L1*), *CKM*, *LHCGR*, *SCML2* and *LEPR* respectively (Jacqueline Smith and Dave Burt, personal communication). This brings the number of mapped genes for which the map location of the human orthologue is known to 216. The genes that have been added to the map include *IFNG, PPARA* and *SOD1* on chromosome 1; *MOS* on chromosome 2; *SULT1A2, TNFR1* (*TNFRSF1A*) and *MFAP1* on chromosome 3; *CAT* and *CALM1* on chromosome 5; *INSR* on chromosome 28 (E53) and *FBN1* on chromosome 10. In all cases except for *PPARA*, the new mapping data extends or confirms known regions of conserved synteny between the chicken and human genomes. With regard to *PPARA*, other genes are located on chicken chromosome 1 that like *PPARA* have been mapped in human to 22q13 (*NAGA, ADSL, H5*), but these form a separate block of conserved synteny.

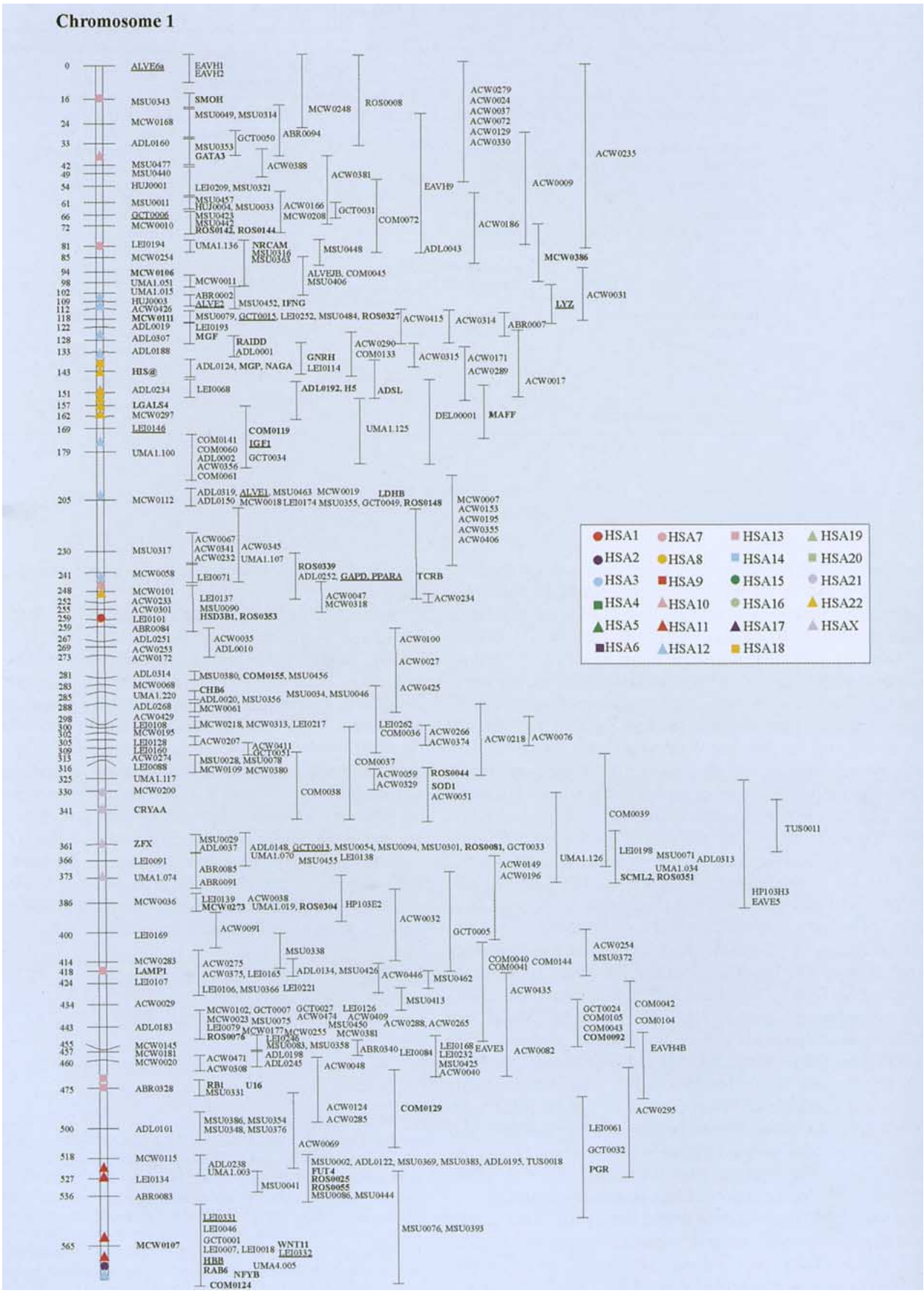
As discussed in the section on the integration of the genetic and physical maps (Fillon and Vignal, in this report), many of the small linkage groups of the consensus map have now been assigned to specific microchromosomes. Therefore, in Fig. 1 the names of these linkage groups have been changed accordingly.

Locus *HSF1* (*MCW0073*) was known to be linked to markers located at the end of chromosome 2 (*ADL0146, LEI0104* and *MCW0324*) but because this was not supported by any other locus on these two linkage groups, these linkage groups were thus far treated as two independent linkage groups. However, fluorescent in situ hybridisation experiments with a BAC clone from *MCW0157* located on linkage group E46C08W18, shows that this small linkage group is indeed the q terminal part of chromosome 2 (A. Vignal, V. Fillon and F. Pitel, personal communication). Therefore, E46C08W18 has now been added to the end of the linkage group of chromosome 2 (Fig. 1).

The mapping information of the new loci has been added to the newly developed chicken AceDB database ChickAce that will be available at the Wageningen chicken web site (http://www.zod.wau.nl/vf/research/chicken/frame_chicken.html).

(Fig. 1 see pages 174–181).

Fig. 1. Linkage map of the chicken genome. The framework loci (loci whose order relative to one another is supported by odds larger than 3) have been ordered and their position is indicated by the number to the left. The possible location of the loci whose order is not supported by odds > 3 is indicated by an error bar. The loci that have been mapped cytogenetically are underlined, whereas those known to represent expressed sequences (identified genes and ESTs) are shown in bold. For genes whose map location on the human map is known, the human chromosome is indicated with a coloured symbol on the vertical bar (Adapted from Groenen et al., 2000).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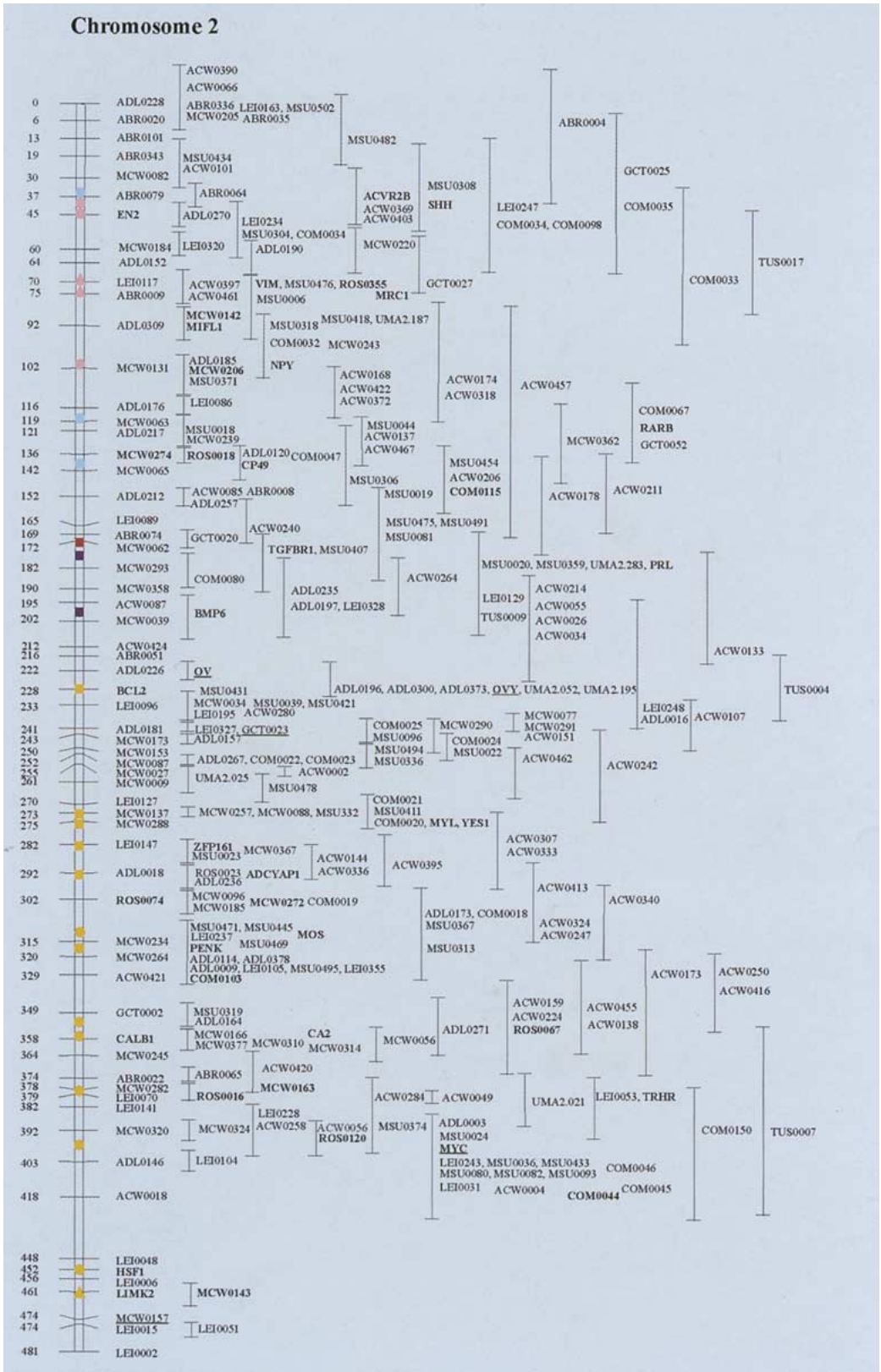
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30 MCW0082
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45 EN2
60 MCW0184
64 ADL0152
70 LEI0117
75 ABR0009
92 ADL0309
102 MCW0131
116 ADL0176
119 MCW0063
121 ADL0217
136 MCW0274
142 MCW0065
152 ADL0212
165 LEI0089
169 ABR0074
172 MCW0062
182 MCW0293
190 MCW0358
195 ACW0087
202 MCW0039
212 ACW0424
216 ABR0051
222 ADL0226
228 BCL2
233 LEI0096
241 ADL0181
243 MCW0173
250 MCW0153
252 MCW0087
255 MCW0027
261 MCW0009
270 LEI0127
273 MCW0137
275 MCW0288
282 LEI0147
292 ADL0018
302 ROS0074
315 MCW0234
320 MCW0264
329 ACW0421
349 GCT0002
358 CALB1
364 MCW0245
374 ABR0022
378 MCW0282
379 LEI0070
382 LEI0141
392 MCW0320
403 ADL0146
418 ACW0018
448 LEI0048
452 HSF1
456 LEI0006
461 LIMK2
474 MCW0157
474 LEI0015
481 LEI0002
ACW0390
ACW0066
ABR0336
MCW0205
LEI0163, MSU0502
ABR0035
MSU0482
ABR0004
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ACW0101
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ABR0064
ACVR2B
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MCW0220
LEI0320
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VIM, MSU0476, ROS0355
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LEI0129
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ACW0034
BMP6
ACW0133
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MCW0034, MSU0039, MSU0421
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ACW0280
LEI0248
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ACW0107
LEI0327, GCT0023
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ZFP161
MSU0023
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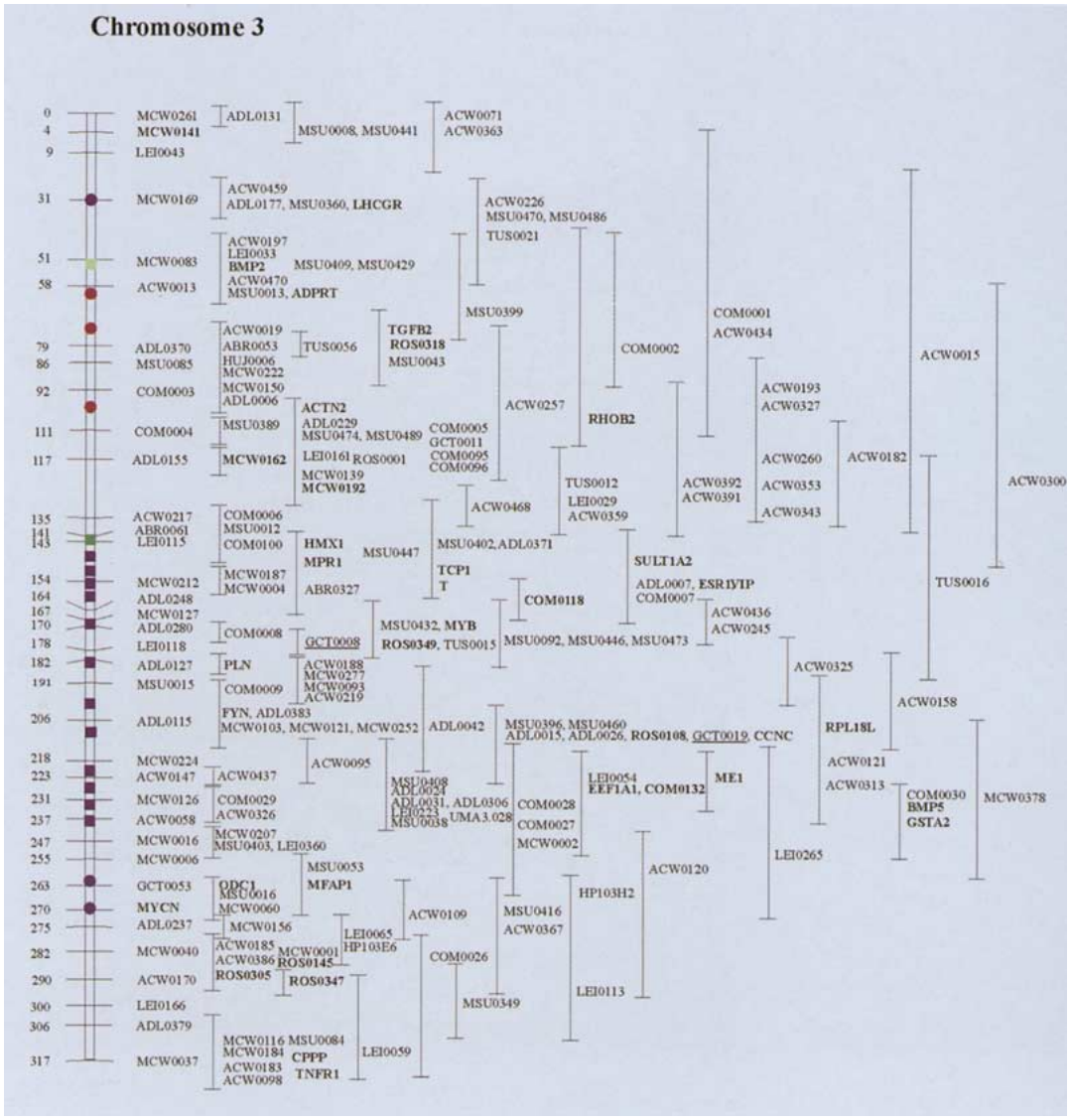
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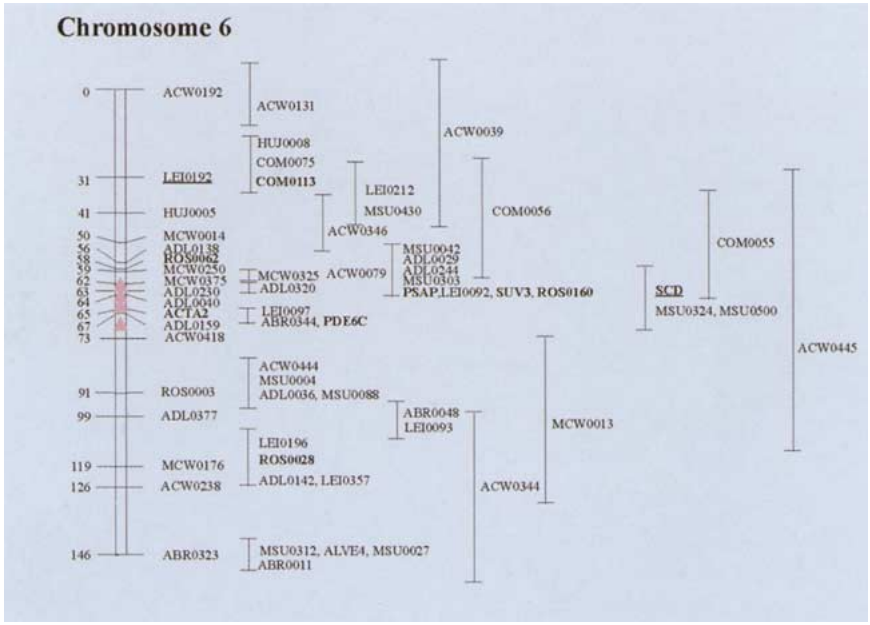
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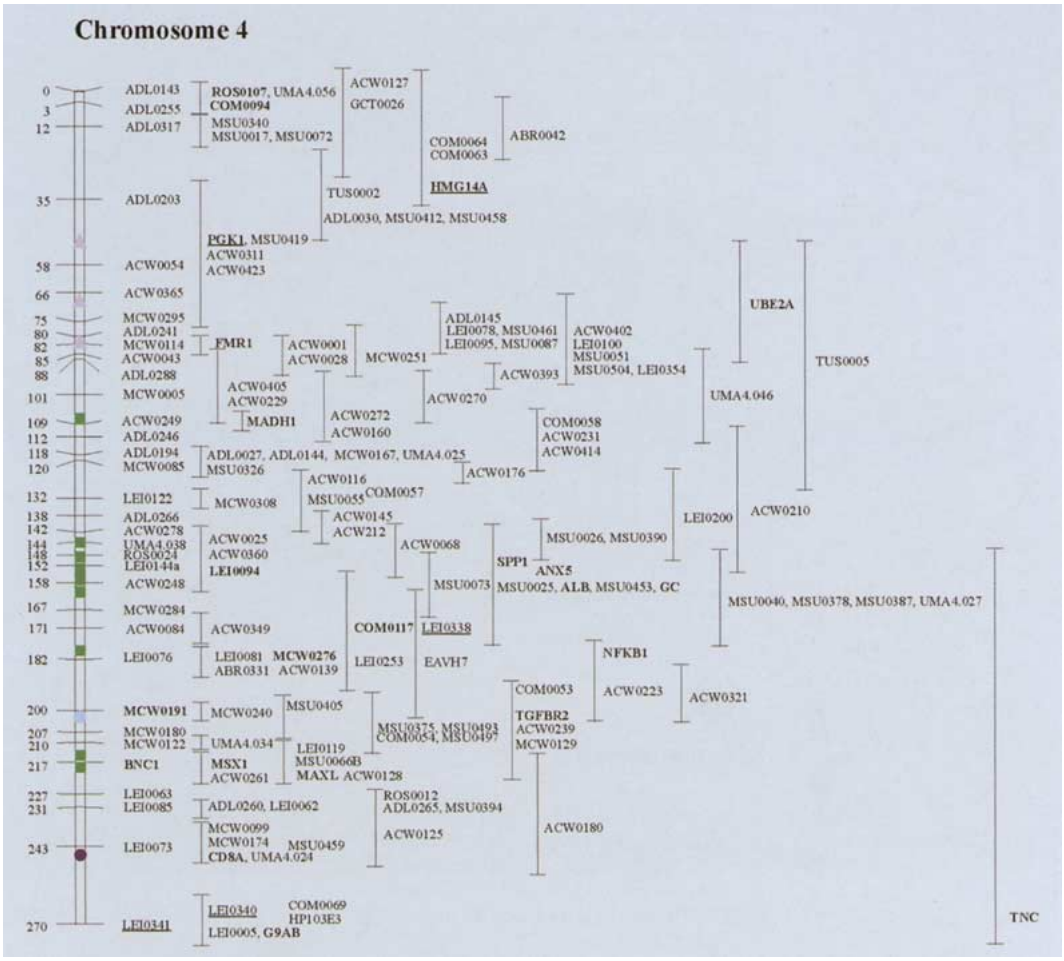
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200 MCW0191
207 MCW0180
210 MCW0122
217 BNC1
227 LEI0063
231 LEI0085
243 LEI0073
270 LEI0341
ROS0107, UMA4.056
COM0094
MSU0340
MSU0017, MSU0072
ACW0127
GCT0026
COM0064
COM0063
ABR0042
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HMG14A
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PGK1, MSU0419
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ADL0145
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LEI0095, MSU0087
ACW0402
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TUS0005
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ACW0160
ACW0270
COM0058
ACW0231
ACW0414
ACW0176
ADL0027, ADL0144, MCW0167, UMA4.025
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MCW0308
ACW0145
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MSU0026, MSU0390
ACW0025
ACW0360
LEI0094
SPP1
ANX5
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MSU0073
MSU0040, MSU0378, MSU0387, UMA4.027
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LEI0081
MCW0276
ABR0331
ACW0139
LEI0253
EAVH7
NFKB1
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ACW0321
COM0053
TGFBR2
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UMA4.034
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MAXL ACW0128
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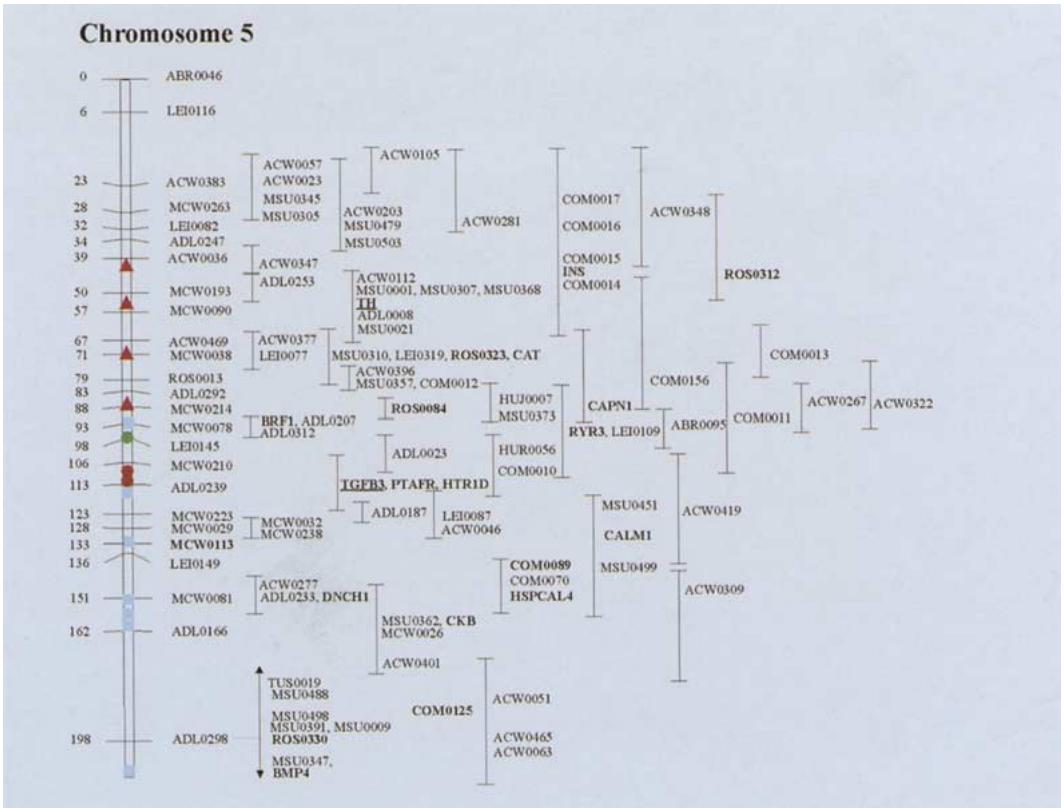
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0 ABR0046
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28 MCW0263
32 LEI0082
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57 MCW0090
67 ACW0469
71 MCW0038
79 ROS0013
83 ADL0292
88 MCW0214
93 MCW0078
98 LEI0145
106 MCW0210
113 ADL0239
123 MCW0223
128 MCW0029
133 MCW0113
136 LEI0149
151 MCW0081
162 ADL0166
198 ADL0298
ACW0057
ACW0023
MSU0345
MSU0305
ACW0105
ACW0203
MSU0479
MSU0503
ACW0281
COM0017
COM0016
COM0015
INS
COM0014
ACW0348
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ACW0347
ADL0253
ACW0112
MSU0001, MSU0307, MSU0368
TH
ADL0008
MSU0021
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LEI0077
MSU0310, LEI0319, ROS0323, CAT
ACW0396
MSU0357, COM0012
COM0156
COM0013
ROS0084
HUJ0007
MSU0373
CAPN1
RYR3, LEI0109
ABR0095
COM0011
ACW0267
ACW0322
BRF1, ADL0207
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HUR0056
COM0010
TGFB3, PTAFR, HTR1D
ADL0187
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COM0089
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HSPCAL4
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ACW0277
ADL0233, DNCH1
MSU0362, CKB
MCW0026
ACW0309
ACW0401
TUS0019
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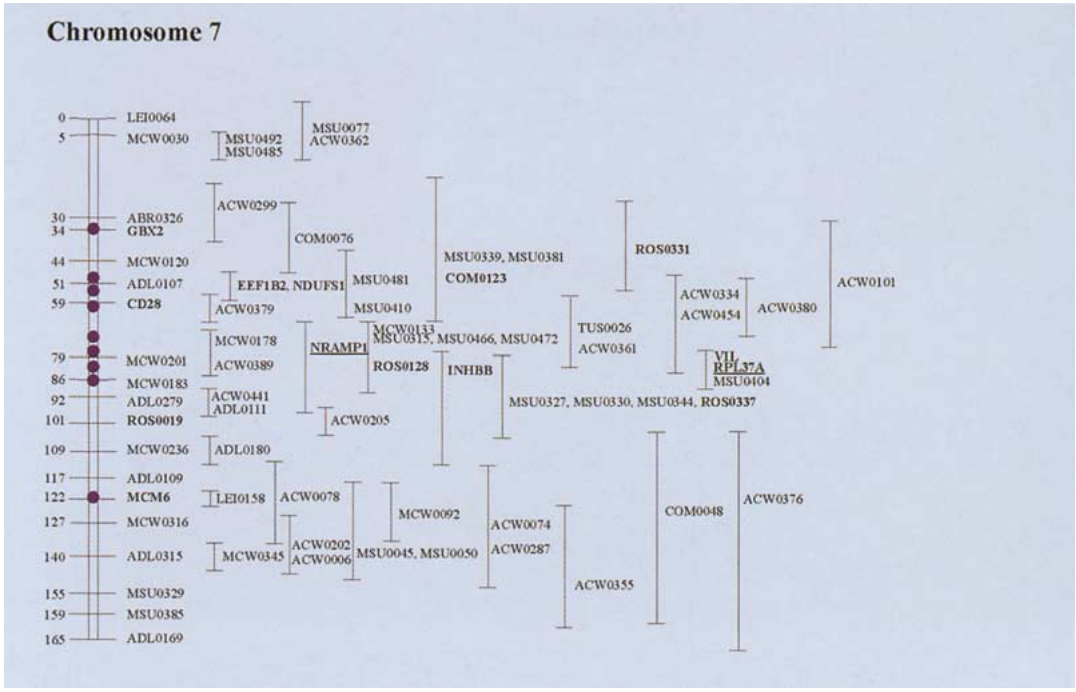
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51 ADL0107
59 CD28
79 MCW0201
86 MCW0183
92 ADL0279
101 ROS0019
109 MCW0236
117 ADL0109
122 MCM6
127 MCW0316
140 ADL0315
155 MSU0329
159 MSU0385
165 ADL0169
MSU0492
MSU0485
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ACW0362
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COM0076
EEF1B2, NDUFS1
MSU0481
MSU0410
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NRAMP1
MCW0133
MSU0313, MSU0466, MSU0472
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INHBB
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COM0123
ROS0331
TUS0026
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VIL
RPL37A
MSU0404
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ADL0111
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MSU0327, MSU0330, MSU0344, ROS0337
ADL0180
LEI0158
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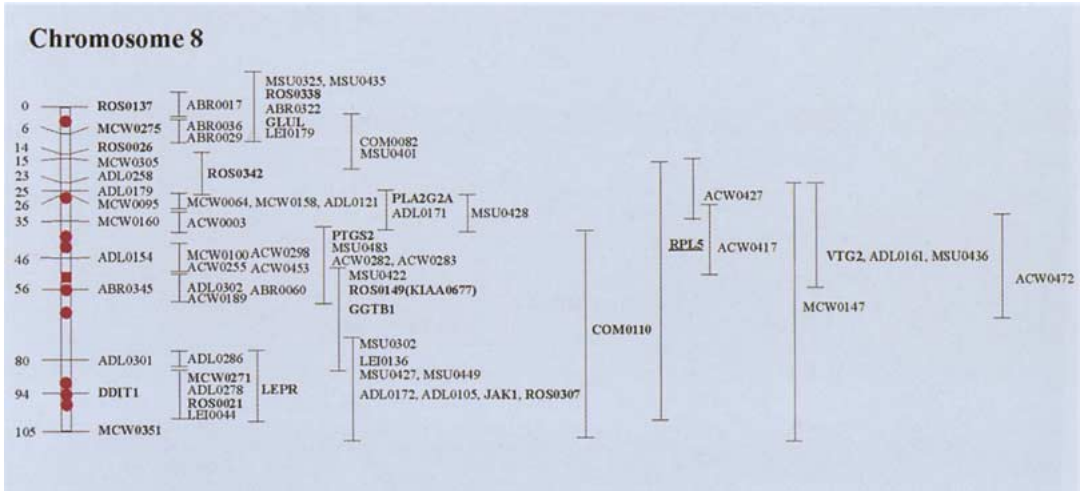
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6 MCW0275
14 ROS0026
15 MCW0305
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25 ADL0179
26 MCW0095
35 MCW0160
46 ADL0154
56 ABR0345
80 ADL0301
94 DDIT1
105 MCW0351
ABR0017
ABR0036
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ROS0338
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GLUL
LEI0179
COM0082
MSU0401
ROS0342
MCW0064, MCW0158, ADL0121
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GGTB1
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MCW0271
ADL0278
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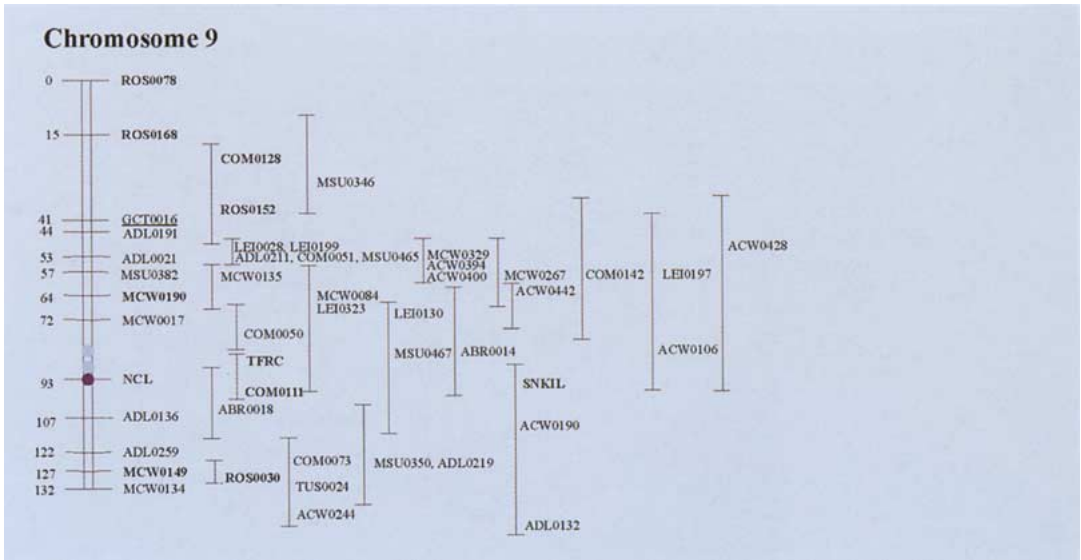
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15 ROS0168
41 GCT0016
44 ADL0191
53 ADL0021
57 MSU0382
64 MCW0190
72 MCW0017
93 NCL
107 ADL0136
122 ADL0259
127 MCW0149
132 MCW0134
COM0128
MSU0346
ROS0152
LEI0028, LEI0199
ADL0211, COM0051, MSU0465
MCW0135
MCW0084
LEI0323
COM0050
TFRC
COM0111
ABR0018
ROS0030
COM0073
TUS0024
ACW0244
MSU0350, ADL0219
LEI0130
MSU0467
ABR0014
MCW0329
ACW0394
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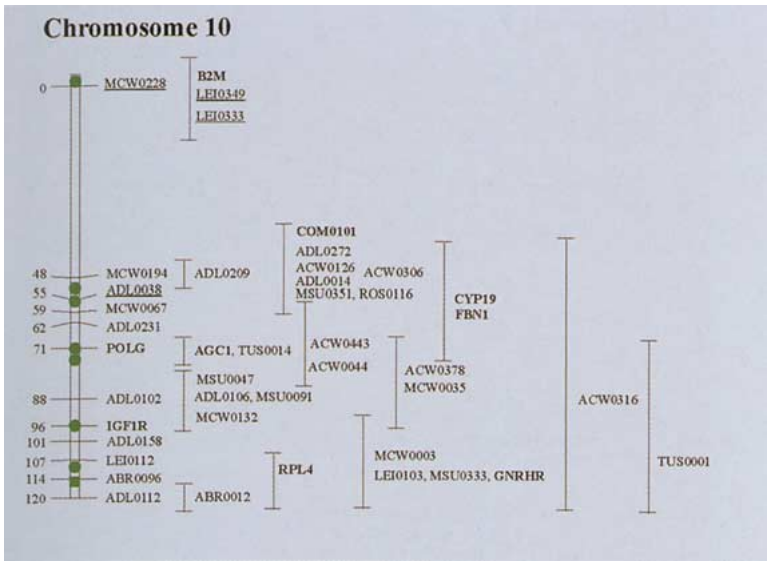
Chromosome 10
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LEI0349
LEI0333
48 MCW0194
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59 MCW0067
62 ADL0231
71 POLG
88 ADL0102
96 IGF1R
101 ADL0158
107 LEI0112
114 ABR0096
120 ADL0112
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AGC1, TUS0014
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ADL0106, MSU0091
MCW0132
ABR0012
COM0101
ADL0272
ACW0126
ADL0014
MSU0351, ROS0116
ACW0306
ACW0443
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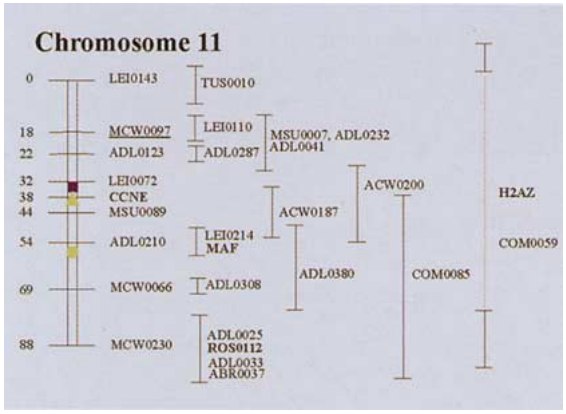
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0 LEI0143
18 MCW0097
22 ADL0123
32 LEI0072
38 CCNE
44 MSU0089
54 ADL0210
69 MCW0066
88 MCW0230
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ADL0287
MSU0007, ADL0232
ADL0041
LEI0214
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ADL0025
ROS0112
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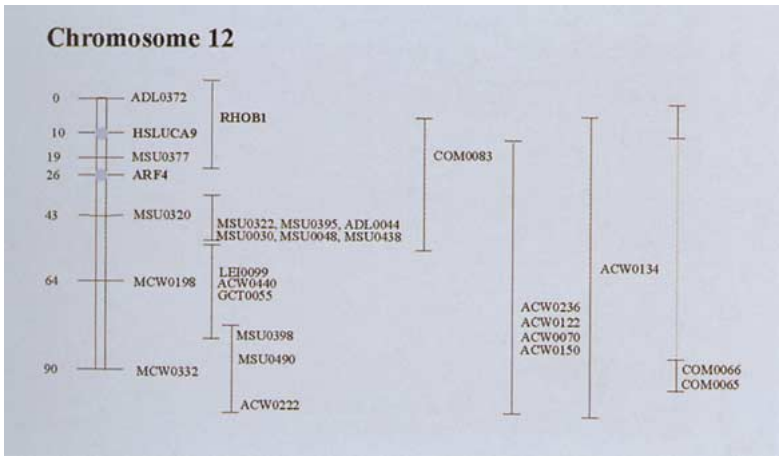
Chromosome 12
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10 HSLUCA9
19 MSU0377
26 ARF4
43 MSU0320
64 MCW0198
90 MCW0332
RHOB1
MSU0322, MSU0395, ADL0044
MSU0030, MSU0048, MSU0438
LEI0099
ACW0440
GCT0055
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COM0083
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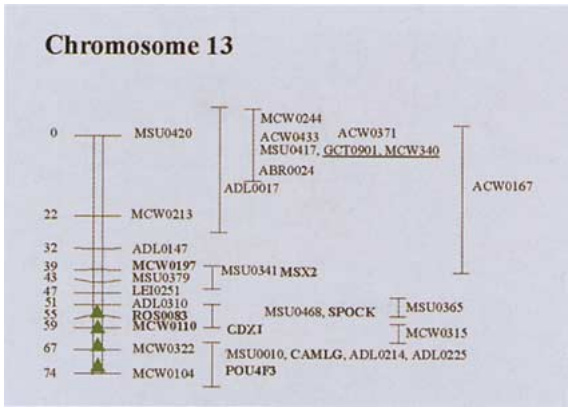
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22 MCW0213
32 ADL0147
39 MCW0197
43 MSU0379
47 LEI0251
51 ADL0310
55 ROS0083
59 MCW0110
67 MCW0322
74 MCW0104
MCW0244
ACW0433
ACW0371
MSU0417, GCT0901, MCW340
ABR0024
ADL0017
ACW0167
MSU0341 MSX2
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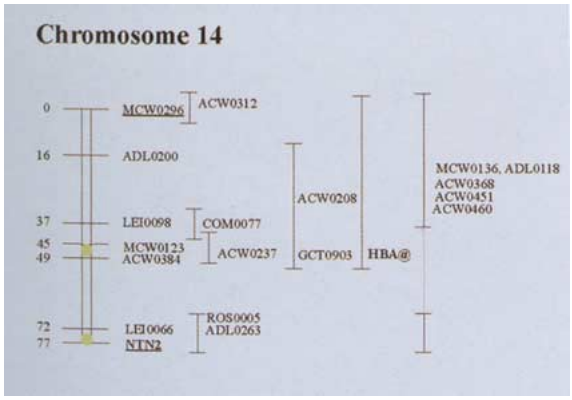
POU4F3


Chromosome 14
0 MCW0296
16 ADL0200
37 LEI0098
45 MCW0123
49 ACW0384
72 LEI0066
77 NTN2
ACW0312
COM0077
ACW0237
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GCT0903
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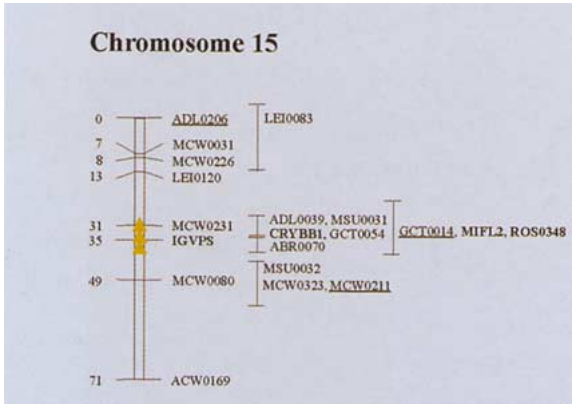
ACW0460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0 ADL0206
7 MCW0031
8 MCW0226
13 LEI0120
31 MCW0231
35 IGVPS
49 MCW0080
71 ACW0169
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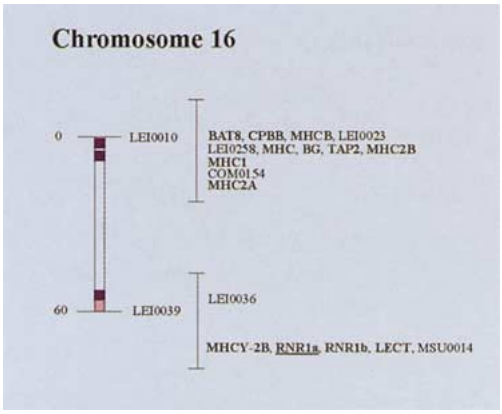
MSU0032
MCW0323, MCW0211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LEI0258, MHC, BG, TAP2, MHC2B
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MHC2A
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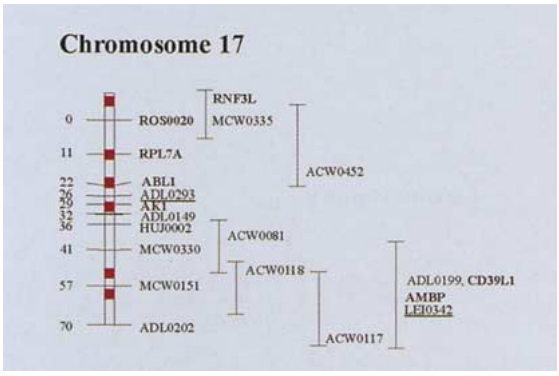
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MCW0335
ACW0452
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RPL7A
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ABL1
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ADL0293
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AK1
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ADL0149
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HUJ0002
ACW0081
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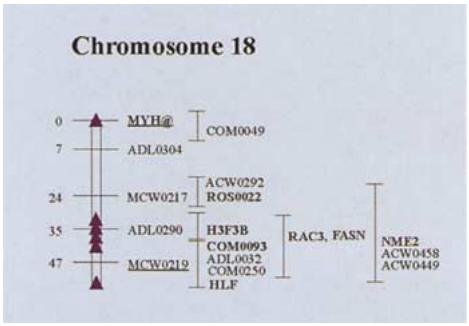
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COM0049
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ADL0304
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MCW0217
ACW0292
ROS0022
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ADL0290
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COM0250
HLF
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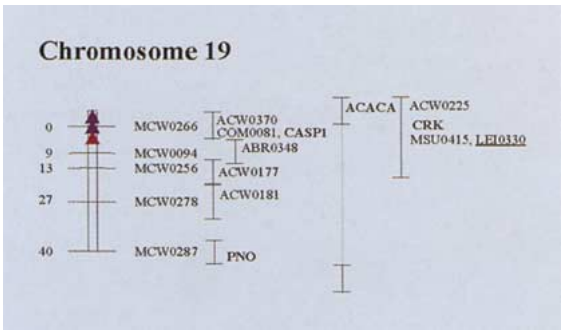
MCW0219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CRK
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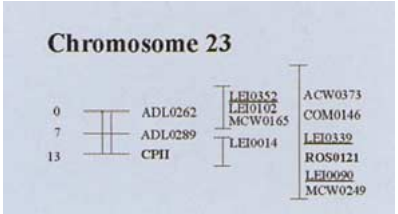
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CPII
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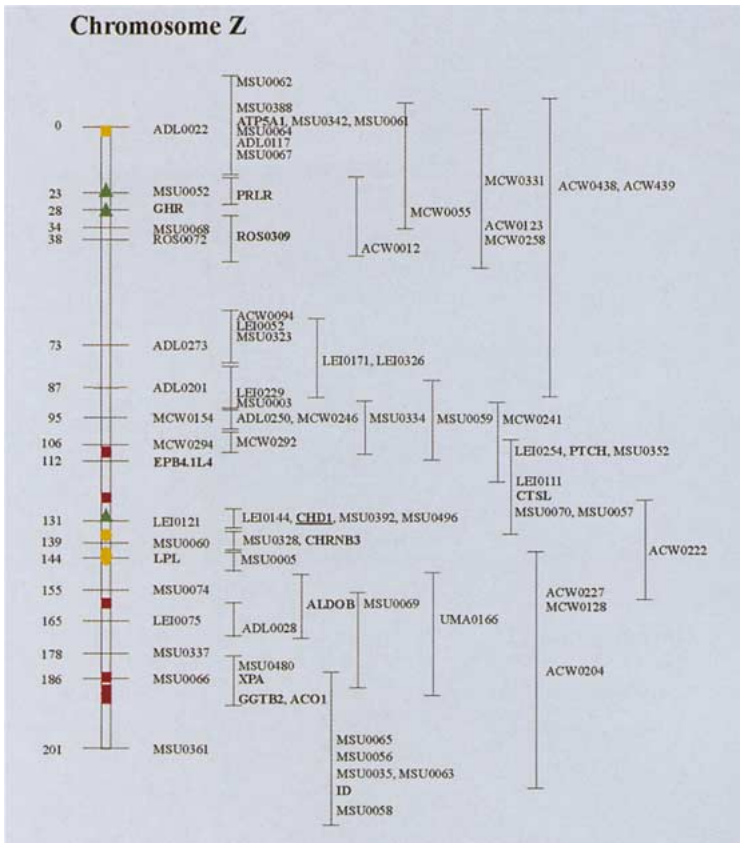
MCW0249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ATP5A1, MSU0342, MSU0061
MSU0064
ADL0117
MSU0067
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MSU0052
PRLR
28
GHR
34
MSU0068
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ROS0072
ROS0309
ACW0012
MCW0055
MCW0331
ACW0438, ACW439
ACW0123
MCW0258
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ADL0273
ACW0094
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MSU0323
LEI0171, LEI0326
87
ADL0201
LEI0229
MSU0003
95
MCW0154
ADL0250, MCW0246
MSU0334
MSU0059
MCW0241
106
MCW0294
MCW0292
112
EPB4.1L4
LEI0254, PTCH, MSU0352
LEI0111
CTSL
MSU0070, MSU0057
ACW0222
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LEI0121
LEI0144, CHD1, MSU0392, MSU0496
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MSU0060
MSU0328, CHRNB3
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LPL
MSU0005
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MSU0074
ALDOB
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ACW0227
MCW0128
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LEI0075
ADL0028
UMA0166
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MSU0337
MSU0480
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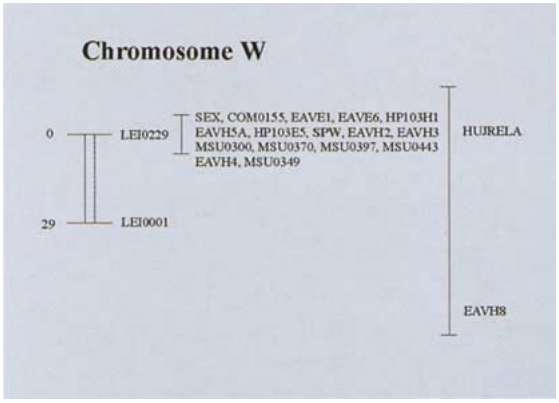
MSU0058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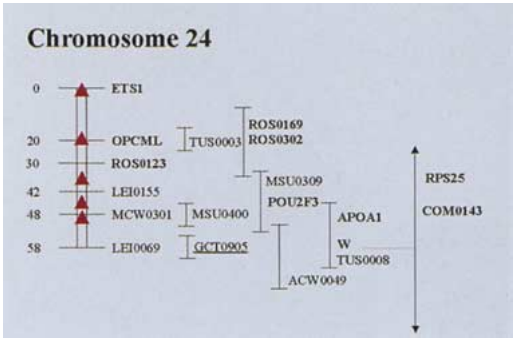
EAVH4, MSU0349
HUJRELA
29
LEI0001
EAVH8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ETS1
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OPCML
TUS0003
ROS0169
ROS0302
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ROS0123
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POU2F3
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LEI0069
GCT0905
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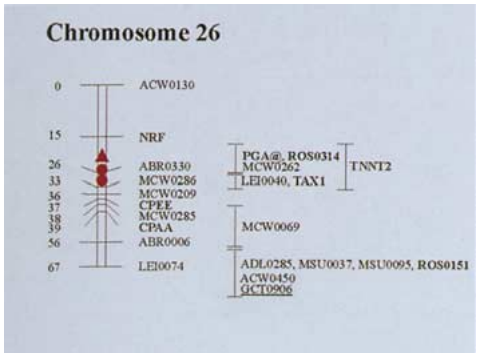
TUS0008
ACW0049


Chromosome 26
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15 NRF
26 ABR0330
33 MCW0286
36 MCW0209
37 CPEE
38 MCW0285
39 CPAA
56 ABR0006
67 LEI0074
PGA@, ROS0314
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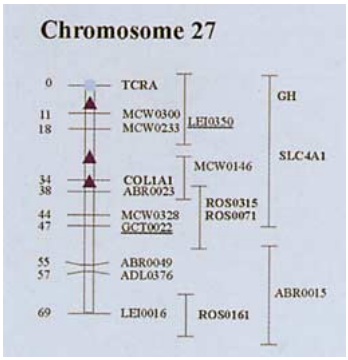
ACW0450
GCT0906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18 MCW0233
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MCW0146
SLC4A1
34 COL1A1
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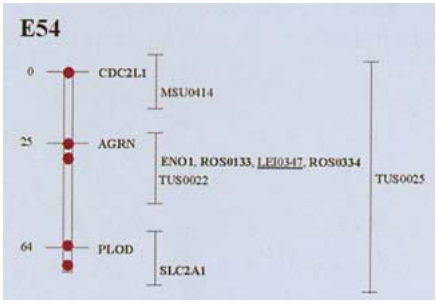
ROS0161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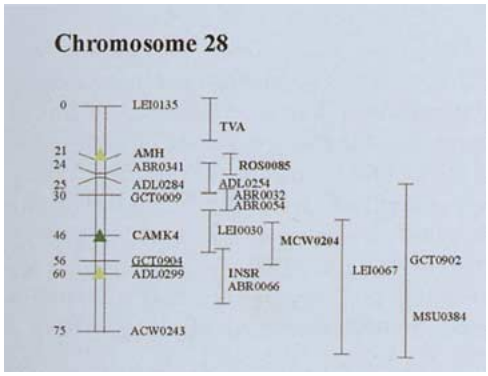
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TVA
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ROS0085
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ADL0254
ABR0032
ABR0054
46 CAMK4
LEI0030
MCW0204
56 GCT0904
60 ADL0299
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ABR0066
LEI0067
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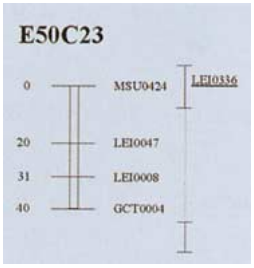
75 ACW0243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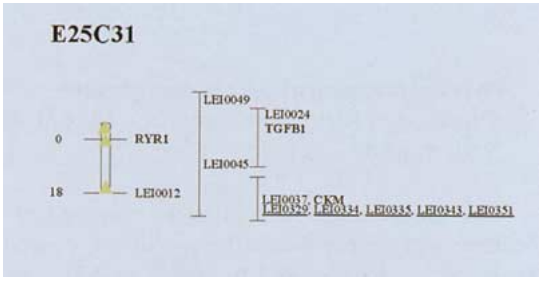
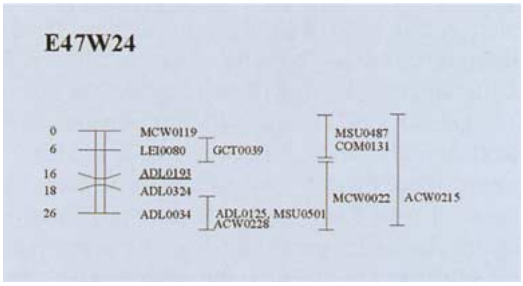
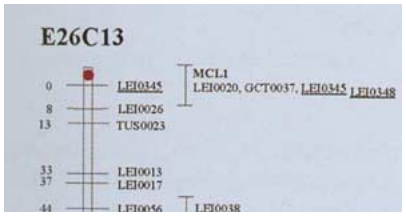
LEI0336
20 LEI0047
31 LEI0008
40 GCT0004


E25C31
LEI0049
LEI0024
TGFB1
0 RYR1
LEI0045
18 LEI0012
LEI0017, CKM
LEI0329, LEI0334, LEI0335, LEI0343, LEI0351


E47W24
0 MCW0119
6 LEI0080
GCT0039
MSU0487
COM0131
16 ADL0193
18 ADL0324
26 ADL0034
ADL0125, MSU0501
ACW0228
MCW0022
ACW0215


E26C13
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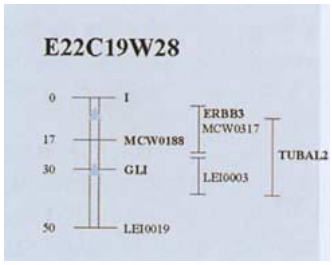
MCL1
LEI0020, GCT0037, LEI0345 LEI0348
8 LEI0026
13 TUS0023
33 LEI0013
37 LEI0017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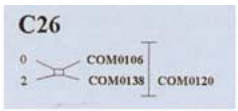
ERBB3
MCW0317
17 MCW0188
TUBAL2
30 GLI
LEI0003
50 LEI0019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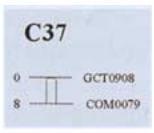
COM0120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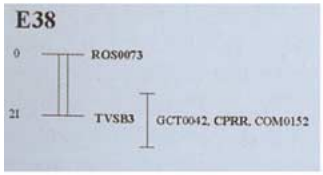
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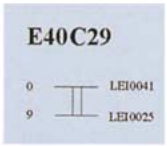
21 TVSB3
GCT0042, CPRR, COM0152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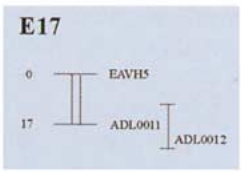
9 LEI0025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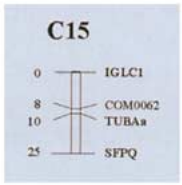
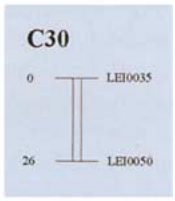
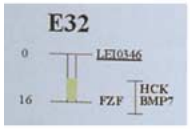
0 EAVH5
17 ADL0011
ADL0012


C15
0 IGLC1
8 COM0062
10 TUBA8
25 SFPQ


C30
0 LEI0035
26 LEI0050


E32
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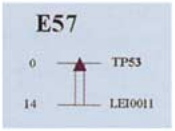
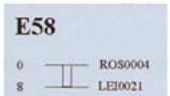
16 FZF
HCK
BMP7


E57
0 TP53
14 LEI0011


E58
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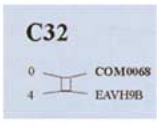
0 HSP5A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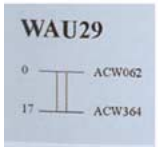
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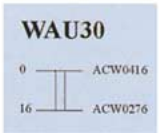
WAU30
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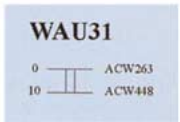
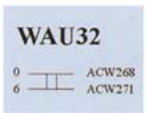
16 ACW0276


WAU31
0 ACW263
10 ACW448


WAU32
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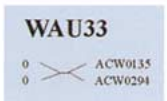
6 ACW271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0 ACW0135
0 ACW0294

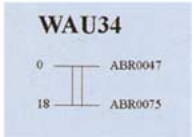

WAU34
0 ABR0047
18 ABR0075

Table 3. Current status of physical mapping with BAC clones

Chromosome	Size (cM)	Markers on the linkage map	BACs isolated	STSs markers developed	Genes identified	Estimated coverage	Homology found with
1 (NK-region)	–	–	20	–		1 Mb	HSA12
5 (Sall-region)	–	–	78	–		2 Mb	HSA14
8 (qtel)	30	12	107	44	10	20%	HSA1
10	120	41	600	240	100	40%	HSA15
11	88	27	16	–	3	5%	HSA19
13	74	33	131	30	7	13%	HSA5
15	71	19	101	40	8	10%	HSA22
24	58	16	102	22	9	10%	HSA11
28	75	20	157	57	23	20%	HSA19

Physical mapping of the chicken genome

(Prepared by R.P.M.A. Crooijmans, M.A.M. Groenen and N. Bumstead)

The ultimate goal for physical mapping of the chicken genome will be the establishment of the complete sequence analogous to human and mouse. The development of large insert libraries in chicken will enable the generation of a complete contig needed to achieve this goal. Two types of large insert libraries have been developed in chicken. A chicken yeast artificial chromosome (YAC) library has been developed by Toye et al. (1997) and this library provides an 8.5-fold redundant coverage and consists of 16,000 clones with an average insert size of 634 kb. YAC clones are efficient in covering large physical areas and are easier to develop. This system has several drawbacks, in particular, insert instability. Therefore, the establishment of another type of large insert library, the bacterial artificial chromosome (BAC) library was constructed.

Two BAC libraries have been constructed in chicken. In the first library (Crooijmans et al., 2000) the *Hin*dIII cloning site was used and consists of almost 50,000 clones with an average insert size of 134 kb and genome coverage of 5.5×. Screening of this library is possible by two-dimensional PCR and by filter hybridization (see webpage http://www/zod.wau.nl/vf/research/chicken). The other library was constructed using the *Bam*HI cloning site, consists of 38,000 clones with an average insert size of 150 kb and has a genome coverage of 5× (J. Dodgson, personal communication). Screening of this library is possible by filter hybridization (see webpage http://hbz.tamu.edu and http://poultry.mph.msu.edu).

Using the Wageningen BAC library, at least one BAC clone has been isolated for markers that have been mapped at 10-cM intervals on the chicken linkage map. Furthermore, in order to be able to integrate the linkage and cytogenetic maps, BAC clones have been isolated with markers from almost every linkage group of the consensus linkage map.

For several linkage groups BAC contigs are currently being developed (Table 3). Much effort is being put into building a complete BAC contig for chicken chromosome 10 (former linkage group E29C09W09) by chromosome walking. This has already resulted in the identification of 600 BAC clones, assembled into 30 contigs and covering almost 40% of this chromosome.

In total more than 1,500 BAC clones have been isolated from the Wageningen BAC library and assigned to linkage groups, which represents a genome coverage of almost 5%. Recently, physical mapping of the complete Wageningen chicken BAC library was started, which will result in a BAC contig map of the complete chicken genome by the end of 2001. Physical mapping of the chicken *Bam*HI BAC library is also performed by fingerprinting (J. Dodgson, personal communication). To date, already 10,000 clones have been fingerprinted.

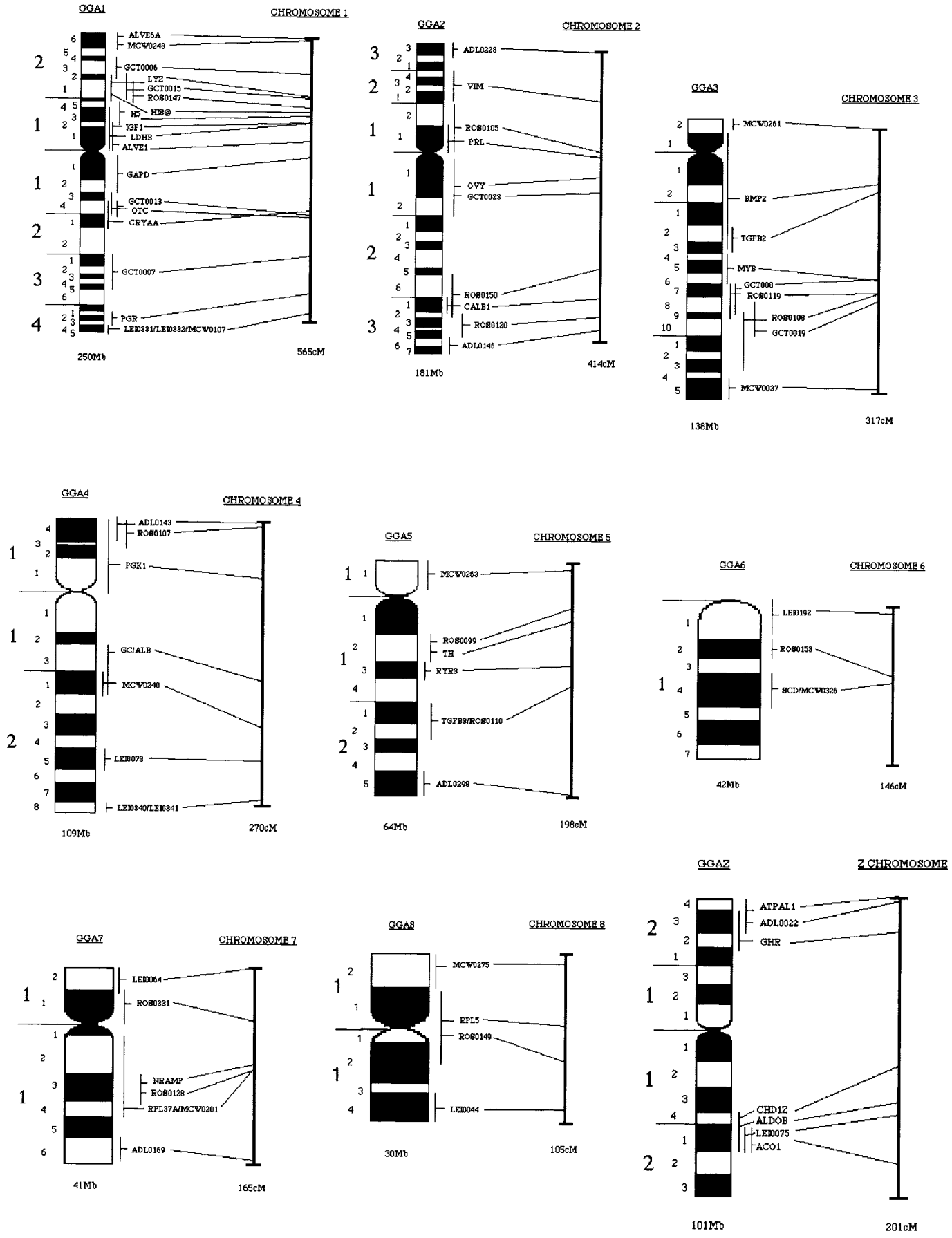
Integration of the genetic and physical maps of the chicken macrochromosomes

(Prepared by J. Smith, V. Fillon, R.P.M.A. Crooijmans and D.W. Burt)

The chicken karyotype comprises 39 pairs of chromosomes which are divided into eight pairs of cytologically distinct chromosomes 1–8 along with the Z and W sex chromosomes and 30 pairs of small, cytologically indistinguishable "microchromosomes". Standardization of the cytogenetic banding patterns has been established for the eight largest chromosomes and the sex chromosomes (Ladjali-Mohammedi et al., 1999), which means we can now relate genetic mapping data to the cytogenetic maps for each macrochromosome. An alignment of genetic and physical maps of the eight macrochromosomes and the Z chromosome are presented here (Fig. 2). Physically mapped clones are shown orientated with the consensus linkage maps (Groenen et al., 2000). The orientation of linkage groups E01C01C11W01 (chr 1), E06C02W02 (chr 2), E02C03W03 (chr 3), E05C04W04 (chr 4), E07E34C05W05 (chr 5), E11C10W06 (chr 6), E45C07W07 (chr 7), E43C12W11 (chr 8) and the Z chromosome has been established. The clones, which have been both genetically and physically mapped for each chromosome are shown in Table 4.

We have correlated genetic (cM) to physical distance (FLpter) for the macrochromosomes as shown in Fig. 3 that allows

Fig. 2. Integration of the genetic and physical maps of the chicken macrochromosomes. Physical ideograms represent the RBG banding pattern. Physical sizes are taken from Smith and Burt (1998) and the genetic sizes represent the current status of the consensus linkage groups.


GGA1
CHROMOSOME 1
ALVE6A
MCW0248
GCT0006
LYZ
GCT0015
ROS0147
H5
HBG@
IGF1
LDHB
ALVE1
GAPD
GCT0013
OTC
CRYAA
GCT0007
PGR
LEI0331/LEI0332/MCW0107
250Mb
565cM
GGA2
CHROMOSOME 2
ADL0228
VIM
ROS0105
PRL
OVY
GCT0023
ROS0150
CALB1
ROS0120
ADL0146
181Mb
414cM
GGA3
CHROMOSOME 3
MCW0261
BMP2
TGFB2
MYB
GCT008
ROS0119
ROS0108
GCT0019
MCW0037
138Mb
317cM
GGA4
CHROMOSOME 4
ADL0143
ROS0107
PGK1
GC/ALB
MCW0240
LEI0073
LEI0340/LEI0341
109Mb
270cM
GGA5
CHROMOSOME 5
MCW0263
ROS0099
TH
RYR3
TGFB3/ROS0110
ADL0298
64Mb
198cM
GGA6
CHROMOSOME 6
LEI0192
ROS0153
SCD/MCW0326
42Mb
146cM
GGAZ
Z CHROMOSOME
ATPAL1
ADL0022
GHR
CHD1Z
ALDOB
LEI0075
ACO1
101Mb
201cM
GGA7
CHROMOSOME 7
LEI0064
ROS0331
NRAMP
ROS0128
RPL37A/MCW0201
ADL0169
41Mb
165cM
GGA8
CHROMOSOME 8
MCW0275
RPL5
ROS0149
LEI0044
30Mb
105cM

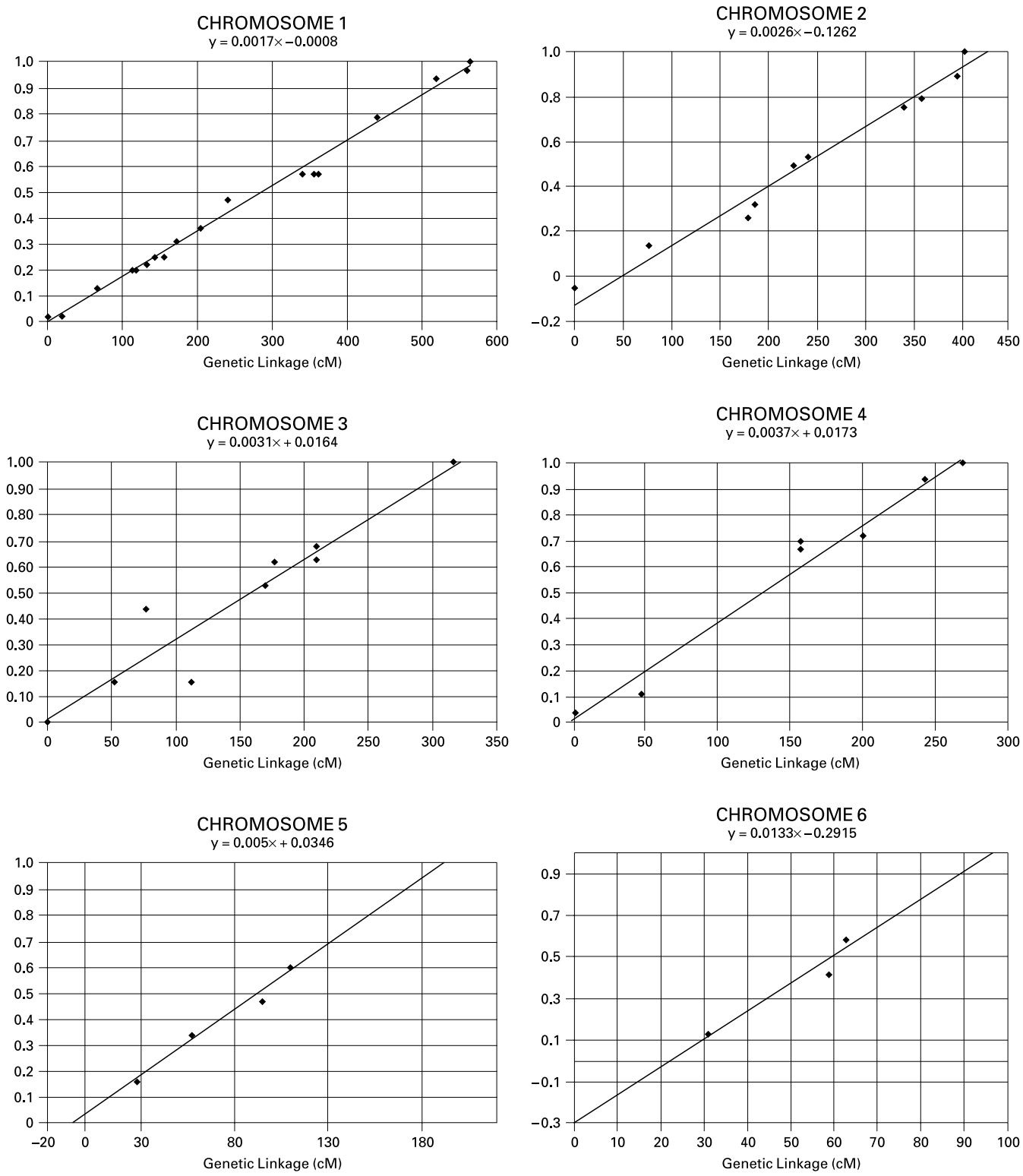
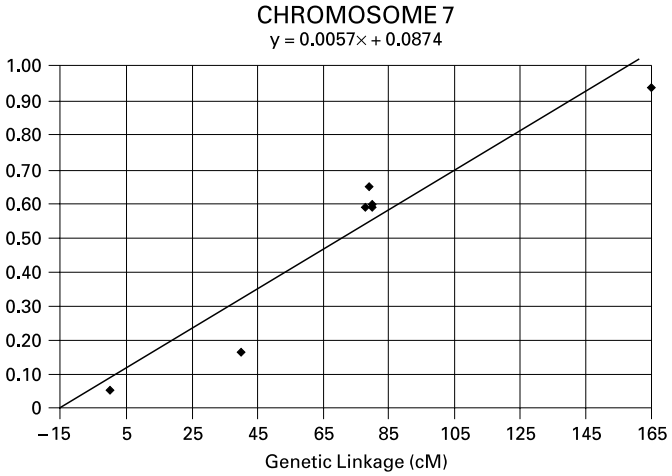
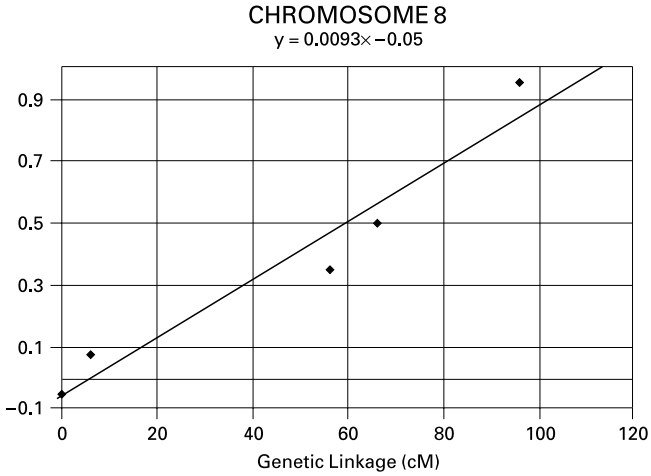
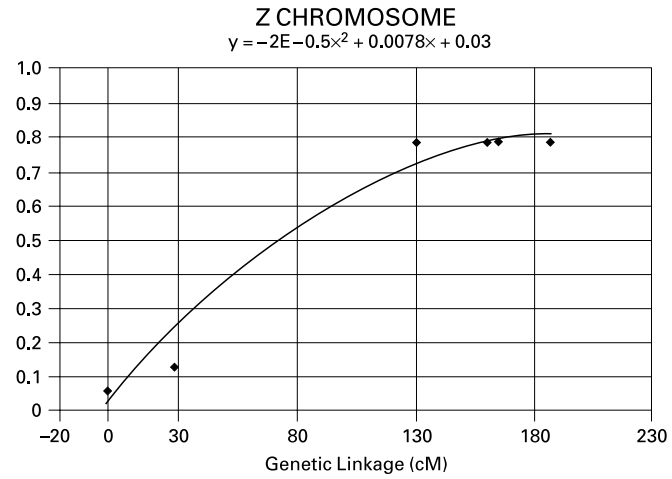


Fig. 3. Correlation of genetic linkage (cM) with physical distance (FLpter) for the eight macrochromosomes and the Z chromosome of chicken.







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. Some of this work has been recently published in Smith et al. (2000a). Current physical and genetic mapping data on the chicken is available at http://www.ri.bbsrc.ac.uk/chickmap/.

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. It is preferable if physical positions can be given relative to the RBG standard, as this is the reference that most of the current data has been measured against.

Integration of genetic and physical maps of chicken microchromosomes reveals high rates of recombination

(Prepared by V. Fillon and A. Vignal)

The standard GTG- and RBG-karyotypes of chicken have been established by the International Committee for the Standardization of the Avian Karyotype for the eight pairs of macrochromosomes and the Z and W sexual chromosomes (Ladjali-Mohammedi et al., 1999). Since they cannot be distinguished individually, the 30 pairs of microchromosomes are ordered arbitrarily by decreasing size and only an estimation of the chromosome number can be given. The physical size of chicken microchromosomes has been estimated to be 7–23 Mb (Bloom et al., 1993). Based on fluorescence measurement, it has been calculated that microchromosomes represent 23% of the female genome (Smith and Burt, 1998). Electron microscopic analyses of chicken synaptonemal complexes show they are mostly acrocentric (Kaelbling and Fechheimer, 1983). Although it is impossible to obtain characteristic banding patterns for microchromosome pairs, certain features of their structure and functions have been studied (for review, see Rodionov, 1996; Fillon, 1998). They have been shown to have an early replicating pattern (Schmid et al., 1989; Ponce de Leon et al., 1992; McQueen et al., 1998), results consistent with their high (G+C) content, as suggested by DAPI or chromomycin A3 staining (Fritschi and Stranzinger, 1985; Auer et al., 1987). The existence of a higher gene density on microchromosomes than on macrochromosomes has been suggested (McQueen et al., 1996; McQueen et al., 1998; Smith et al., 2000b; Smith, in this report).

The chicken consensus linkage map is composed of a few large linkage groups, that have been assigned to macrochromosomes, and numerous small linkage groups or independent markers probably corresponding to microchromosomes (Groenen et al., 2000), but for which a cytogenetic localisation had to be defined to enable a precise localisation of genes and markers on the microchromosome fraction with a correct chromosome identification. This is also necessary for the integration and completion of genetic maps. For this purpose, a collection of large insert BAC and PAC clones (Zoorob et al., 1996; Crooijmans et al., 2000) were used as microchromosome tags for identification in two-colour FISH experiments (Fillon et al., 1998) from which 22 individual microchromosome pairs can be identified (Fig. 4a). A nomenclature, based on the estimated size of each labeled microchromosome pair is proposed (Table 5) and is used throughout this report. The genetic marker-containing clones led to the integration of genetic and cytogen-

Table 4. Summary of loci which have been genetically and physically mapped in the chicken

Locus	Physical position	FLpter	cM (consensus)	Reference
Chromosome 1				
ALVE6A	1p26	0.02	0	Smith et al., 2000a
MCW0248	1p26	0.01	0	Fillon and Crooijmans[b]
GCT0006	1p24–p22	0.13	66	Morisson et al.,1998
LYZ	1p22–p15	0.20[a]	112	Smith et al., 2000a
GCT0015	1p22–p21	0.21	118	Morisson et al.,1998
ROS0147	1p21–p15	0.22[a]	132	Smith et al., 2000a
HIS@	1p22–p21	0.25[a]	143	Suzuki et al., 1999a
H5	1p14–p13	0.25[a]	155	Smith et al., 2000a
IGF1	1p12–p11	0.31	172	Smith et al., 2000a
LDHB	1p13–p11	0.36[a]	204	Suzuki et al., 1999a
ALVE1	1p12–p11	0.36[a]	204	Smith et al., 2000a
GAPD	1q11–q12	0.47[a]	241	Smith et al., 2000a
CRYAA	1q14–q21	0.57[a]	341	Suzuki et al., 1999a
OTC	1q13–q14	0.57[a]	356	Smith et al., 2000a
GCT0013	1q14	0.57	362	Morisson et al.,1998
GCT0007	1q31–q35	0.79	441	Morisson et al.,1998
PGR	1q42–q44	0.94[a]	520	Smith et al., 2000a
LEI0331	1qter	0.97[a]	561	Groenen et al., 2000
MCW0107	1q45	0.99	565	Fillon and Crooijmans[b]
LEI0332	1qter	1.00[a]	566	Groenen et al., 2000
Chromosome 2				
ADL0228	2p33	0.02	0	Fillon and Crooijmans[b]
VIM	2p24–p21	0.14	76	Groenen et al., 2000
ROS0105	2p12–p11	0.26	179	Smith et al., 2000a
PRL	2p11	0.32[a]	186	Suzuki et al., 1999a
OVY	2q11–q12	0.49[a]	226	Smith et al., 2000a
GCT0023	2q11–q12	0.53	241	Morisson et al., 1998
ROS0150	2q26–q32	0.75	336	Smith et al., 2000a
CALB1	2q31	0.79	358	Suzuki et al., 1999a
ROS0120	2q32–q35	0.89	395	Smith et al., 2000a
ADL0146	2q36–q37	0.98	403	Fillon and Crooijmans[b]
Chromosome 3				
MCW0261	3p12	0	0	Fillon and Crooijmans[b]
BMP2	3p11–q11	0.16[a]	52	Smith et al., 2000a
TGFB2	3q22–q23	0.23	77	Groenen et al., 2000
MYB	3q24–q26	0.53[a]	170	Smith et al., 2000a
GCT0008	3q27–q28	0.62	177	Morisson et al., 1998
ROS0119	3q27–q29	0.63	210	Smith et al., 2000a
ROS0108	3q29–q33	0.63[a]	210	Smith et al., 2000a
GCT0019	3q28–q2.10	0.68	210	Morisson et al., 1998
MCW0037	3q35	0.98	317	Fillon and Crooijmans[b]
Chromosome 4				
ADL0143	4p14	0.03	0	Fillon and Crooijmans[b]
ROS0107	4p14–p13	0.04[a]	1	Smith et al., 2000a
PGK1	4p14–p11	0.11[a]	48	Smith et al., 2000a
GC	4q13–q21	0.67[a]	158	Suzuki et al., 1999a
ALB	4q13–q21	0.70[a]	158	Suzuki et al., 1999a
MCW0240	4q21	0.76	201	Fillon and Crooijmans[b]
LEI073	4q25	0.97	243	Fillon and Crooijmans[b]
LEI0340	4qter	1.00	269	Groenen et al., 2000
LEI0341	4qter	1.00	270	Groenen et al., 2000
Chromosome 5				
MCW0263	5q11	0.17	28	Fillon and Crooijmans[b]
INS	5q12	0.34	57	Smith et al., 2000a
TH	5q12	0.34[a]	57	Smith et al., 2000a
RYR3	5q13	0.47	95	This paper
TGFB3	5q21–q22	0.60[a]	110	Smith et al., 2000a
ROS0110	5q21–q22	0.60	110	Smith et al., 2000a
ADL0298	5q25	0.98	198	Fillon and Crooijmans[b]
Chromosome 6				
LEI0192	6q11	0.22	31	Fillon and Crooijmans[b]
ROS0153	6q12	0.41	59	Groenen et al., 2000
SCD1	6q14	0.58	63	Fillon et al., 1997
MCW0326	6q14-q15	0.72	63	Fillon and Crooijmans[b]
Chromosome 7				
LEI0064	7p12	0	0	Fillon and Crooijmans[b]
ROS0331	7p11	0.17	40	Smith et al., 2000a
NRAMP	7q13	0.59	78	Smith et al., 2000a
ROS0128	7q13	0.59	80	Smith et al., 2000a
RPL37A	7q12–q14	0.60	80	Smith et al., 2000a
MCW0201	7q13–q14	0.66	79	Fillon and Crooijmans[b]
ADL0169	7q16	0.94	165	Fillon and Crooijmans[b]
Chromosome 8				
MCW0275	8p12	0.11	6	Fillon and Crooijmans[b]
RPL5	8cen	0.35[a]	56	Smith et al., 2000a
ROS0149	8cen	0.50[a]	66	Smith et al., 2000a
LEI0044	8q14	0.99	96	Fillon and Crooijmans[b]
Z chromosome				
ATPAL1	Zp24–p23	0.06[a]	-1	Smith et al., 2000a
ADL0022	Zp24–p23	0.03	0	Fillon and Crooijmans[b]
GHR	Zp23–p22	0.13	28	Suzuki et al., 1999c
CHD1Z	Zq14–q21	0.78[a]	131	Fridolfsson et al., 1998
ALDOB	Zq14–q21	0.78[a]	160	Suzuki et al., 1999c
LEI0075	Zq14–q21	0.75	165	Fillon and Crooijmans[b]
ACO1	Zq14–q21	0.78[a]	187	Nanda et al., 1999

[a] FLpters estimated from given cytogenetic band positions.
[b] Unpublished results.

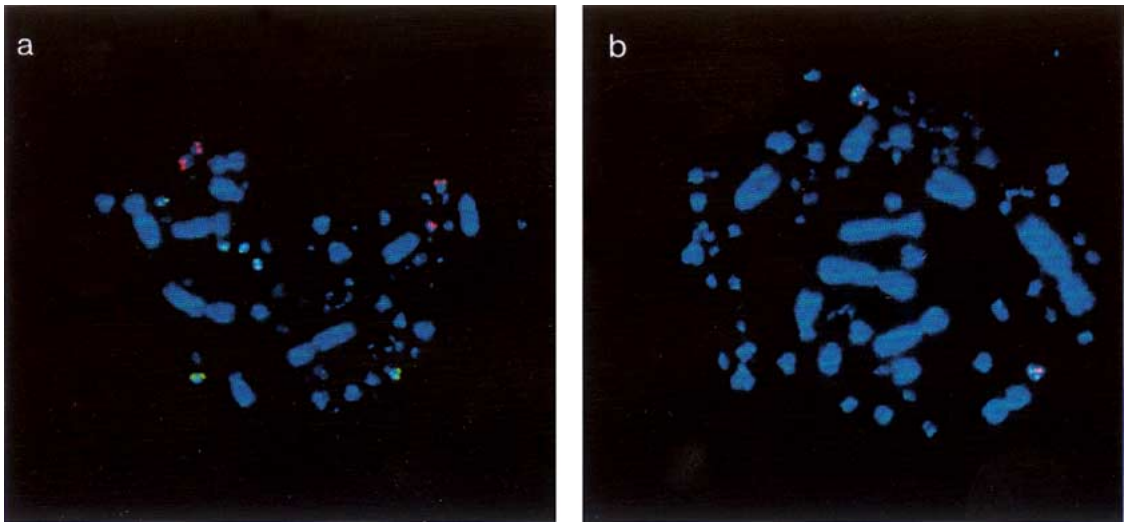


Fig. 4. (**a**) Hybridisation of P1-8 (red), P3-1 (red), P3C6 (green), P5H12 (green) and FAS (green) that label five different microchromosome pairs of the chicken. (**b**) Hybridisation of P1-8 (GCT903) (red) and P6V11 (GCT908) (green) on the same microchromosome 14.

Table 5. Microchromosome FISH tags which enabled the microchromosome identification. A microchromosome number has been proposed, based on the approximative size estimation of each microchromosome pair. The genetic markers containing clones led to the integration of genetic and cytogenetic maps for microchromosomes (Fillon et al., in preparation).

FISH tags	Genetic markers	Consensus linkage groups	Genetic sizes (cM)	Microchromosome numbers
P1A6	GCT16	E36C06W08	132	9
8G10	MCW132	E29C09W09	120	10
29L10	MCW0097	E30C14W10	88	11
11C21 n°1	MCW332	E16C17W22	90	12
P3-1	GCT907	E48C28W13W27	74	13
P1-8	GCT903	E35C18W14	77	14
P3C6	GCT14	E18C15W15	71	15
MHC	MHC	Ch16	60	16
8L2	ADL293	E41W17	70	17
FAS	FAS	E31E21C25W12	47	18
ACC	ACC	E52W19	40	19
P5H12	no	no	no	21
P7E4	no	no	no	22
28L18 n°1	MCW249	E27C36W25W26	13	23
P2-4	GCT905	E49C20W21	58	24
Cos 5A	no	no	no	25
P2-7	GCT906	E60C04W23	67	26
P10G12	GCT22	E59C35W20	75	27
P2-3	GCT904	E53C34W16	75	28
gB5F5	no	no	no	29
pB5F5	no	no	no	30
c19-12	no	no	no	31

etic maps for 16 linkage groups (Morisson et al., 1998; see Table 5). Most of the linkage groups corresponding to microchromosomes are longer than 50 cM (Table 5). The genetic to physical ratio of macrochromosomes is on average 396 kb/cM (Smith and Burt, 1998; Smith et al., 2000a). Our data suggest a minimum of 150 to 250 kb/cM for microchromosomes. This suggests a higher rate of recombination on microchromosomes. In accordance with these findings, previous studies suggested a difference in recombination rates for microchromosomes. Chiasmata analyses on chicken lampbrush chromosomes demonstrated that in microchromosomes one or two crossing-over events might occur (Rahn and Solari, 1986; Rodionov et al., 1992a, b). Moreover, the two genetically independent Major Histocompatibility Complexes *B@* and *Rfp-Y@* (Miller et al., 1996) have been located on the same microchromosome 16 (Fillon et al., 1996). Likewise, the genetic marker GCT908 segregates independently, whereas it has been localised by FISH with P1-8 (GCT903) on microchromosome 14 (Fig. 4b) (Morisson et al., 1998). These two results clearly demonstrate recombination hot spots in microchromosomes. Rodionov et al. (1992a, 1992b) suggest that this is necessary to ensure chromosome pairing during meiosis and mitosis, thus explaining the stability of the avian karyotype.

Differences in gene density on chicken macrochromosomes and micro-chromosomes

(Prepared by J. Smith)

The microchromosomes of chicken constitute 23% of the female genome[1] (Smith and Burt, 1998), are GC-rich (Auer et al., 1987) and have a higher CpG content than the macrochromosomes (McQueen et al., 1996). Since 60–70% of known chicken genes are associated with a CpG-island, it has been postulated that the microchromosomes may represent a gene-

[1] In this instance, chromosomes 1–5 and the Z chromosome are referred to as "macrochromosomes" and chromosomes 6–38 and the W chromosome as "microchromosomes". This is because this was the nomenclature used in the studies on gene density.

dense fraction of the chicken genome. This idea is supported by acetylation studies of histone H4 and analysis of CpG-island-like sequences in cloned genomic DNA (McQueen et al., 1998), which indicate that microchromosomes are also associated with higher gene activity. This hypothesis has been tested by directly comparing the gene densities on the macrochromosomes and microchromosomes, based on sequence sampling of cosmid DNA and from the analysis of the distribution of genes mapped by physical means and by genetic linkage. From these different approaches it is estimated that the microchromosomes are twice as gene-dense as macrochromosomes (Smith et al., 2000b).

In sequence sampling approaches, randomly selected cosmids that were known to map to either a macrochromosome or a microchromosome, by FISH, have been examined. From the number of genes found in the cosmid clones isolated from macrochromosomes and from microchromosomes and the amount of DNA sequenced it has been estimated that the gene density of the microchromosomes is from 1.3 times (Smith et al., 2000b) to 2.4 times (Clark et al., 1999) that of macrochromosomes.

The average CpG content of cosmid clones from macrochromosomes and microchromosomes has also been analysed as an indirect measure of the abundance of CpG islands. From a sample of cloned chicken genomic DNA it was estimated that microchromosomal DNA has a CpG content 1.6 times that of macrochromosomal DNA (Smith et al., 2000b). Whether the differences in CpG content are related to gene content or a structural feature of microchromosomes has still to be determined. However, from sequence sampling studies, it is estimated that there is a gene every 22-kb on the macrochromosomes and once every 17-kb on microchromosomes. From the size of the chicken macrochromosomes and microchromosomes (Smith and Burt, 1998) it can therefore, be estimated that there are a total of 60,000 genes in the chicken genome.

The number of genes that have been physically mapped at random in the chicken has also been examined. The number of genes mapped to macrochromosomes and to microchromosomes has been compared. From this physical mapping data, it is estimated that the microchromosomes are 1.3 times as dense as the macrochromosomes (Smith et al., 2000b). So far, 171 genes have been mapped by FISH but many target specific chromosomes were selected based on comparative maps with human.

The number of genes genetically mapped to macrochromosomes and microchromosomes in the chicken has also been used to determine gene density on each set of chromosomes. Excluding microsatellite-based markers, which have been shown to be biased in their distribution (Smith et al., 2000b), gene markers which have been assigned randomly to the genetic maps of macrochromosomes have been studied. When this genetic mapping data is compared to the physical size of the genome represented by the two sets of chromosomes, it is estimated that the microchromosomes are 2.3 times as gene-dense as the macrochromosomes.

Accurate estimates for the relative gene density were not possible in early studies (McQueen et al., 1996; McQueen et al., 1998) but suggested a 6-fold difference in gene density (an estimate based mostly on CpG-island-like sequences in cloned genomic DNAs) on macrochromosomes and microchromosomes, and that 75% of all chicken genes were located on the microchromosomes. If true, this should have been reflected in significantly more gene homologies being found by the sequence sampling approach. This contrasts with the more recent and more conservative estimate of around 48% of all chicken genes being located in the microchromosome fraction, thus explaining the lower number of sequence homologies found by sequence sampling (Smith et al., 2000b). If the gene density difference is 2.5 fold, then the numbers of genes on the macro- and microchromosomes can be estimated to be 31,000 and 29,000, respectively.

Molecular cytogenetic studies to facilitate physical mapping of the avian genome

(Prepared by D.K. Griffin)

A fundamental requirement for all physical gene mapping strategies is the accurate assignment of the chromosomes. Among eukaryotic species, genes and anonymous clones are placed on the genome map with specific reference to their position on a particular chromosome. The proximity of clones on a chromosome (e.g. as indicated by a fluorescence in-situ hybridisation [FISH] experiment) is clear evidence of close physical distance and, in addition indicates genetic linkage. Therefore, in order for physical gene mapping strategies to be meaningful, each chromosome must be clearly identifiable and the sequences contained upon it assigned correctly in relation to one another and in relation to an agreed convention (Burt et al., 1995a).

Most animals, including humans, have chromosomes that can be distinguished by simple banding techniques. Moreover the position of sequences on the map is indicated by the chromosome, the chromosome arm and finally the chromosome band. In chickens however, as with virtually all birds, a significant obstacle to the physical mapping of genes is the inability to distinguish many of the chromosomes. This is due to (a) the large number of chromosomes (2n = 78) and (b) the presence of microchromosomes. Depending on the definition given by different authors, *Gallus domesticus* has 6–10 pairs of macrochromosomes including the Z and smaller W in the heterogametic female (e.g. Kaelbing and Fechheimer, 1983; Fritschi and Stranzinger, 1985; Auer et al., 1987; Schmid et al., 1989; Ponce de Leon et al., 1992). In addition there are 28–32 pairs of microchromosomes that have met with varying degrees of karyotype definition. G-banding studies have identified chromosomes 1–10 plus the Z and W whereas studies using counterstain enhanced fluorescence were successful in identifying chromosomes 1–18 (Auer et al., 1987). There are a number of drawbacks to using these banding techniques in physical mapping however, (a) because their efficacy has yet to be assessed in combination with FISH experiments and (b) chromosomes smaller than 18 remain indistinguishable. Clearly therefore molecular cytogenetic means are essential for full karyotype definition. Two strategies have been adopted for distinguishing

the chromosomes of the chicken, particularly the microchromosomes: (1) The use of individual clones as landmarks and (2) Chromosome painting.

Use of individual clones

The underlying strategy of this approach is that clones e.g. BACs, PACs and cosmids, once isolated and mapped to a microchromosome (by FISH), are used as a landmark for that chromosome. Other clones either cohybridise to that chromosome or hybridise to other chromosomes and can be used as landmarks for them. In this way putative chromosome numbers can be assigned and a low resolution genome map built based around accurate chromosome identification. Fillon et al. (1998) initially were able to distinguish 16 microchromosomes using 17 different BAC, PAC and cosmid clones. BACs and PACs were selected from large insert containing libraries (Zoorob et al., 1996). In more recent experiments this has now been extended to 22 chromosomes and putative chromosome numbers have been assigned (Fillon and Vignal, in this report; see Table 5). That is, there are 22 landmark clones assigned and available from chromosomes 9–31. The advantage to this approach is that it is a relatively simple and cost effective means of defining the smaller chromosomes in the karyotype. Moreover, when the clones are isolated from sequences that are assigned to a genetic linkage group, or when cohybridisation with sequences in a known linkage group is established, full integration of the genetic and physical map can be performed. Indeed 17 of the clones have already been assigned to known linkage groups (Table 5).

Chromosome painting

An alternative to the use of landmark clones for chromosome assignment is to generate a chromosome specific genomic library for each chromosome, label it with a hapten and use it as a chromosome paint. This is technically more demanding than clone isolation but has a number of advantages. First, unlike individual clones, chromosome paints cover the entire length of the chromosome and can identify rearrangements such as translocations. These rearrangements can potentially be between individuals of the same species, between different strains of the same species or between different species. Second they provide a resource of chromosome specific sequences from which more clones can be isolated. For instance, recent experiments have been initiated to generate chromosome specific normalised cDNA libraries from this material (Masabanda and Griffin, unpublished results).

Strategies that have, to date, been used to generate chromosome-specific paints involve amplification and labeling by DOP-PCR of isolated chromosome specific material. In initial experiments chromosomes were isolated by flow cytometry (e.g. Carter et al., 1992). Eleven individual chromosomes were isolated in the flow karyotype, four hundred of each were flow-sorted and the template amplified and labeled. Eleven colour chromosome painting (Fig. 5) in combination with DAPI banding revealed that genomic libraries had been successfully generated from chromosomes 1–9, Z and one microchromosome. In addition, three paints recognised two pairs of microchromosomes and two paints recognised three pairs (Griffin et al., 1999). Thus, although a flow cytometry strategy was an excellent one for generating paints from the larger chromosomes, it was limited in its ability to distinguish the microchromosomes. In a separate set of experiments a means of generating chromosome paints from the microchromosomes was developed. In order to do this it was essential to be able to amplify template DNA from a single microchromosome. This is because it is not practicable to relocate the same chromosome on a different metaphase and thus increase the number of chromosomes in the template (Griffin et al., 1999). To date these experiments have been successful in generating chromosome paints from 20 microchromosomes of which at least 14 are different (Fig. 6). Recently these approaches have been combined i.e. single microchromosomes have been isolated by flow-cytometry and amplified/labeled producing paints for 14 microchromosomes (Masabanda and Griffin, unpublished results). Thus, in total, chromosome paints from approximately 46 chromosomes have been generated from the chicken karyotype. As there are only 39 chromosome pairs in the karyotype, it is certain that some of the paints will be of the same chromosome. Moreover we feel it is likely that there remains some chromosomes from which paints have still to be isolated.

Although the two approaches (use of individual clones as landmarks and chromosome painting) are presented as alternatives to one another, they are, in many ways, complementary. For instance, cohybridisation (by FISH) of paint and clone can lead to unequivocal cytogenetic assignment by two independent means. Moreover, particularly for the smaller chromosomes (that may be as small as 7 Mb), chromosome paints may be developed by the pooling of several individual clones. Therefore it seems reasonable to suggest that a combination of single clone mapping and chromosome painting will lead to (a) to full definition of all chicken chromosomes and (b) to integration of the physical and genetic maps in chicken and other avian genomes.

Comparative chromosome painting

(Prepared by M. Guttenbach, I. Nanda, C. Steinlein, D.K. Griffin and M. Schmid)

Comparative chromosome painting has been shown to be a rapid and comprehensive method for the detection of chromosome homologies between different species. Zoo-FISH can detect conservation of synteny, chromosome rearrangements such as translocations or fissions and provides clues on genome evolution. Thus, data obtained by conventional banding analyses can be confirmed or refined. Zoo-FISH was initially used for comparison between human and other mammalian karyotypes (Scherthan et al., 1994; Rettenberger et al., 1995; Raudsepp et al., 1996; Chowdhary et al., 1998; Iannuzzi et al., 1999), but more recently Chowdhary and Raudsepp (2000) applied a human chromosome 4 painting probe to chicken metaphases and could demonstrate conserved synteny over 300 Myr divergence by distinct hybridization to chicken chromosome 4cen → q26.

Meanwhile chicken painting probes especially for macrochromosomes are available. By microdissection and DOP-

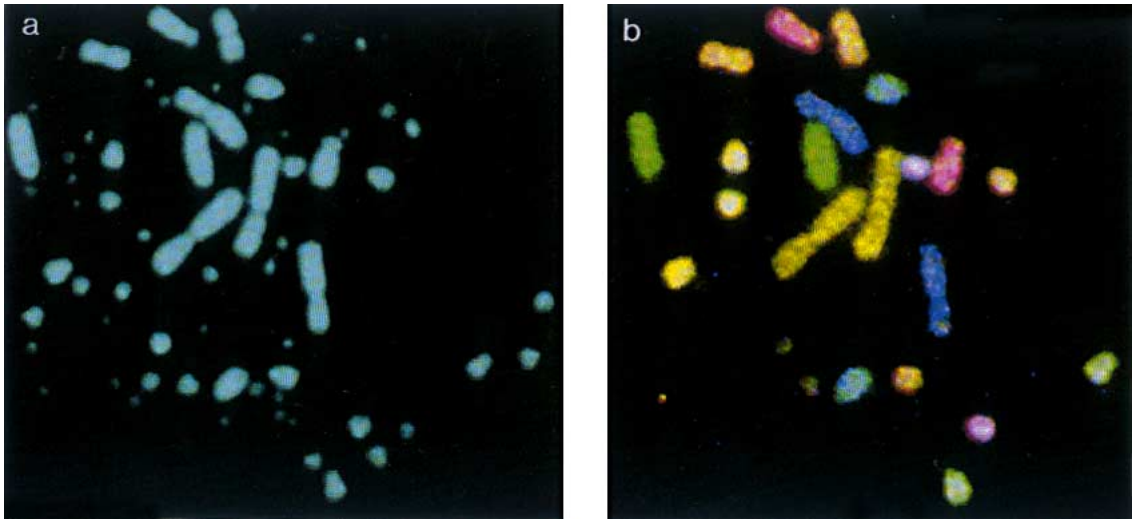


Fig. 5. Eleven-colour chromosome painting of chromosomes 1–9, Z and one large microchromosome of the chicken.

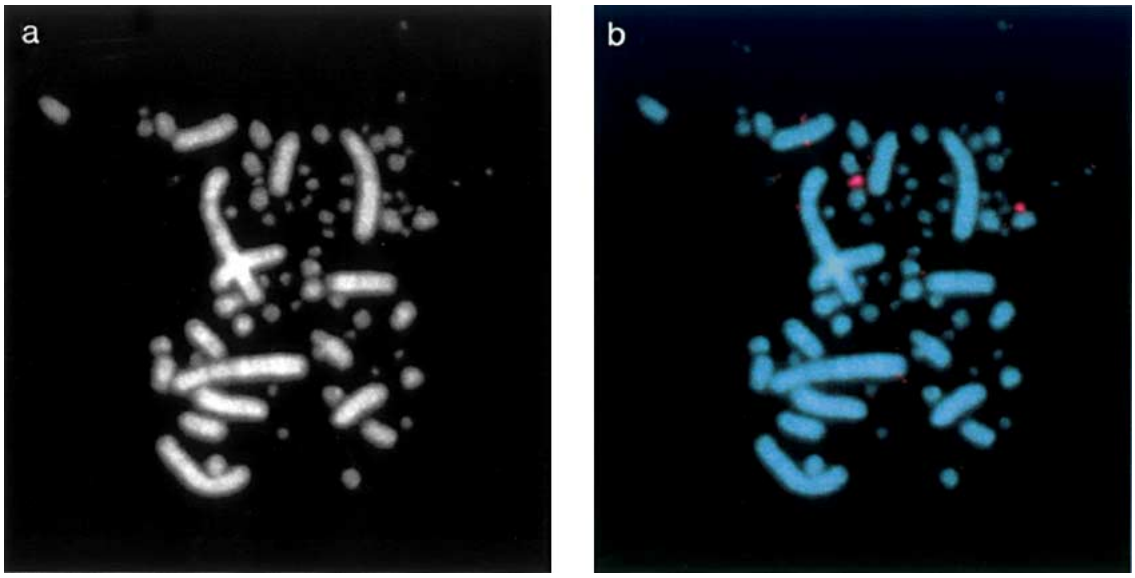


Fig. 6. Chromosome painting of a small microchromosome of the chicken. Probe isolated by microdissection of a single chromosome followed by DOP-PCR amplification and labeling.

Shuttle-PCR Zimmer et al. (1997) prepared a Z-painting probe in order to screen BAC-libraries for Z-specific clones. Guillier-Gencik et al. (1999) generated whole chromosome paints for chicken chromosomes 1–8, Z and W by microdissection and DOP-PCR. An alternative approach, flow-sorting and DOP-PCR amplification, was used by Griffin et al. (1999) to generate chicken painting probes 1–9 and Z. Application of these probes to other avian species will reveal chromosome rearrangements which occurred during avian evolution. In the first Zoo-FISH study between bird species, Shetty et al. (1999) hybridized chicken paints 1–9 and Z to emu chromosomes and found strong chromosome homology in these distantly related species. With the exception of chicken paint 4, each autosomal probe detected a single emu chromosome of corresponding size and morphology. Besides emu chromosome 4 a pair of additional microchromosomes was labeled by paint 4. The Z-probe detected both, Z and W in the emu, confirming extensive homology between these poorly differentiated sex chromosomes. Using the same set of chicken painting probes (Griffin et al., 1999), we have started Zoo-FISH analyses in several avian species belonging to six different orders: *Rhea americana* (Struthioniformes), *Phasianus colchicus, Chrysolophus pictus, Lophura nycthemera, Coturnix coturnix* (Galliformes), *Strix nebulosa, Bubo bubo* (Strigiformes), *Streptopelia roseogrisea* (Columbiformes), *Turdus merula* (Passeriformes), *Anser anser* and *Cairina moschata* (Anseriformes). Preliminary data is shown in

Table 6. Comparative chromosome painting of chicken chromosome paints 1–9 and Z to chromosomes of 11 bird species

Species	Chromosome[a]									
Chicken *(Gallus domesticus)* 2n = 78	1	2	3	4	5	6[b]	7[b]	8[b]	9[b]	Z
Japanese quail *(Coturnix coturnix japonica)* 2n = 78	x	x	x	x	x	x	x	x	x	x
Pheasant *(Phasianus colchicus)* 2n = 82	x	3 + 6	2	4 + micro	x	x		x		x
Golden pheasant *(Chrysolophus pictus)* 2n = 82	x	3 + 6	2	4 + micro	x	x	x	x	x	
Silver pheasant *(Lophura nycthemera)* 2n = 80	x	3 + 6	2	4 + micro	x	x	x	x	x	x
African collared dove *(Streptopelia roseogrisea)* 2n = 78	x		x	These probes bind to either the short or long arm of one of the chromosomes 4-7.						
Blackbird *(Turdus merula)* 2n = 80			x			x		x	x	
Great grey owl *(Strix nebulosa)* 2n = 82	x		x	4 + micro ?	x	x	x	x		
Eagle owl *(Bubo bubo)* 2n = 80				1p ?				x		
Duck *(Cairina moschata)* 2n = 78						x				
Greylag goose *(Anser anser)* 2n = 80						x		x		
Rhea *(Rhea americana)* 2n = 80						x	x	x	x	

[a] x Indicates probe detects corresponding chromosomes.
[b] The exact target chromosome of these paints in the species analysed is to be determined.

Table 6. With few exceptions, the individual chicken paints labeled single chromosomes or chromosome arms in all other species. If not otherwise indicated, chromosome paints hybridized to chromosomes of comparable size. The exact chromosome number, however, has yet to be determined (especially for paints 6–9) by detailed karyotype analyses and will be published elsewhere (manuscript in preparation). In the different pheasant species, chicken paint 2 hybridizes to two telocentric chromosomes (Nos. 3 and 6), thus confirming the origin of these chromosomes by fission of the ancestral chromosome 2 of the Galliformes (for review, see Christidis, 1990). Consequently, paint 3 binds to chromosome 2 in these species (Fig. 7). Comparable to the observations in emu, the telocentric chromosome 4 as well as a microchromosome are detected by chicken paint 4, suggesting that chicken chromosome 4 arose by fusion of the ancestral chromosome 4 and a microchromosome.

In the African collared dove chromosome pairs 1–3 are easily identifiable, whereas pairs 4–7 are very similar in size and morphology (meta- to slightly submetacentric chromosomes). Paints 6–9 each hybridized to either a short or long arm of one of the chromosomes 4–7. The exact position has to be evaluated by double hybridization of probes and/or karyotype analyses.

These preliminary Zoo-FISH results clearly demonstrate the occurrence of homologous chromosomal segments in the genomes of the bird species examined and reveal the chromosome rearrangements in the course of avian evolution. Additional application of single chicken microchromosome paints to other species (Griffin et al., 1999) will further contribute to our understanding of karyotype evolution in birds.

Comparative chromosome G-banding in poultry – Ideograms

(Prepared by V. Fillon)

Most avian karyotypes share the same typical organization, comprising a few macrochromosome pairs and a lot of tiny microchromosomes. The presence of microchromosomes makes classical cytogenetics more difficult than in mammals.

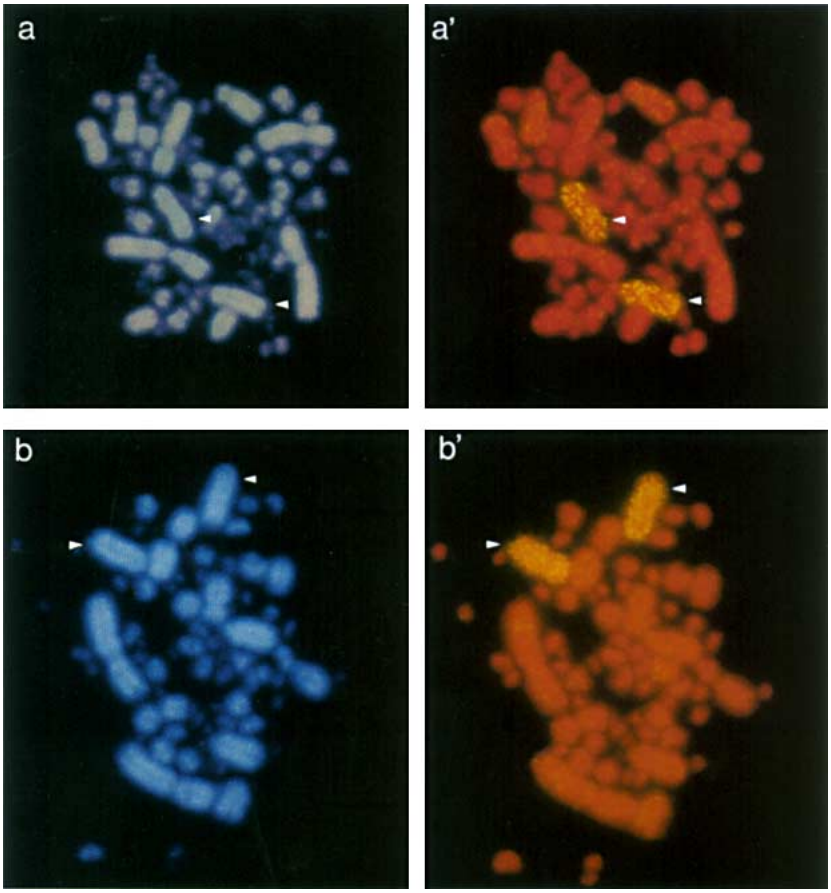


Fig. 7. Fluorescent in situ hybridization of chicken autosomal paint 3 to metaphase plates from chicken (**a'**) and golden pheasant (**b'**). The DAPI-counterstained chromosomes are depicted in (**a**) and (**b**). Labeled chromosomes are indicated by arrowheads. In contrast to the chicken, in the golden pheasant paint 3 hybridizes to chromosome 2.

Table 7. Correspondences between G-banded macrochromosomes of chicken *(Gallus gallus domesticus)*, quail *(Coturnix coturnix)*, turkey *(Meleagris gallopavo)* and duck *(Anas platyrhynchos)*

Macrochromosomes			
Chicken	Quail	Turkey	Duck
1	1 (with inversion)[a]	1	1
2	2 (with inversion)	3 + 6	2
3	3	2	3
4	4 (with inversion)	4 (without p arm)	4 (with rearrangments and without p-arm)
5	5	5 (with p arm)	Rearrangments ?
6	6	8	Rearrangments
7	7 with rearrangments ?	7	Rearrangments
8	8 (with inversion)	9	Rearrangments
Z	Z (without the pter positive G-band)	Z (with inversion)	Z (with rearrangments)

[a] Inversion: pericentric rearrangement.

The order Galliformes is one of the best studied, and many chromosome homologies have been shown (Ryttman and Tegelström, 1981, 1983; Stock and Bunch, 1982). Recently, a standard has been defined for the eight macrochromosomes plus the Z and W sex chromosomes of the chicken (Fig. 8) (Ladjali-Mohammedi et al., 1999). Considering the chicken standard karyotype as a reference (Carlenius et al., 1981; Ponce de Leon et al., 1992; Ladjali et al., 1995; Ladjali-Mohammedi et al., 1999), ideograms based on banding patterns previously described have been established for the duck (*Anas platyrhynchos*) (Mayr et al., 1989a; Denjean et al., 1996), the quail (*Coturnix coturnix*) (Stock and Bunch, 1982; Mayr et al., 1989b; Schmid et al., 1989; Suzuki et al., 1999c) and the turkey (*Meleagris gallopavo*) (Ryttman and Tegelström, 1981, 1983; Stock and Bunch, 1982). All chromosomes have been classified by decreasing size (Figs. 9–11). These ideograms will be used for the cytogenetic localisation of genes and markers in poultry and comparative mapping studies. Tentative correspondences between macrochromosomes for chicken and others species inferred from classical cytogenetic banding studies are reported in Table 7. Heterologous hybridisations with painting probes of chicken chromosomes or with BACs from the chicken cytogenetic map will improve the detection of rearrangments and help to define breakpoint boundaries.

Comparative maps between chicken, mouse and human

(Prepared by D.W. Burt, J. Smith, N. Bumstead, M.A.M. Groenen, R.P.M.A. Crooijmans, I. Nanda, H.H. Cheng, S. Mizuno, A. Vignal and F. Pitel)

This report summarises the current status (August, 2000) of the comparative maps for genes and anonymous loci that have been mapped in chicken, mouse and human. Table 8 provides a listing of loci that make up the comparative map based on

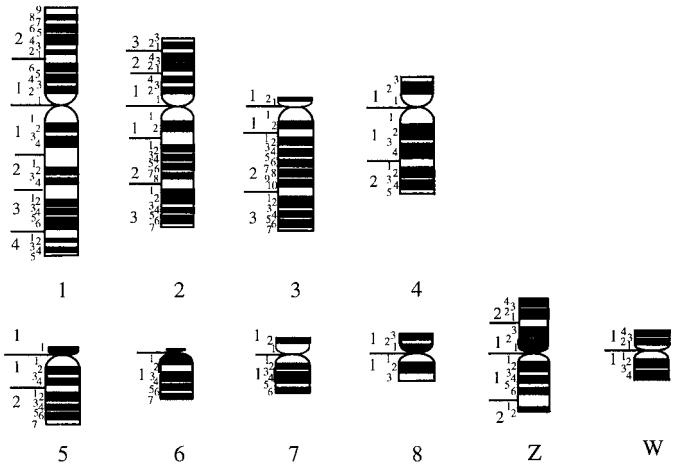


Fig. 8. Standard ideograms for GTG-banded macrochromosomes of the chicken (Ladjali-Mohammedi et al., 1999).

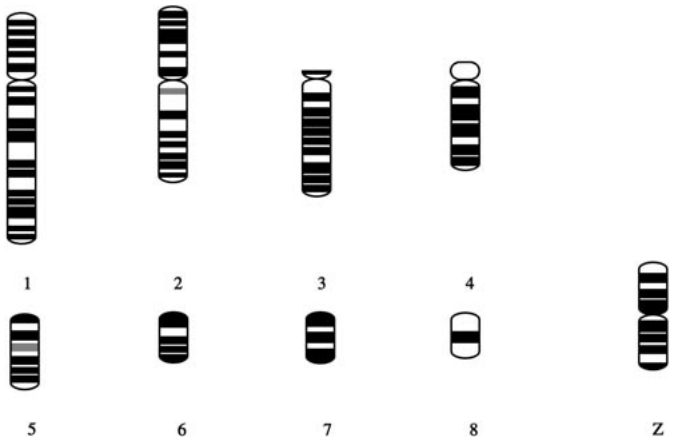


Fig. 9. Ideograms of GTG-banded macrochromosomes of the quail (*Coturnix coturnix*). (Stock and Bunch, 1982).

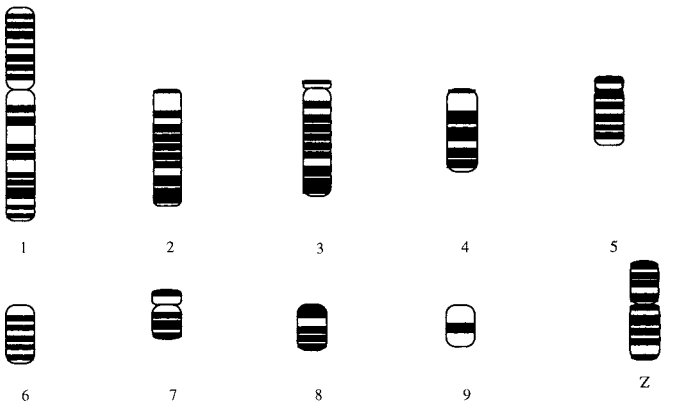


Fig. 10. Ideograms of GTG-banded macrochromosomes of the turkey (*Meleagris gallopavo*). (Stock and Bunch, 1982).

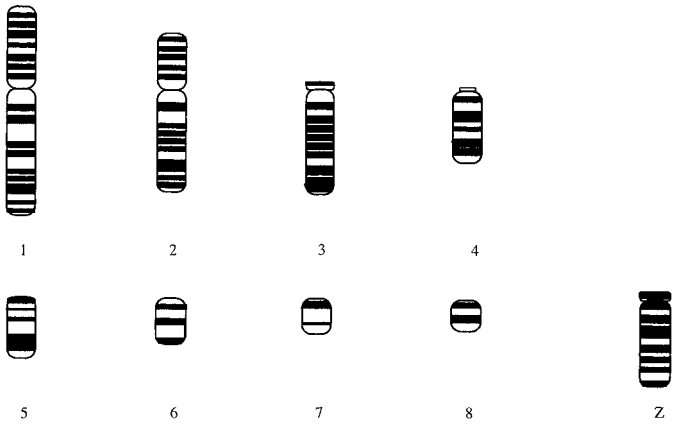


Fig. 11. Ideograms of GTG-banded macrochromosomes of the duck (*Anas platyrhynchos*). (Denjean et al., 1996).

genetic and physical mapping data for 342 orthologues (More details can be found at http://www.ri.bbsrc.ac.uk/chickmap/). The key criteria for orthology are based on the HUGO recommendations (Andersson et al., 1997). Conserved segments in the mouse and human gene maps are indicated by bold outlines. Map positions of loci are mostly based on information available from the genome databases for the mouse (http://www.informatics.jax.org/), human (http://gdbwww.gdb.org/gdb/; http://www.ncbi.nlm.nih.gov/genemap/; http://www.ncbi.nlm.nih.gov/omim/) and chicken (http://www.ri.bbsrc.ac.uk/chickmap/). The location of chicken loci are shown relative to the consensus genetic linkage map (Groenen et al., 2000) and using the current microchromosome numbering system (Fillon and Vignal, in this report). Chicken genes mapped by FISH were integrated into the consensus genetic map using the integrated genetic-physical maps of chicken macrochromosomes (Smith et al., 2000a; and this report). The construction of the comparative map used the principles described by Sankoff et al. (1997). Due to experimental error in both genetic linkage and FISH mapping the precise order of genes in the chicken map was not always clear. In these cases we used the maps of the human and mouse to predict the most likely order.

There have been a number of early reports suggesting extensive conservation of synteny between chicken and mammalian genomes for both chicken macrochromosomes and microchromosomes (Burt et al., 1995a; Klein et al., 1996; Girard-Santosuosso et al., 1997 and Smith et al., 1997). This conclusion has now been confirmed by many recent studies based on both genetic linkage (Smith and Cheng, 1998; Burt and Cheng, 1998; Kaiser et al., 1999 and Groenen et al., 1999) and physical mapping methods (Masabanda et al., 1998; Pitel et al., 1998; Sazanov et al., 1998, 2000; Nanda et al., 1999, 2000 and Suzuki et al., 1999a, b, c).

Table 8. Comparative map of chicken, mouse and human based on loci mapped by genetic linkage and physical mapping

Chick locus	Chr.	FISH (RBG)	Consensus mid-point[a]	Error	T[b]	Human locus	Chr.[c]		Mouse locus	Chr.[c]		P[d]	References
SMOH	1		16	6		SMOH	7	q31-q32	*Smoh*	6	7.00		
GATA3	1		38	7		GATA3	10	p15	*Gata3*	2	7.00		
NRCAM	1		87	14		NRCAM	7	q31.1-q31.2	*Nrcam*	12	22.00		
G22P1	1	p22–p21	95	6		G22P1	22	q13	*G22p1*	15	47.50		
IFNG	1	p23–p21	105	6		IFNG	12	q14	*Ifng*	10	67.00		
HMGIC	1	p21–p13	116	21		HMGIC	12	q13.3-q14	*Hmgic*	10	67.50		
LYZ	1	p14–p13	112	12		LYZ	12	q13.3-q14	*Lyzs*	10	66.00		
MYF6	1	p21	106	6		MYF6	12	q21	*Myf6*	10	59.00		
MGF	1		126	5		MGF	12	q22	*Mgf*	10	57.00		
DCN	1	p21–p13	116	21		DCN	12	q21.3-q23	*Dcn*	10	55.00		
CRADD	1	p21–p15	132	7		CRADD	12	q21.33-q23.1	*Cradd*	10	52.00		
GNRH1	1		137	7		GNRH1	8	p21-p11.2	*Gnrh*	14	39.50		
HIS@	1	p21	143	1		H1F1	6	p22.2-p22.1	*Hist1*	13	12.00		
MGP	1		143	3		MGP	12	p13.1-p12.3	*Mglap*	6	S		
NAGA	1		143	3		NAGA	22	q11	*Naga*	15	S		
ADSL	1	p21–p13	151	10		ADSL	22	q13.1	*Adsl*	15	S		
H5	1	p14–p13	155	10		H1F0	22	q13.1	**H1f0**	15	S	M	
LGALS4	1		157	1		LGALS4	22	q12-q13	*Lgals4*	7	4.00	H	
MAFF	1		163	13		MAFF	22	q12.2-q13.2	*Maff*	7	S	M	
ASCL1	1	p15–p11	160	30		ASCL1	12	q22-q23	*Mash1*	10		M	
IGF1	1	p12–p11	172	17		IGF1	12	q22-q23	*Igf1*	10	48.00		
TRA1	1		174			TRA1	12	q22-q23	*Tra1*	10	49.00		
ITPR2	1		S		2	ITPR2	12	p11	*Itpr2*	6	S	M	Bumstead, unpublished
LDHB	1	p11	204	3		LDHB	12	p12.2-p12.1	*Ldh2*	6	62.00		
CCND2	1	p11–q11	230	35		CCND2	12	p13	*Ccnd2*	6	60.00		
GAPD	1	q11–q12	241	13		GAPD	12	p13	*Gapd*	6	56.00		
TCRB@	1		230	25		TRB@	7	q35	*Tcrb*	6	20.50		
PPARA	1		241	13		PPARA	22	q13.31	*Ppara*	15	48.80		Vignal and Pitel, unpublished
HSD3B1	1		254	10		HSD3B1	1	p13.1	*Hsd3b1*	3	49.10		
EPHA3	1	q11	274	9		EPHA3	3	p11.2	*Epha3*	16	S	M	
IFNAR2	1	q12–q13	313	18		IFNAR2	21	q22.1	*Ifnar2*	16	61.00	M	
IL10RB	1	q12–q13	313	18		IL10RB	21	q22.1-q22.2	*Il10rb*	16	61.00		
IFNAR1	1	q12–q13	313	18		IFNAR1	21	q22.1	*Ifnar*	16	64.00		
GART	1	q12–q13	313	18		GART	21	q22.1	*Gart*	16	63.00		
SOD1	1		330	13		SOD1	21	q22.2	*Sod1*	16	61.00		Cheng, unpublished
CRYAA	1	q14	341	1		CRYAA	21	q22.3	*Crya1*	17	17.40		
FABP7	1	q14–q21	357	27		FABP7	6	q22-q23	*Fabp7*	10	S	M	
OTC	1	q13–q14	356			OTC	X	p21.1	*Otc*	X	3.00		
NR0B1	1	q21	363	33		NR0B1	X	p21.3-p21.2	*Nr0b1*	X	33.00		
ZFX	1		361	1		ZFX	X	p22.2-p21.3	*Zfx*	X	34.60		
SCML2	1		365	12		SCML2	X	p22	*Scml2*	X	S	M	
SCYC1	1		365			SCYC1	1	q23-q25	*Lptn*	1	87.00		
LAMP1	1		418	1		LAMP1	13	q34	*Lamp1*	8	1.00		
HOXC@	1	q31	444	2		HOXC@	12	q12-q13	*Hoxc*	15	57.40		
RB1	1		474	3		RB1	13	q14.2	*Rb1*	14	41.00		
U16	1		474	3		U16	13	q14.2	**U16**	14	41.00		
HMG1	1		S		2	HMG1	13	q12	*Hmg1*	5	86.00		
PGR	1	q42–q44	520	26		PGR	11	q22.1-q22.3	*Pgr*	9	S		
FUT4	1		527	12		FUT4	11	q21	*Fut4*	9	3.00		
TYR	1	q42–q44	557	15		TYR	11	q21	*Tyr*	7	44.00		
UCP2	1	q23–q41	524	18		UCP2	11	q13	*Ucp2*	7	50.00		
WNT11	1		564	18		WNT11	11	q13	*Wnt11*	7	48.00		
HBB@	1		567	18		HBB	11	p15.4	*Hbb*	7	50.00		
RAB6	1		569	18		RAB6	2	q14-q21	*Rab6*	2	S	M	
NFYB	1		570	18		NFYB	12	q22-q24.1	*Nfyb*	10	43.70		
WNT3A	2		-1			WNT3A	17	S	*Wnt3a*	11	32.00	H	
ACVR2B	2		36	10		ACVR2B	3	p22-p21.3	**Acvr2b**	9	S	M	
SHH	2		38	16		SHH	7	q36	*Shh*	5	16.00		
EN2	2		45	1		EN2	7	q36	*En2*	5	15.00		
ADL0190	2		62	6		Hs.70333	10	p11.2	*Mm.1310632*	18	S	M	
VIM	2	p31–p21	76	12		VIM	10	p13	*Vim*	2	7.00		
MRC1	2		77	12		MRC1	10	p13	*Mrc1*	2	5.00		
NPY	2		98	11		NPY	7	p15.1	*Npy*	6	22.00		
HOXA@	2	p21	116	17		HOXA@	7	p15-p14	*Hoxa*	6	26.30		
CP49	2		138	8		CP49	3	q21.2-q22.3	*Cp49*	UN			
RARB	2		120	15		RARB	3	p24.3-p24.2	*Rarb*	14	1.50		
THRB	2	p12	140	13		THRB	3	p24.1-p22	*Thrb*	14	S	M	
EGFR	2	p11	176	6		EGFR	7	p12	*Egfr*	11	9.00		
TGFBR1	2	p12–p11	179	12		TGFBR1	9	q33-q34.1	*Tgfbr1*	4	19.30		
PRL	2	p11	186	19		PRL	6	p22.2-p22.1	*Prl*	13	14.00		
BMP6	2		200	8		BMP6	6	p24-p23	*Bmp6*	13	20.00		
ALDH1A5	2	q11–q21	245	24		ALDH1A1	9	q21	*Aldh1a2*	19	12.00		

Table 8 (continued)

Chick locus	Chr.	FISH (RBG)	Consensus mid-point[a]	Error	T[b]	Human locus	Chr.[c]		Mouse locus	Chr.[c]		P[d]	References
BCL2	2		228	1		BCL2	18	q21.33	*Bcl2*	1	59.80		
YES1	2		274	6		YES	18	p11.31-p11.22	*Yes*	5	18.00		
MYL	2		274	6		Hs.118167	18	p11.32		5	S	M	
ZFP161	2		282	4		ZFP161	18	pter-p11.21	*Zfp161*	17	41.00		
ADCYAP1	2		294	7		ADCYAP1	18	p11	*Adcyap1*	18	S	M	
CDH2	2	q24	309	9		CDH2	18	q12.1	*Cdh2*	18	6.00		
RYR2	2		302	5		RYR2	1	q42.1-q43	*Ryr2*	13	7.00		Groenen and Crooijmans, unpublished
PRKDC	2	q24–q25	325	7		PRKDC	8	q11	*Prkdc*	16	9.20		
PENK	2		322	13		PENK	8	q11.23-q12	*Penk2*	9	7.00		
MOS	2		320	13		MOS	8	q11	*Mos*	4	0.00		
LYN	2	q26	356	7		LYN	8	q13	*Lyn*	4	0.00		Groenen and Crooijmans, unpublished
CALB1	2	q26	358	1		CALB1	8	q21.3-q22.1	*Calb1*	4	10.50		
CA2	2		358	4		CA2	8	q13-q22.1	*Car2*	3	10.50		
TRHR	2		383	12		TRHR	8	q23	*Trhr*	15	24.70		
MYC	2		401	16		MYC	8	q24.12-q24.13	*Myc*	15	32.00		
HSF1	2		452	1		HSF1	8	q24.3	*Hsf1*	15	S	M	
LIMK2	2		461	1		LIMK2	22	q12	*Limk2*	1	S		
LHCGR	3		32	10		LHCGR	2	p21	*Lhcgr*	17	46.50		
BMP2	3	q11–q21	52	14		BMP2	20	p12	*Bmp2*	2	76.00		
ADPRT	3		54	14		ADPRT	1	q41-q42	*Adprp*	1	98.60		
TGFB2	3	q11–q21	77	12		TGFB2	1	q41	*Tgfb2*	1	101.50		
ACTN2	3		110	15		ACTN2	1	q42-q43	*Actn2*	13	7.00		
SULT1A2	3		150			SULT1A2	16	p12-p11.2	*Stp2*	17	34.00		Cheng, unpublished
HMX1	3		151	11		HMX1	4	p16.1	*Hmx1*	5	18.00		
T	3		146	15		T	6	q27	*T*	17	4.00		
TCP1	3		146			TCP1	6	q25-q27	*Tcp1*	17	7.50		
MPR1	3		153	11		IGF2R	6	q25.3	*Igf2r*	17	7.35		
VIP	3		153	15		VIP	6	q24-q27	*Vip*	10	S	M	
ESR1	3		153	15		ESR1	6	q25.1	*Esr1*	10	12.00		
MYB	3	q23–q25	170	10		MYB	6	q23.3-q24	*Myb*	10	16.00		
PLN	3		182	3		PLN	6	q22.1	*Pln*	10	S	M	
FYN	3		200	14		FYN	6	q21	*Fyn*	10	25.00		
CCNC	3	q26–q29	210	10		CCNC	6	q21	*Ccnc*	10	S	M	
EEF1A1	3		231	20		EEF1A1	6	q14	*Eef1a*	4	S	M	
ME1	3		222	12		ME1	6	q12	*Mod1*	9	48.00		
BMP5	3		238	13		BMP5	6	q12-q13	*Bmp5*	9	42.00		
GSTA2	3		239	13		GSTA2	6	p12	*Gsta*	9	43.00		
HOXB@	3	q31	243	14		HOXB@	17	q21-q22	*Hoxb*	11	56.00		
MFAP1	3		263	11		MFAP1	15	q15-q21	*Nfm*	14	28.50		
ODC1	3		265	7		ODC1	2	p25	*Odc*	12	6.00		
MYCN	3		270	1		MYCN	2	p24.3	*Nmyc1*	12	4.00		
TNFRSF1A	3		320			TNFRSF1A	12	p13	*Tnfrsf1a*	6	57.10		Hsiao-Ching, unpublished
HPRT1	4	p14	-9	17		HPRT1	X	q26.1	*Hprt*	X	17.00		
BTK	4	p14	-9	17		BTK	X	q21.33-q22	*Btk*	X	51.00		
PGK1	4	p14–p11	48	24		PGK1	X	q13.3	*Pgk1*	X	44.80		
CUL4B	4		75			CUL4B	X	q23	*Cul4b*	X	S	M	Groenen and Crooijmans, unpublished
UBE2A	4		66	20		UBE2A	X	q24-q25	*Ube2a*	X	S	M	
FMR1	4		82	2		FMR1	X	q27.3	*Fmr1*	X	24.50		
FGB	4	q11	90	7		FGB	4	q28	*Fgb*	3	48.20		
IRF2	4	q12–q24	157	52		IRF2	4	q35.1	*Irf2*	8	S	M	
MADH1	4		109	3		MADH1	4	q28	*Madh1*	13	35.00		
IL8	4	q13–q14	118			IL8	4	q13-q21	*Il8*	5	S	M	
FGF2	4	q21–q23	144	16		FGF2	4	q25-q27	*Fgf2*	3	19.90		
ANXA5	4		157	16		ANXA5	4	q26-q28	*Anxa5*	3	20.00		
IL2	4		157			IL2	4	q24	*Il2*	3	68.90		
NFKB1	4		189	14		NFKB1	4	q24	*Nfkb1*	3	68.90		
SPP1	4		156	16		SPP1	4	q11-q21	*Spp1*	5	56.00		
ALB	4	q23	158	16		ALB	4	q11-q13	*Alb1*	5	50.00		
GC	4	q22–q23	158	16		GC	4	q12-q13	*Gc*	5	50.00		
PPAT	4		S		3	PPAT	4	q12	*Ppat*	5	S	M	
KDR	4		S		1	KDR	4	q12	*Flk1*	5	42.00		
KIT	4	q23–q24	180	29		KIT	4	q12	*Kit*	5	42.00		
PAICS	4		S		3	PAICS	4	q12	*Paics*	5	S	M	
PGM2	4		S		3	PGM2	4	p11-q12	*Pgm1*	5	38.00		
BNC1	4		217	1		BNC1	4	p16	*Bnc1*	5	S	M	
MSX1	4		217	6		MSX1	4	p16.3-p16.1	*Msx1*	5	21.00		
TGFBR2	4		209	16		TGFBR2	3	p22	*Tgfbr2*	9	52.00		
CD8A	4		244	7		CD8A	2	p12	*Cd8a*	6	30.50		
CTNNA1	4	q27	251	4		CTNNA1	5	q31	*Catna1*	18	11.00		
MAX	5	p11	4	8		MAX	14	q23	*Max*	12	33.00		

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Table 8 (continued)

Chick locus	Chr.	FISH (RBG)	Consensus mid-point[a]	Error	T[b]	Human locus	Chr.[c]		Mouse locus	Chr.[c]		P[d]	References
PAX6	5	q11	26	12		PAX6	11	p13	*Pax6*	2	58.00		
RAG2	5	q11–q12	46	12		RAG2	11	p13	*Rag2*	2	56.00		
TH	5	q11–q12	57	12		TH	11	p15.5	*Th*	7	67.30		
INS	5	q11–q12	57	12		INS	11	p15.5	*Ins2*	7	69.20		
IGF2	5	q11–q12	57			IGF2	11	p15.5	*Igf2*	7	69.00		
MYOD1	5	q11–q12	44	19		MYOD1	11	p15.1	*Myod1*	7	23.50		
CAT	5		71			CAT	11	p13	*Cas1*	2	57.00		Cheng, unpublished
ROS0323E	5		73	9		Hs.151050	11	p11.12		UN			
CCND1	5	q11–q12	61	23		CCND1	11	q13.3	*Ccnd1*	7	72.30		
CAPN1	5		76	15		CAPN1	11	q13	*Capn1*	19	3.00		
MCW0214	5		88			Hs.82426	14	q22		UN			Groenen and Crooijmans, unpublished
BRF1	5		92	4		BRF1	14	q22-q24	*Brf1*	12	S	M	
RAD51	5		S		1	RAD51	15	q15.1	*Rad51*	2	66.80		Groenen and Crooijmans, unpublished
THBS1	5		S		1	THBS1	15	q15	*Thbs1*	2	65.00		Groenen and Crooijmans, unpublished
RYR3	5	q13	95	15		RYR3	15	q14-q15	*Ryr3*	2	S		
PTAFR	5	q21–q22	110	10		PTAFR	1	p35-p34.3	*Ptafr*	4	62.40		
HTR1D	5	q21–q22	110	10		HTR1D	1	p36.3-p34.3	*Htr1d*	4	66.00		
TGFB3	5	q21–q22	110	10		TGFB3	14	q24	*Tgfb3*	12	41.00		
CALM1	5		128	20		CALM1	14	q24-q31	*Calm*	7	4.50		Vignal and Pitel, unpublished
HSPCAL4	5		151	10		HSPCAL4	14	q32.3	*Hspcal4*	12	S	M	
IGH@	5		S		2	IGH@	14	q32.33	*Igh*	12	58.00		Bumstead, unpublished
DNCH1	5		151	6		DNCH1	14	q32.3-qter	*Dnchc1*	12	55.00		
CKB	5		160	20		CKB	14	q32.3	*Ckb*	12	55.00		
HSPA2	5		170			HSPA2	14	q22	*Hsp70-2*	12	34.00		Bumstead, unpublished
SOS2	5	q24–q25	192	11		SOS2	14	q21-q22	*Sos2*	12	30.00		
BMP4	5		202	10		BMP4	14	q22-q23	*Bmp4*	14	14.00		
TFAM	6	q11–q12	43	8		TFAM	10	q21	*Tfam*	10	38.00		
SUPV3L1	6	q12	59	6		SUPV3L1	10	q22.1	**Supv3L1**	10	S	M	Smith, unpublished
PSAP	6		59	6		PSAP	10	q22.1	*Psap*	10	35.00		
PLAU	6		59			PLAU	10	q44	*Plau*	14	2.50		Groenen and Crooijmans, unpublished
ACTA2	6		65	1		ACTA2	10	q22-q24	*Actvs*	7	S	M	
PDE6C	6	m	65	2		PDE6C	10	q24	*Pde6c*	7	68.00		
SCD	6	q14	63	5		SCD	10	q24	*Scd1*	19	43.00		
CYP17	6	q14–q15	75	6		CYP17	10	q24.3	*Cyp17*	19	46.00		
ROS0028E	6		111	12		Hs.11859	1	q25-q31		1	S	M	
RXRG	7	p12–p11	13	35		RXRG	1	q22-q23	*Rxrg*	1	88.10		
COL3A1	7		-12			COL3A1	2	q31-q32.3	*Col3a1*	1	21.10		
NAB1	7		0			NAB1	2	q32.3-q33	*Nab1*	1	27.00		Groenen and Crooijmans, unpublished
FN1	7		5			FN1	2	q34	*Fn1*	1	36.10		
GDF8	7	p11	33	15		GDF8	2	q32.1	*Mstn*	1	27.70		
GBX2	7		34	1		GBX2	2	q37	*Gbx2*	1	65.00		
NDUFS1	7		51	6		NDUFS1	2	q33-q34	*Ndufs1*	1	S	M	
EEF1B2	7		51	6		EEF1B2	2	q33-q34	*Eef1b2*	1	S	M	
CD28	7		59	1		CD28	2	q33	*Cd28*	1	30.10		
SLC11A1	7	q13	78	18		SLC11A1	2	q35	*Slc11a1*	1	39.20		
VIL1	7		79	8		VIL1	2	q35	*Vil*	1	40.80		
INHBB	7		92	22		INHBB	2	cen-q13	*Inhbb*	1	64.10		
ROS0019E	7		101	1		Hs.11360	3	q13.3		16	S	M	
HOXD@	7	q13–q14	106	22		HOXD@	2	q31	*Hoxd*	2	45.00		
MCM6	7		122	1		MCM6	2	q14-q21	*Mcm6*	2	S	M	
PTPRC	8	p12	4	13		PTPRC	1	q31-q32.3	*Ptprc*	1	74.00		
GLUL	8		1	12		GLUL	1	q25	*Glns*	1	S	M	
PLA2G2A	8		32	7		PLA2G2A	1	p36.1-p35	*Pla2g2a*	4	66.60		
PTGS2	8		50	14		PTGS2	1	q25.2-q25.3	*Ptgs2*	1	76.20		
AT3	8	p11	50	13		AT3	1	q23-q25.1	*At3*	1	84.60		
RPL5	8	p11–q11	56	40		RPL5	1	p13.3-p11	*Rpl5*	3	S	M	
ROS0149E	8		66	16		Hs.155983	1	p33-p31		4	S	M	
B4GALT2	8		67	16		B4GALT2	1	p33-p34	*B4galt2*	4	S	M	
MCW0271	8		90			Hs.12413	1	p33-p31.1		UN			Groenen and Crooijmans, unpublished
LEPR	8		90	20		LEPR	1	p31.2-p31.1	*Lepr*	4	46.70		
JAK1	8		92	20		JAK1	1	p32.3-p31.3	*Jak1*	4	46.30		
GADD45A	8		94	1		GADD45A	1	p31.2-p31.1	*Gadd45a*	3	70.50		
ZNF265	8		94			ZNF265	1	p22.1-p21.3	*Zfp265*	UN			Groenen and Crooijmans, unpublished
TFRC	9		90	10		TFRC	3	q26.2-qter	*Trfr*	16	22.50		
EIF4A2	9		93			EIF4A2	3	q24-q27	*Eif4a2*	16	S	M	
NCL	9		93	1		NCL	2	q12-qter	*Ncl*	1	48.40		
PAX3	9		99			PAX3	2	q36	*Pax3*	1	44.00		
SKIL	9		105	30		SKIL	3	q24-q27	*Skir*	3	13.00		
MCW0134	9		132	1		Hs.9414	3	q25.2		3	S	M	

Table 8 (continued)

Chick locus	Chr.	FISH (RBG)	Consensus mid-point[a]	Error	T[b]	Human locus	Chr.[c]		Mouse locus	Chr.[c]		P[d]	References
CKMT	10		S		1	CKMT	15	q15	*Ckmt1*	2	67.60		Groenen and Crooijmans, unpublished
B2M	10	m10–11	0	10		B2M	15	q21-q22.2	*B2m*	2	69.00		
FBN1	10	m	60	20		FBN1	15	q21.1	*Fbn1*	2	71.00		Groenen and Crooijmans, unpublished
CYP19	10		60	20		CYP19	15	q21	*Cyp19*	9	31.00		
ANXA2	10	m	S		1	ANXA2	15	q21-q22	*Anxa2*	9	S	M	Groenen and Crooijmans, unpublished
NEO1	10		S		1	NEO1	15	q22-q23	*Neo1*	9	S	M	Groenen and Crooijmans, unpublished
CRABP1	10	m8–9	S		1	CRABP1	15	q22-qter	*Crabp1*	9	31.00		Groenen and Crooijmans, unpublished
TPM1	10		S		1	TPM1	15	q22	*Tpm1*	9	40.00		Groenen and Crooijmans, unpublished
HMX3	10		71	1		HMX3	UN		*Hmx3*	7	61.00		
AGC1	10		71	7		AGC1	15	q26	*Agc*	7	39.00		
POLG	10		71	1		POLG	15	q25	*Polg*	7	S		
IGF1R	10	m10–11	88	10		IGF1R	15	q26.1-q26.3	*Igf1r*	7	33.00		
NR2F2	10		S		1	NR2F2	15	q26	*Nr2f2*	7	33.00		Groenen and Crooijmans, unpublished
RPL4	10		111	9		RPL4	15	cen-qter	*Rpl4*	UN			
GNRHR	10		109	13		GNRHR	4	q21.2	*Gnrhr*	5	44.00		
CSNK2A2	11		0			CSNK2A2	16	q13	*Csnk2a2*	8	50.00		
CCNE	11	m10–15	38	1		CCNE	19	q12-q13	*Cnne*	7	16.00		
MAF	11		55	4		MAF	16	q22-q23	*Maf*	8	61.00		
HZAF2	11	m	36	60		HZAF2	4	q24	**H2az**	3	S	M	
ADL0240	12		10	1		**HSLUCA9**	3	p21.3	**Hsluca9**	9	S	M	
ARF4	12		10	1		ARF4	3	p21.2-p21.1	*Arf4*	9	S	M	
MSX2	13		41	5		MSX2	5	q34-q35	*Msx2*	13	32.00		
SPOCK	13		47	3		SPOCK	5	q31	*Spock*	18	S	M	
CDX1	13		56	6		CDX1	5	q31-q33	*Cdx1*	18	30.00		
POU4F3	13		71	7		POU4F3	5	q31	*Pou4f3*	18	24.00		
CAMLG	13	m8–12	70	7		CAMLG	5	q23	*Caml*	13	35.00		
IRF1	13	m	S		2	IRF1	5	q23-q31	*Irf1*	11	29.00		
HBA@	14	m8–12	26	30		HBA1	16	p13.3	*Hba*	11	16.00		
NTN2	14		77	1		NTN2L	16	p13.3	*Ntn2*	11	S	M	
CRYBB1	15	m	31	3		CRYBB1	22	q11.2	*Crybb1*	5	59.00		
CRYBA4	15		32	3		CRYBA4	22	q11.2-q13.1	*Cryba4*	5	59.00		
IGVPS	15	m	35	1		IGL@	22	q11.2	*Igl*	16	13.00		
MIF	15		32	10		MIF	22	q11.2	*Mif*	10	40.50		
IGLC1	C15		0	1		IGL@	22	q11.2	*Igl*	16	13.00		
TUBAL2	C15		10	1	2	Hs.112015	22	p11.2-q11.2		10	S	M	
SFPQ	C15		25	1	2	SFPQ	1	p33-p31.1	*Sfpq*	4	S	M	
B@	16	m16	0	20		HLA	6	p21.3	*H2*	17	23.00		
BTN1A1	16		0	20		BTN1A1	6	p22-p21.3	*Btn-rs1*	17	20.30		
G9A	16		0	20		G9A	6	p21.3	*Bat8*	17	18.87		
C4A	16		0	20		C4A	6	p21.3	*C4*	17	18.80		
ABCB3	16		0	20		ABCB3	6	p21.3	*Abcb3*	17	18.62		
ABCB2	16		0	20		ABCB2	6	p21.3	*Abcb1*	17	18.60		
B-DMB	16		0	20		HLA-DMB	6	p21.3	*H2-Mb1*	17	18.58		
B-DMA	16		0	20		HLA-DMA	6	p21.3	*H2-Ma*	17	18.56		
TAPBP	16		0	20		TAPBP	6	p21.3	*Tapbp*	17	18.41		
BRD2	16		0	20		BRD2	6	p21.3	*Brd2*	17	18.53		
RNF3L	17		-4	7		RNF3L	9	q34	*Rnf3l*	2	S	M	
RPL7A	17	m8–12	11	1		RPL7A	9	q34.1	*Rpl7*	2	15.50		
ABL1	17	m8–12	22	1		ABL1	9	q34.1	*Abl*	2	21.00		
AK1	17	m8–12	29	1		AK1	9	q34.1	*Ak1*	2	21.60		
ENTPD2	17		56	19		ENTPD2	9	q34	*Entpd2*	2	S		
HXB	17		S			HXB	9	q33	*Tnc*	4	32.20		
AMBP	17		58	19		AMBP	9	q32-q33	*Ambp*	4	30.60		
MYH@	18	m16–18	0	1		MYH1	17	p13.1	*Myhse*	11	35.00		
HLF	18		48	8		HLF	17	q21-q22	*Hlf*	11	52.00		
NME2	18		31	20		NME2	17	q21-q22	*Nme2*	11	72.00		
H3F3B	18	m16–18	35	5		H3F3B	17	q25	*Hsf3b*	11	S	M	
FASN	18	m16–18	40	12		FASN	17	q25	*Fasn*	11	72.00		
RAC3	18		40	12		RAC3	17	q24-qter	*Rac2*	11	S	M	
ACACA	19	m16–20	-1	45		ACACA	17	q21	*Acac*	11	S	M	
CRK	19		0	17		CRK	17	p13	*Crko*	11	44.15		
CASP1	19		1	5		CASP1	11	q22.2-q22.3	*Casp1*	9	1.00		
ETS1	24	m8–9	0	1		ETS1	11	q24	*Ets1*	9	15.00		
OPCML	24	m18–22	20	1		OPCML	11	q23-qter	*Obcam*	9	10.00		
RPS25	24		40	20		RPS25	11	q23.3	*Rps25*	9	S	M	

➤

Table 8 (continued)

Chick locus	Chr.	FISH (RBG)	Consensus mid-point[a]	Error	T[b]	Human locus	Chr.[c]		Mouse locus	Chr.[c]		P[d]	References
POU2F3	24		43	7		*POU2F3*	11	S	*Pou2f3*	9	23.00	H	
APOA1	24		53	10		APOA1	11	q23.3	*Apoa1*	9	27.00		
NFASC	26		-7			*NFASC*	1	q31-q32.3	*Nfasc*	1	70.00	H	
NRF1	26		15	1		NRF1	7	q31-q32	*Nrf1*	6	S		
PGA@	26	m19–23	25	6		PGA@	11	q13	*Pga@*	UN			
ROS0314E	26		25	6		Hs.80464	1	p13.3-q12		3	S	M	
TNNT2	26		27	8		TNNT2	1	q32	*Tnnt2*	1	S	M	
CNTN2	26	m19–23	33	3		CNTN2	1	q32-q33	*Cntn2*	1	S	M	
TCRA@	27		0	1		TRA@	14	q11.2	*Tcra*	14	19.50		
DAD1	27		0	1		DAD1	14	q11-q12	*Dad1*	14	36.50		
GH	27		10	40		GH1	17	q22-q24	*Gh*	11	65.00		
SLC4A1	27		20	30		SLC4A1	17	q12-q21	*Slc4a1*	11	62.00		
COL1A1	27		36	1		COL1A1	17	q21.3-q22	*Cola1*	11	56.00		
SF3A2	28	m	21		4	SF3A2	19	p13.3	*Sf3a2*	10	43.00		
AMH	28	m	21	1	4	AMH	19	p13.3	*Amh*	10	43.00		
ROD1	28		30	4	4	ROD1	19	p13.3	*Rod1*	10	S	M	Groenen and Crooijmans, unpublished
TRAP95	28		30	4	4	TRAP95	19	p13.3	*Trap95**	10	S	M	Groenen and Crooijmans, unpublished
PTBL1	28		30	4	4	Hs.101750	19	p13.3	*Ptb*	10	43.00		Groenen and Crooijmans, unpublished
HNRPL	28		30	1	4	HNRPL	19	p13.3	*Hnrpl*	10	S	M	Groenen and Crooijmans, unpublished
CAMK4	28		46	1		CAMK4	5	q21-q23	*Camk4*	18	12.00		Groenen and Crooijmans, unpublished
RENT1	28		48	10	4	RENT1	19	p12	*Rent1*	8	33.50		Groenen and Crooijmans, unpublished
GDF1	28		48	10	4	GDF1	19	p12	*Gdf1*	8	S	M	Groenen and Crooijmans, unpublished
COMP	28		48	10	4	COMP	19	p13.1	*Comp*	8	33.0		Groenen and Crooijmans, unpublished
JUND1	28		48	10	4	JUND1	19	p13.1	*Jund1*	8	33.00		Groenen and Crooijmans, unpublished
INSR	28		58	10		INSR	19	p13.3	*Insr*	8	1.00		
PTPRS	28		60	2		PTPRS	19	p13.3	*Ptprs*	17	33.80		Groenen and Crooijmans, unpublished
ERBB3	E22C19W28		14	10		ERBB3	12	q13	*Erbb3*	10	70.00		
TUBAL1	E22C19W28		25	20		TUBAL1	12	S	*Tubal1*	10	S	M	
GLI	E22C19W28		30	1		GLI	12	q13.2-q13.3	*Gli*	10	69.00		
SNRPD2	E25C31		-2			SNRPD2	19	q13.3	*Snrpd2*	7	S	M	
TGFB1	E25C31		-1	12		TGFB1	19	q13.2	*Tgfb1*	7	6.50		
RYR1	E25C31		0	1		RYR1	19	q13.1	*Ryr1*	7	10.00		
CKM	E25C31		20	10		CKM	19	q13.2	*Ckmm*	7	4.00		Smith, unpublished
MCL1	E26C13		-1	6		MCL1	1	q21		3	43.6?		
HCK	E32	m10–15	15	6		HCK	20	q11-q12	*Hck*	2	86.00		
BMP7	E32	m10–15	16	6		BMP7	20	q13	*Bmp7*	2	102.00		
CDC2L1	E54	m	0	1		CDC2L1	1	p36.3	*Cdc2l1*	4	79.40		
AGRN	E54	m	25	1		AGRN	1	p36.3-p32	*Agrn*	4	S		
ENO1	E54		30	15		ENO1	1	p36	*Eno1*	4	79.00		
PLOD	E54		64	1		PLOD	1	p36.3-p36.2	*Plod*	4	76.50		
SLC2A1	E54		70	15		SLC2A1	1	p35-p31.3	*Slc2a1*	4	52.00		
TP53	E57		14	1		TP53	17	p13.1	*Trp53*	11	39.00		
ATP5A1	Z	p24–p23	-1	15		ATP5A1	18	q11-q21	*Atp5a1*	18	51.00		
IFN1	Z	p24–p23	-3	8		IFNA1	9	p22	*Ifna1*	4	42.60		
IFN2	Z	p24–p23	-3	8		IFNB1	9	p22	*Ifnb*	4	42.60		
RPS6	Z		S		1	RPS6	9	p21	*Rps6*	4	S	M	Nanda, unpublished
PRLR	Z	p23–p22	24	4		PRLR	5	p14-p13	*Prlr*	15	4.60		
GHR	Z	p23–p22	28	1		GHR	5	p14-p12	*Ghr*	15	4.60		
DMRT1	Z	p21–p13	37	8		DMRT1	9	p24.3	*Dmrt1*	19	C2-C3		
VLDLR	Z	p13–p12	57	12		VLDLR	9	p24	*Vldlr*	19	20.00		
SMARCA2	Z	p12–p11	66	9		SMARCA2	9	p24-p23	*Smarca2*	19	17.00		
HINT	Z	q11	102	15		HINT	5	q31.2	*Hint*	UN			Mizuno, unpublished
NTRK2	Z	p12–p11	80	12		NTRK2	9	q22.1	*Ntrk2*	13	36.00		Nanda, unpublished
SPIN	Z	q11	102	15		SPIN	9	q22.1-q22.3	*Spin*	13	S	M	Mizuno, unpublished
SYK	Z	q11	106	5		SYK	9	q22	*Syk*	13	37.00		
PTCH	Z		116	18		PTCH	9	q22.3	*Ptch*	13	34.00		
CTSL	Z		119	18		CTSL	9	q22.1-q22.2	*Ctsl*	13	30.00		
CHD1	Z	q14–q21	131	6		CHD1	5	q15-q21	*Chd1*	17	7.45		
CHRNB3	Z		139	4		CHRNB3	8	p11.2	*Chrm3*	8	S	M	
LPL	Z		144	1		LPL	8	p22	*Lpl*	8	33.00		
MUSK	Z	q14–q21	150	13		MUSK	9	q31.3-q32	*Nsk1*	4	26.30		
ALDOB	Z	q14–q21	160	10		ALDOB	9	q22.3-q31	*Aldo2*	4	22.30		
XPA	Z		186	6		XPA	9	q22.3	*Xpa*	4	21.50		
TMOD	Z	q21	175	32		TMOD	9	q22.2-q22.3	*Tmod*	4	21.50		
ACO1	Z	q14–q21	187	6		ACO1	9	p22-p13	*Aco1*	4	20.90		
B4GALT1	Z		187	5		B4GALT1	9	p21-p13	*B4galt1*	4	18.60		

The comparative maps between chicken, mouse and human were used to estimate the number of autosomal conserved segments between these species (Burt et al., 1999 and Waddington et al., 2000a, 2000b). The number of autosomal conserved segments between chicken-human is 154, of which 100 (65%) have been defined. In contrast, the number of autosomal conserved segments between chicken-mouse is 312, of which 144 (46%) have been defined. Only small conserved segments remain to be identified and will require larger numbers of genes to be mapped to detect them with more than one marker (so far, we have assumed that conserved segments marked by a single homologue are real, this needs to be confirmed). Overall these results at first were a surprise – and showed that the genomes of chicken and human are more alike than that of mouse and human (Burt et al., 1999). These results may be explained if the genomes of chicken and human evolve (rearrange) at a slower rate than that of the mouse. Why – is unknown, but possible mechanisms have been proposed (Burt et al., 1999). The stability of the avian genome has also been demonstrated from Zoo-FISH studies showing conservation of synteny between chicken and emu macrochromosomes (Shetty et al., 1999), species that diverged over 80 million years ago. Recently, Zoo-FISH was even possible with human chromosome 4-specific paint on chicken chromosomes and showed that the human chromosome corresponds largely to chicken chromosome 4 (Chowdhary and Raudsepp, 2000).

WWW sites:
http://www.ri.bbsrc.ac.uk/chickmap/table_contents.html
http://poultry.mph.msu.edu/resources/Resources.htm
http://www.zod.wau.nl/vf/research/chicken/frame_chicken.html

Gene content and evolutionary conservation of avian sex chromosomes

(Prepared by I. Nanda, T. Haaf, M. Schartl, R. Fries and M. Schmid)

Birds possess a ZZ/ZW sex chromosome system in which the female is the heterogametic sex. The average Z chromosome is a medium-sized macrochromosome, representing 7–10% of the entire genome. The W of most modern birds is considerably smaller than the Z and largely heterochromatic. In primitive birds, the W chromosome resembles the Z in size and morphology, and most likely also in gene content (Fridolfsson et al., 1998; Ogawa et al.,1998). The absence of Z chromosome dosage compensation in modern birds with highly differentiated sex chromosomes implies a higher expression for Z-linked genes in ZZ males than in ZW females (Baverstock et al., 1982; Schmid et al., 1989). However, it is not clear whether this dosage difference between the two sexes also plays a role in avian sex determination. There are several lines of evidence for a dominant (dosage-insensitive) ovary-determining factor on the W chromosome, as well as for a dosage-sensitive testis-determining factor on the Z. More details on avian sex chromosomes can be found in three recently published reviews (Piggozi, 1999; Ellegren, 2000; Nanda et al., 2000).

Sex-linked genes with housekeeping and possible sex-specific functions

Despite the fact that the *Gallus gallus* (GGA) Z covers almost 210 cM of the chicken genome (Levin et al., 1993), our knowledge on its gene content is still rather limited. Classical linkage studies led to the assignment of several loci, mainly morphological traits, to the Z chromosome (Bitgood, 1993), and probably of a single locus with possible sex-specific function, histoantigen H-W, to the W (Wachtel et al., 1975). Concerted mapping efforts in the past few years have significantly improved chicken maps (Burt et al., 1999). In particular, the laboratories of S. Mizuno (Japan), Y. Matsuda (Japan), M. Schmid (Germany), D. Burt (UK), and H. Cheng (USA) mapped a number of type I (functional) loci to the chicken sex chromosomes. Taken together, 28 genes were assigned to the GGA Z (Fig. 12a) and four to the W (Fig. 12b). Five Z-linked genes were genetically mapped through classical linkage analysis. All other genes were localized on metaphase chromosomes by fluorescence in situ hybridization (FISH) with cDNA or genomic DNA probes. Most of the currently known sex-linked genes have housekeeping functions. The few candidate genes which may be involved, directly or indirectly, in sex determination will be discussed in more detail in the section on DNA sequences and gene functions of the Z and W chromosomes (Mizuno, in this report). In addition to type I loci, several anonymous DNA sequences, including repetitive DNAs and pseudogenes, were mapped to both Z and W.

By comparing the location of Z-linked genes in chicken and human genomes, 17 Z-linked genes were found to have orthologs on the human (HSA) chromosome 9pter→q31, representing one of the largest and oldest regions of conserved chromosome synteny among vertebrates. Additional smaller segments showing synteny with GGA Z were identified on HSA 5 and HSA 8 (Nanda et al., 2000). In this context, it is important to emphasize that comparative gene mapping did not detect any homology between the avian Z and mammalian X, which makes it very unlikely that they have a common ancestral origin.

Information on chromosome homologies in the chicken, which is a relatively poorly mapped species compared with humans, can greatly facilitate the assignment of additional

Footnote to Table 8

[a] S: syntenic.
[b] T: (1) FISH and comparative gene mapping. (2) COM map and comparative gene mapping. (3) Somatic cell genetics and comparative gene mapping. (4) Genetic linkage and comparative gene mapping.
[c] UN: unassigned loci.
[d] P: H, M; predictions based on human and mouse conserved segments.

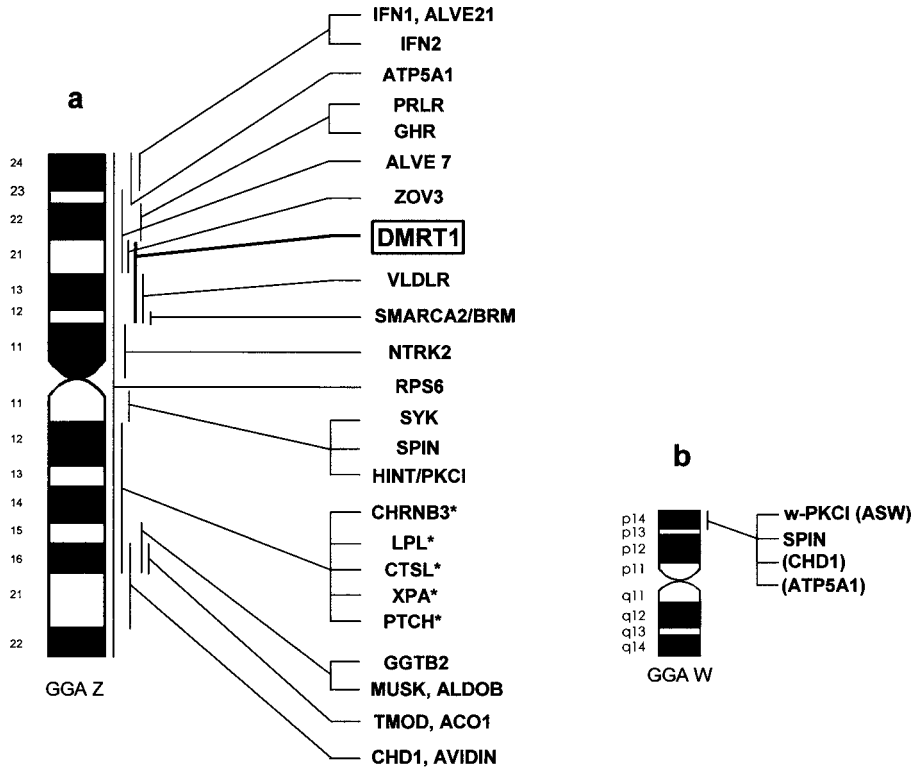

Fig. 12. (**a**) G-banded ideogram of the chicken Z chromosome showing the cytogenetic locations of 28 Z-linked genes. The five genes indicated by stars were mapped by genetic linkage analysis. Z linkage of RPS6 is based on quantitative Southern hybridization. All other genes were FISH-mapped. DMRT1 was earlier assigned to Zp21 (Nanda et al., 1999), but recent mapping suggests that it is positioned at Zp12→p13. The map position of individual genes may slightly differ when cytogenetic and genetic information are integrated (see also contribution of Burt et al., in this report). (**b**) G-banded ideogram of the chicken W chromosome with four W-linked genes. The chromosome positions of *CHD1* and *ATP5A1* have not yet been resolved. Considering the heterochromatic nature of large W parts including the entire long arm, both genes are positioned provisionally on the short arm.

chicken genes to the same syntenic region (Burt et al., 1999). For example, based on the identified chromosome homology between GGA Z and HSA 9, we were able to isolate a candidate testis-determining gene, *DMRT1*, from the short arm of the chicken Z chromosome (Nanda et al., 1999; 2000). *DMRT1* is structurally and functionally related to male sexual regulatory genes from different phyla, i.e. *Drosophila melanogaster doublesex* and *Caenorhabditis elegans mab-3* (Raymond et al., 1998). The human ortholog is located within the critical region for sex reversal in patients with monosomy 9p. Similar to the situation in humans, where haploinsufficiency for a dosage-sensitive gene in HSA 9p24.3 interferes with normal testis development (Raymond et al., 1999b), two Z-linked *DMRT1* dosages may be required for testis formation in male chickens, whereas expression from a single Z chromosome in ZW females leads to female sexual differentiation. Higher *DMRT1* expression levels in the male than in the female genital ridges during early stages of chicken embryogenesis as well as testis-specific expression after onset of sexual differentiation provide further strong evidence for a functional role of *DMRT1* in avian sex determination (Raymond et al., 1999a; Shan et al., 2000).

Recently another possible sexual regulatory gene, tentatively called avian sex-specific W-linked factor (*ASW*), was detected on the chicken W chromosome. Since *ASW* is expressed exclusively in early female chicken embryos (O'Neill et al., 2000), it could act as a dominant ovary-determining factor in birds. Its nucleotide sequence is identical with W-linked *PKCI*, which has a homolog on the Z chromosome (Mizuno, in this report).

Z-W homology and evolution of avian sex chromosomes

Based on similar Z chromosome sizes and Z linkage of a common genetic trait, albinism, in different bird species, Ohno (1967) proposed that the Z sex chromosome has been conserved during avian evolution. Biochemical studies (Baverstock et al., 1982) which consistently revealed higher levels of the Z-linked aconitase gene product in male than in female birds from three different orders first confirmed Ohno's hypothesis on the conservation of Z-linked genes. Moreover, classical G-banding (Christidis, 1990) and DNA replication banding (Schmid et al., 1989) demonstrated extensive structural and functional homology between different avian Z chromosomes. During meiosis both Z and W in carinate birds are paired to some extent, and at least one recombination nodule is formed in the synapsed region (Pigozzi, 1999), suggesting partial homology of an evolutionarily conserved segment between Z and W.

At least three Z-linked genes, *IREBP*, *ZOV3*, and *CHD1*, and one Z-linked DNA marker, *EE0.6,* were mapped to the Z in widely different bird species, including the primitive Ratitae (Saitoh et al., 1993; Ogawa et al., 1998; Ellegren et al., 2000). By quantitative Southern hybridization, two genes, *PKCI* (*HINT*) and *SPIN*, also showed Z linkage in several species (Hori et al., 2000; Itoh et al., unpublished). Furthermore, as in chicken, the *IFN* is found to be on the distal long arm tip of the duck Z (Nanda et al., 1998). Comparative hybridization of Z-linked chicken probes directly visualized four loci on the quail Z (Suzuki et al., 1999c). Similarly, a chicken cosmid was used to localize the candidate testis-determining factor, *DMRT1*, to the Z chromosomes of three galliform birds (Nanda et al., 2000). Comparatively mapped genes are distributed along the entire

length of the chicken Z. This makes it unlikely that only particular Z segments have been conserved during avian evolution. In addition, a chicken Z-chromosome-specific DNA library painted both the Z and the W chromosomes of the primitive emu (Shetty et al., 1999). This argues in favor of the notion that Z and W differentiated from an ancestral homologous chromosome pair and that despite highly variable morphology, the avian Z has been conserved in its entirety. Comparative chromosome painting studies in a larger number of bird species are underway.

Although the W chromosome is largely heterochromatic in most modern birds, at least some W-linked sequences appear to be evolutionarily conserved. Most importantly, *ASW*, up to now the strongest candidate for an ovary-determining factor, is present on the W chromosome of 17 tested avian species (O'Neill et al., 2000). Two loci, *CHD1* and *EE0.6*, which were mapped to the W chromosome of many different birds, have homologs on the Z (Itoh et al., 1997; Fridolfsson et al., 1998; Ogawa et al., 1998). They are particularly useful to determine the sex of carinate birds by restriction or PCR analysis. In addition, a turkey anonymous cDNA was located to both, Z and W chromosomes in chicken, pheasant, and turkey (Dvořák et al., 1992). Altogether, four different genes and a few other DNA sequences are shared by Z and W (Fridolfsson et al., 1998; Ellegren et al., 2000), reflecting the common evolutionary history of Z and W. Heterochromatinization and subsequent deletions of "degenerated" W chromosome regions, and extensive intrachromosomal rearrangements of the Z may have contributed to the evolution of highly differentiated sex chromosomes in modern birds. Contrary to the high conservation of the sex chromosomes within birds, it seems beyond any doubt that avian and mammalian sex chromosomes evolved independently from each other, and that they should harbor fundamentally different sex-determining genes leading to different sex determining mechanisms.

DNA sequences and gene functions of the Z and W chromosomes

(Prepared by S. Mizuno)

Hunt for a sex-related gene on the chicken W chromosome

The positive role of the W chromosome in the early differentiation of the female was suggested strongly by the observation that initial differentiation of the 3A+ZZW triploid chicken embryo was similar to that of the normal female (Sheldon and Thorne, 1995). It may thus be expected that some unknown gene on the avian W chromosome exhibits a positive female-determining function. We have been searching for such a gene using two different approaches. The first approach is based on the organization of the chicken W chromosome. About 65% of DNA sequences in the W chromosome are *Xho*I-family and *Eco*RI-family repetitive sequences, which occupy most of the long arm and about half of the short arm (Mizuno and Macgregor, 1998). These repetitive sequence-occupying regions form a conspicuous W-heterochromatic body in the interphase nucleus (Suka et al., 1993). The distal part of the W short arm escapes from the formation of heterochromatin (Suka et al., 1993) and is expected to contain a very short pseudoautosomal paring region (PAR), because the tip of this region was shown to pair with the tip of the non-heterochromatic short arm of the Z chromosome in the lampbrush ZW bivalent at diplotene (Hori et al., 1996; Mizuno and Macgregor, 1998), and a single recombination nodule was observed in this region at pachytene (Rahn and Solari, 1986). Except for the PAR, the distal non-heterochromatic region of the W chromosome may contain non-repetitive sequences including active gene(s). We isolated a genomic clone located in this region from a W chromosome–specific genomic library (Ogawa et al., 1997), and starting with this cloned region, contigs of cosmid and BAC genomic clones covering about 480 kb non-repetitive sequence region were selected subsequently. By applying the exon-trapping procedure on this entire region, an exon-like sequence, *CPE15,* was obtained. cDNA clones obtained from the chicken ovary or testis library using *CPE15* as a probe showed extensive sequence similarity to the mouse *spindlin*, which encodes a 30-kDa protein which is abundant in the mouse oocyte and zygote and associates with the spindle and undergoes cell-cycle-dependent phosphorylation (Oh et al., 1997). The chicken cDNA sequence also showed a relatively high-level similarity to mouse *Ssty*, a multi-copy gene on the long arm of the Y chromosome and expressed during spermatogenesis (Burgoyne et al., 1992). The chicken *spindlin* genes are present on both W and Z chromosomes, and the protein-encoding sequences of these genes are nearly identical. However, by utilizing the sequence difference in the 3′-UTR, we could demonstrate that both genes are actively transcribed (Y. Itoh et al., manuscript in preparation).

The other approach we adopted was to search for a W-linked cDNA clone in the subtracted (female minus male) cDNA library, constructed from the mixed tissues of undifferentiated gonads and mesonephroi of the 5-day (stages 26 to 28) chicken embryos. The *Wpkci* gene, thus obtained, is a multicopy gene located near the end of the distal non-heterochromatic region of the W chromosome and encodes an altered form of PKCI (protein kinase C interacting protein). The chicken *PKCI* gene (a homologous gene for that of the mammalian PKCI; Brzoska et al., 1996) was cloned and located close to the centromere on the long arm of the Z chromosome. The W-linkage and the multicopy nature of *Wpkci* are conserved in every species of birds tested except for the Ratitae species (emu and ostrich). In the latter species, the probe for the third exon of chicken *Wpkci*, which was 73% similar to that of *PKCI*, detected a male, female common band in Southern blot hybridization but reiteration of the sequence in the female was not detected, and thus, it was not possible to conclude if the *Wpkci* gene was present in the female genome of these Ratitae species. We propose a model that Wpkci interferes with the PKCI function by forming a heterodimer with it, which may lead to female sex determination (Hori et al., 2000). We noticed on the DNA database that the protein-encoding sequence of *Wpkci* was identical to that of *ASW* (O'Neill et al., 2000).

Sex-related genes on the Z chromosome

Genes for spindlin (*cSpin-Z*) and PKCI (*chPKCI*) are located close to the centromere on the long arm of the chicken Z chromosome (Y. Itoh, manuscript in preparation; Hori et al.,

2000). These two genes are located near the end of the distal, non-heterochromatic region on the short arm of the W chromosome. It thus suggests that significant morphological changes occurred during differentiation of the present day Z and W chromosomes of the Carinatae birds (Ogawa et al., 1998). The *ZOV3* gene on the short arm of the Z chromosome encodes an immunoglobulin-superfamily glycoprotein consisting of 327 amino acid residues (Saitoh et al., 1993), which is structurally related to the mouse GP-70 and chicken HT7, all having two Ig-like loops of the C2 type in the extracellular domain. Immunocytological detection indicates that ZOV3 is present in the plasma membrane of granulosa cells and of islets of estrogen-producing cells in the theca externa layer of ovarian follicles, implying that ZOV3 expression on the membrane may be important for the intercellular contacts of cells producing estrogen or its precursor steroids (Kunita et al., 1997). The *VLDLR* gene on the short arm of the chicken Z chromosome (Nanda et al., 1999) encodes the 95-kDa VLDL (very low density lipoprotein) receptor on the plasma membrane of oocytes, which has been shown to be responsible for the accumulation of the two major yolk precursor proteins, VLDL and vitellogenin, by endocytosis during the oocyte maturation (Barber et al., 1991). The *DMRT1* gene on the short arm of the chicken Z chromosome was implied to be involved in the differentiation of the male gonads as suggested for mammals (Nanda et al., 1999). In chickens, transcripts of the *DMRT1* gene are detectable in the genital ridge area of the stage-25 embryo in both male and female. Levels of expression of the *DMRT1* gene in embryos of stages 28 to 31 are significantly higher in the male, probably reflecting the two-times higher gene dosage in the male (Raymond et al., 1999a). Recently, we found an approximately 460-kb long region, designated MHM (male hypermethylated) region, which is located just adjacent to the *DMRT1* gene on the short arm of the Z chromosome. This region consists of tandem repeats of a 2.2-kb unit. On the two Z chromosomes in the male, the cytosines of CpG dinucleotides in this region are highly methylated and transcriptionally inactive. On the other hand, this region is much less methylated and actively transcribed on the single Z chromosome in the female. The transcripts are of high molecular weight and heterogeneous in size and accumulate at the site of transcription, which is adjacent to the *DMRT1* locus in the nucleus. We speculate that the accumulation of the heterogeneous transcripts may be one of several factors to cause suppression of the *DMRT1* gene in the female (M. Teranishi et al., manuscript in preparation).

Overall, a gene on the W chromosome is likely to trigger the cascade of gene expression toward the female determination but functions of several genes on the Z chromosome are also required for the process of differentiation and the maintenance of functions in the female gonad.

Homeobox genes

(Prepared by K. Ladjali-Mohammedi)

Homeobox genes are involved in the genetic control of development and are thought to organize the body plan. They are present in the genomes of enteropneusts, tunicates, amphioxus, hagfish, lampreys and jawed vertebrates (reviewed by Holland and Garcia-Fernàndez, 1996).

Table 9. Chromosome location of HOX gene clusters in the human, mouse, porcine and chicken genomes

Species	HOXA@	HOXB@	HOXC@	HOXD@
Human	7p15–p14	17q21–q22	12q12–q13	2q31
Mouse	6 (26.3)	11 (56.0)	15 (57.4)	2 (45.0)
Pig	18q21–q24	12p12–p11	5p12–p11	15q22–q23
Chicken	2p21	3q31	1q31	7q13–q14

The first homeobox genes and also the best known, are the homeotic selector genes of *Drosophila melanogaster*. These genes are organized into two complexes, the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C) (McGinnis et al., 1984; Akam, 1989; Duboule and Dollé, 1989; Graham et al., 1989). In mammals, *Hox* genes are organized into four complexes designated A, B, C and D, that each consist of about 10 genes extending over 100 kb (reviewed by Ruddel et al., 1987; Kessel and Gruss, 1990; McGinnis and Krumlauf, 1992). Genes have been assigned to 13 paralogous groups highly related by sequence homology and position in the clusters. A characteristic of these clusters is that the order of the genes within the cluster generally parallels the position along the body where the genes are expressed. In a given complex, the *Hox* genes located at the 3′ end have the most anterior expression (Gaunt et al., 1988). The order of *Hox* genes within each cluster is highly conserved throughout evolution suggesting that the physical organization of *Hox* genes is essential for their expression and accounts for major biological functions (Cillo, 1995).

The four HOX clusters map to four different chromosomes in mammals (reviewed in Duboule, 1994; Lahbib-Mansais et al., 1996; Rabin et al., 1985; Apiou et al., 1996). The *Hox*A cluster was assigned to mouse chr 6, human chr 7 and pig chr 18, *Hox*B to mouse chr 11, human chr 17 and pig chr 12, *Hox*C to mouse chr 15, human chr 12 and pig 5 and *Hox*D to mouse chr 2, human chr 2 and pig chr15 (Table 9). In chicken, the four *Hox* gene clusters were assigned to four distinct macrochromosomes (Ladjali-Mohammedi et al., in press) (Table 9). The *Hox*A cluster mapped to chromosome 2p21, *Hox*B to 3q31, *Hox*C to 1q31, and *Hox*D to 7q13→q14.

Genomic sequencing

(Prepared by N. Bumstead and J. Kaufman)

Although the sequence of the chicken ovalbumin gene was among the first genomic sequences to be investigated (O'Hare et al., 1979) so far relatively little genomic sequencing has been carried out in chickens. Currently database entries total 1.5 Mb of chicken genomic sequence from 200 entries, however this includes only three substantial regions: the core region of the major histocompatibilty complex (92 kb: Kaufman et al., 1999), the class II cytokine receptor cluster (46 kb: Reboul et al., 1999) and the β-globin region (31 kb: Reitman et al., 1993).

Table 10. Chicken genomic sequences

Genomic region	Accession No.	Size (bp)
Chicken MHC region	GGBLOCUS	92863
Class II cytokine receptor cluster	AF082664, AF082665, AF082666, AF082667	46304
T-cell receptor alpha	GGU83833	31793
Myosin heavy chain	GGMYHE	31111
Beta-globin cluster	GGHBBRE	30539
GART-B	AF236855	18384
Band 17 gene	GGU59420	8321
Beta-2 microglobulin	GGB2MGB5, GGB2MGB6, GGB2MGBMR	8233
Interferon-gamma	GGIFNGAMM	6765
Lysozyme	GGGTLYSO, GGGTLYSOZ	6162
Cardiac phospholamban (PLB).	GGPLB1, GGPLB2	5226
Transforming growth factor-beta 3	GGTGFB3, GGTGFB3A, GGTGFB3B, GGTRGFB3, S46000	4855
Histone H5	GGHI03	4845
ZF5 DNA binding protein	GG51640, GG51641	4740
Fatty acid synthase gene	GGFASA	4736
RING3 gene	GGRING3GE	4651
Opsin	GGU87449	4543
Nitric Oxide synthase	GG46503, GG46504	4418
Cellular ski novel gene c-sno	S78393, S78394, S78395, S78398, S78402,S78406	4288
c-ets (p54)	GGCETS, S68254, S68255	4032
Interleukin-2	GGA224516	3818
Drebrin	S65279, S65280, S65281, S65288, S65289, S65290, S65291, S65292, S65294, S65296	3769
Interleukin-8	GGA9800	3617
GATA-3	S78786, S78787, S78788, S78789	3386
Alpha-globin region	GGY18681	3319
Phospholamban	GDPLB	3312
Erythrocyte anion transport protein	GGEATP, GGEATPA, GGEATPB	3281
Estrogen receptor gene	GGU60211	3000
c-Rmil	GGCRMIL	2678
Ovalbumin	GGALB2, S82572, S82576	2607
A1 adenosine receptor	S78199	2555
cAMP-dependent protein kinase inhibitor (PKI)	AF139056	2508
Slow myosin heavy chain 2 (SM2)	GGU85023	2413
Nuclear factor CTCF gene	AF077830	2285
Alpha-1 type-II collagen	GGC2A101, GGC2A102, GGC2A103, GGC2A104, GGC2A106, GGC2AA2	2157
Telokin	GGTELO	1882
ICSBP gene	GGY15087	1800
Prepro-insulin-like growth factor-II	S82960, S82962	1681
Myosin heavy-chain	GDMYHCG	1680
Prostaglandin G/H synthase PGHS-2	S53041	1610
Protamine gene locus 1	GGPROTAMN	1528
c-mil	GGCMILA	1518
c-src	S43620	1517
Neural cell adhesion molecule.	GGNCAMG	1383
Hypothetical protein walter	GGWALTER	1288
Nerve growth factor beta (NGF)	GGNGFB3E	1156
GHox-8	S64478	1107
Histone H1	GGH11A1	1098
Chicken repetitive sequence GG1-CR1	GDGG1CR1	1049
Translin.	GGTRANSLN	984
Immunoglobulin heavy chain variable region	S64683, S64684, S64685, S64686, S64687, S64688, S64690, S64692, S65968	957
Slow myosin heavy chain 1 (SM1)	GGU85022	917
Heparan sulfate proteoglycan	GGSYN4	876
U2 small nuclear RNA gene.	GGUGU2	840
Rho -globin,embryonic beta-type globin	GS443S1, GS443S2, GS443S3	831
Erythroid type delta-aminolevulinate synthase ALAS-E	S57264	824
Protamine gene locus 2	GGPROTAMI	818
TNF and lymphotoxin homologue	GGTNF	796
Erythroid delta-aminolevulinate synthase ALAS-E	S69605	790
C-erbB	S66408	700
Histone H4	GGH43D8	675
Natural resistance-associated macrophage protein 1 (NRAMP1)	S82465	660
Chicken embryo kinase 5 CEK-5	S81295	646
Cardiac/slow skeletal alkali myosin light-chain MLC1c/1s	S66109	607
Growth hormone regulated gene GHRG-1	S75126	597
Type X collagen	S83255	582
Small nuclear ribonucleoprotein E	GGSNRNPE	519
Scale keratin gene	GGKER01	468
Chicken CR1 repetitive element CR1CMc	GGCM12	459
Vinculin	S52270, S52271, S52272	454
Immunoglobulin light chain variable region	S65967	423
CR1 repetitive element, CR1CMb	GGCM13	409
CR1 repetitive element, CR1CMa	GGCM14	402
Acetylcholine receptor alpha subunit	GGACHRA1, GGACHRA2	400
Kainate-binding protein	S87402	394
Fibronectin	GGNEC1, GGNECT	383
Calmodulin	GGCAM1, GGCAM2	365
Aggrecan chondroitin sulfate CS2 domain	GS656S1, GS656S2	357
Acidic fibroblast growth factor	S63261	349
Feather keratin gene	GGKER02	297
Acetylcholine receptor gamma-subunit	S47175	280
Growth hormone cGH	GS559	203
Cardiac troponin T (TNT)	S82182	190
Hoxb-4	S80955, S80960	176
Alpha-2 type-1 collagen	GGC1A210, GGC1A215	150
Chicken MHC B-G region	GGBGAA, GGBGAB, GGBGAC, GGBGAD, GGBGAF, GGBGAE	135
Alpha-1 type III collagen	GGCO11, GGCO12	126
Chicken-specific tandem repeats	S78225	42

In addition to these more extensive regions the genomic sequences of a considerable number of individual genes have also been investigated in detail, and these are listed in Table 10. Comparison of genomic sequence for these genes has allowed detailed comparison of their structure with that of their human and mice orthologues. This has shown that numbers of introns and the codon phase of intron/exon boundaries are very strongly conserved between these species, even though the sizes of introns or exons may be very dissimilar. For example, the comparison of human, murine and chicken gene carried out by Kaiser and Mariani (1998) showed that chicken interleukin 2 is comprised of four exons, with all three introns interrupting the coding sequence between codons (frame 0) exactly as in its human and murine orthologues. The three introns have consensus splice donor/acceptor sequences but otherwise are widely diverged from the mammalian sequences as well as being substantially smaller in size. Other examples of this preservation of intron and exon structure include NRAMP1 (Hu et al., 1996), interleukin 8 (Kaiser et al., 1999), interferon γ (Kaiser et al., 1998), prostaglandin G/H synthase (Xie et al., 1993), insu-

lin-like growth factor (Darling et al., 1996), δ-aminolevulinate synthase (Yamamoto, 1992) and β2-microglobulin (Riegert et al., 1996). This conservation of exon structure and boundaries can provide a useful tool for distinguishing between possible mammalian orthologues of human genes (Kaiser and Mariani, 1998).

Although intron sizes are frequently smaller in chicken genes than in their mammalian counterparts this is not always the case, for example intron 1 of chicken interferon γ is 50% bigger than that of its human equivalent (1,825 bp:1,238 bp) though intron 3 in the same gene is smaller (641 bp:2,428 bp; Kaiser et al., 1998), and although the total size of the 14 introns of chicken NRAMP1 is smaller than in the human gene, individual introns may be larger or smaller than their equivalents (Hu et al., 1996). Similarly differences in 3′ untranslated regions vary from gene to gene and may be similar to those in humans and mice as for interleukin 8 (Kaiser et al., 1999) or differ between species as for interleukin 2 (Kaiser and Mariani, 1998). Differences in exon sizes for coding sequence and 5′ untranslated regions are in general small in comparison to the variation in intron size.

A notable feature of many of the chicken genes is a very high G+C content, particularly in the first exon, for example in β2-microglobulin (Riegert et al., 1996), protamine gene (Oliva and Dixon, 1989), N-CAM (Colwell et al., 1992) and CTCF transcription factor (Klenova et al., 1993), although this is not universal. It is not clear at present whether this high G+C content relates to chromosomal position or to particular classes of genes although Riegert et al. (1996) suggested that it is related to the extent of sequence divergence from mammals.

Regulatory elements have been identified and characterised for a number of chicken genes, and at least in some cases parallel those of equivalent mammalian genes. For example, Lin et al. (1996) identified NF-κβ, PEA1, PEA3 and C/EBP transcription factor binding sites associated with chicken inducible nitric oxide synthase as in the human gene; similarly Jakowlew et al. (1992) and Burt et al. (1995b) showed that transcription binding sites for TGF-β3 are similar between the chicken and human genes. Consensus TATA and CCAAT boxes are present in some chicken genes, for example protein kainate (Eshhar et al., 1992) but not all (EGF-receptor: Callaghan et al., 1993; c-sno: Givol et al., 1995). Comparison of the genomic sequences with cDNA sequence shows that, as in mammals, chicken genes frequently show alternative splicing, for example c-src (Dorai et al., 1991) drebrin (Kojima et al., 1993) or c-ets-1 (Crepieux et al., 1993).

Chicken genomic sequence frequently contains CR1 repetitive elements. These degenerate retroviral elements are found widely in the chicken genome, with an estimated 30,000 copies per genome (Burch et al., 1993). CR1 elements are often associated with genes, notably in the β-globin region (Reitman et al., 1993), and are typically orientated to point toward the expressed gene (Stumph et al., 1984). At present too little extensive chicken genomic sequence is available to define the relationship between CR1 sequences and genes but it is possible they play a role in regulation of expression (Chen, 1991).

So far information on intergenic spacing and local gene order in chickens is confined to three regions of extensive sequencing. The largest region to be sequenced so far has been the core region of the chicken Major histocompatibility complex (Mhc). Kaufman et al. (1999) sequenced a cosmid contig spanning 92 kb of this region and have carried out detailed comparison of its gene structure with that of mammals (Zoorob et al., 1990; Kaufman et al.,1999; Jacob et al., 2000). The central portion of this region contains 11 genes within roughly 44 kb, with intergenic distances as small as 30 nucleotides and average intron sizes of 200 bp. The region contains a number genes homologous to genes present in mammalian Mhc regions (class I-α chain, class II-β chain, ABCB2, ABCB3, BRD2 and C4A), however gene order differs, and genes such as the LMP genes and class II-β chain are absent from the chicken region, while additional B-G genes and C-type lectins are present. It is unclear whether the striking differences seen between the chicken and mammalian Mhc genomic organisation are due to its immunological function, its location on a microchromosome near the nucleolar organiser region, or both.

In contrast to the Mhc region, gene order in the chicken class II cytokine receptor region is identical to the equivalent human region, with interferon α/β receptor genes 1 and 2 and the interleukin 10 receptor 2 aligned in the same order and in the same transcriptional orientation and with conserved intron/exon structure and boundaries (Reboul et al., 1999). As in the Mhc region, gene size and intergenic distances are much smaller than in the equivalent human region, with the overall size being approximately a third of its human equivalent. Although the receptors in this cluster are immunologically active molecules it seems less likely that interactions between these genes might have led to increased linkage than in the case of the Mhc genes, however the receptor complex is located on chromosome 1 (Table 8), suggesting reduced intron sizes and intergenic distances may not be peculiar to the smaller microchromosomes.

The chicken β-globin cluster is also considerably smaller than that of its human or murine counterparts, and is thought to have evolved by gene duplication after divergence of birds from mammals (Reitman et al., 1993). In contrast to the seven murine β-globin genes, the chicken complex contains only four genes. However gene number varies among mammalian species with the human β-globin complex containing five genes and a pseudogene and the rabbit only four genes. The region is G+C rich and contains a very high representation of the CR1 repeat (16% of the sequence of the cluster). The chicken globin cluster is located on chromosome 1, further indicating that reduced gene sizes and intergenic distances are not restricted to microchromosomes.

Although the extent of genomic sequencing in chickens is limited it seems clear that in general intron structure and regulatory elements are well conserved between chicken genes and their mammalian equivalents, even in portions of genes where sequence homology is weak. In contrast the limited amount of cluster sequencing suggests that although gene order and orientation can be similar between chickens and mammals, this is not necessarily the case, as in the Mhc region or the globin cluster. It is interesting that so far little evidence of pseudogenes has been found in chickens, suggesting these may be rare.

The rapid growth in large insert cloning in chickens and reduced costs of genomic sequencing make it likely that much

more genomic sequence will soon become available for the chicken, making it possible to address the relative frequency of pseudogenes, CR1 elements and G+C rich regions, and to compare gene size and density in different chromosome regions.

Towards a whole genome radiation hybrid panel in chicken

(Prepared by M. Morisson and A. Vignal)

Whole genome radiation hybrid (WGRH) panels provide a complementary approach to the different genome mapping techniques currently used in chicken. The resolution that can be achieved is higher than recombinant mapping, enabling the ordering of markers otherwise clustered on the genetic map. Data thus obtained can be of great help for building the more detailed maps based on large-insert clone contigs. Another major advantage of radiation hybrid mapping, is the ability to use any STS-type marker, without the need to identify polymorphisms as required for the genetic map or the need to isolate large-insert clones as required for FISH mapping. The mapping of genes is thus far simpler, an important point to be taken into consideration with the arrival of a large amount of EST data. WGRH panels are now available for genome mapping in many species including human (Gyapay et al., 1996; Stewart et al., 1997), mouse (McCarthy et al., 1997), rat (Watanabe et al., 1999), dog (Vignaux et al., 1999), cat (Murphy et al., 1999), cow (Womack et al., 1997) and pig (Yerle et al., 1998). The potential resolution of the panel is tailored by the radiation dose and panels of different resolutions can be created depending on the needs: high-resolution transcript maps of a whole genome, contig construction or regional fine mapping of candidate regions for QTL.

Recently, zebrafish WGRH panels and RH maps were published (Kwok et al., 1998; Geisler et al., 1999; Hukriede et al., 1999), demonstrating that RH technology can be used for non-mammalian vertebrates. In 1998, Kwok et al. published the first collection of 48 chicken radiation hybrids. However, these clones were unstable and not suitable for a WGRH panel.

As a first step towards a complete panel, 265 chicken whole genome radiation hybrids were produced by fusing irradiated female embryonic diploid fibroblasts to a deficient HPRT hamster cell line (Wg3hcl2) (Echard et al., 1984). The hybrids were selected in HAT media. A radiation dose of 6,000 rads was chosen as a compromise between resolution power and linkage power to build a first chicken RH map.

After several tests, the ratio of one irradiated chicken fibroblast for one hamster recipient cell was used and one clone was recovered for 720,000 donor cells, corresponding to a fusion efficiency of approximately 1.4×10^{-6} clones per chicken fibroblast. This low fusion efficiency was expected given the evolutionary distance of the two species and results obtained by others (Kwok et al., 1998; Bumstead, personal communication).

The first 176 hybrids were screened for the presence of chicken DNA by using a set of 46 microsatellite markers chosen across the genome from the genetic map. All markers were genotyped in duplicate and scored as positive or negative for chicken PCR product. Clones that were discordant between the two genotypings were not used in the data analysis.

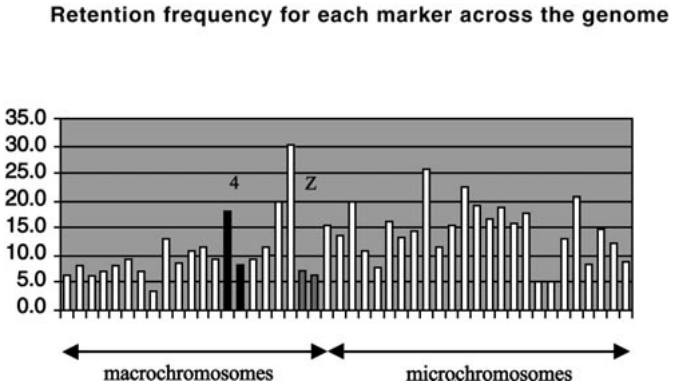

Fig. 13. The retention frequency of 46 markers was tested on a panel of 176 hybrids. As a general trend, a higher retention frequency can be observed for microchromosome markers than for macrochromosome markers.

The average retention rate of the 176 hybrids at this preliminary stage was 12.7%. The retention frequency for each marker across the genome is shown in Fig. 13. The overall retention rate for markers located on microchromosomes is higher (14.4%) than that found on macrochromosomes (10.6%). These results are in accordance with those of Kwok et al. (1998), whose retention frequencies were 17.8% for microchromosomes and 10.6% for macrochromosomes.

It has been observed that smaller chromosomes are generally retained at a higher rate, this trend being clear in the human WGRH panel (Gyapay et al., 1996). In the chicken, where the difference in size between the macrochromosomes and the microchromosomes is exacerbated, this trend is more evident. One possible explanation is that in a microchromosome, markers are always close to a centromere, a region in which retention frequencies have been shown to be high (James et al., 1994).

No higher retention frequency can be observed on the chromosome 4 bearing the HPRT gene (Fukagawa et al., 1999), since no marker closely flanking this gene was used in the study. The two haploid markers located on the chromosome Z showed retention frequencies lower than the others, due to the use of female chicken donor cells.

For both practical and economical reasons, reducing the number of hybrid DNA samples in a panel to the number that can be easily handled in a microplate is highly desirable. Theoretical data has been reported on the consequences of hybrid selection on the mapping power of RH panels (Barrett, 1992; Jones, 1996; Lunetta et al., 1995). A retention frequency over 20% for the hybrids is a minimum requisite for optimal efficiency. Therefore, a subset of 44 hybrids was selected to constitute a part of the final mapping panel. The average retention frequency of these hybrids is 22.9% for the whole genome, 21.9% for the microchromosomes and 24.2% for the macrochromosomes

In this study, only one hybrid out of four was considered as having a suitable retention frequency for the final mapping panel. We expect that 20 more hybrids will be selected among

Table 11. Summary of chicken cDNA libraries and EST collections

Institute/University	Tissue source	No. of ESTs	% Homologies	No. of unique genes
Roslin Institute	5 day old embryo	3414	58	nd
Compton	ConA-stimulated T cells	1726	nd	nd
University of Delaware	ConA-stimulated T cells	6627	82	3706
University of Delaware	Fat, Liver, Oviduct	1826	74	904
University of Hamburg	Bursal lymphocytes	7638	50	4887
EMBL	Various tissues	307	nd	nd
Total		21,538	~65	~12,000

the 89 we haven't characterised yet. This would give us a panel of 65 usable hybrids. Therefore, more fusion experiments will be needed to build a panel of 90 hybrids. Also the efficiency of the panel will be tested by genotyping markers closely linked on the genetic map.

Once finalised and produced on a large scale, the chicken WGRH will be a powerful tool for the production of high density maps, an intermediate step between the available genetic and cytogenetic maps and local or whole genome contig maps. The possibility to integrate large numbers of ESTs in the radiation hybrid map will enhance the precision of comparative mapping and will extend the number of positional candidates in QTL mapping approaches.

Chicken EST resources

(Prepared by D.W. Burt, N. Bumstead, J-M. Buerstedde and J. Burnside)

There has been rapid progress in the construction of a consensus genetic linkage map of the chicken (Groenen et al., 2000; and this report) with almost 2000 mapped loci. The map contains over 200 genes for which the map location of the human orthologue is known. This limited information on gene homologies has been used to demonstrate a high degree of conservation of synteny between the chicken and human genomes (Burt et al., 1999; and this report). To answer questions about the conservation of gene order and to provide a more accurate predictive tool, there is a need to identify and map many more chicken genes.

Partial sequencing of cDNA clones to develop expressed sequence tags (ESTs) is a rapid, simple and efficient approach, now widely used in many farm animal genome projects, to create gene catalogues representing the gene content of any particular genome (Hatey et al., 1998). Only a few studies have described the isolation and mapping of chicken ESTs. Li et al. (1998) described the isolation of liver ESTs and showed they could detect a significant level of DNA polymorphisms, based on RFLP analysis. Smith et al. (2000) isolated ESTs from a turkey pituitary cDNA library and detected SNP variation in five avian species, including chicken and turkey. ESTs have also been used as a source of genetic markers. Spike et al. (1996) and Bumstead et al. (1994, 1995) described ESTs isolated from T and spleen cells, respectively. RFLP polymorphisms were mapped in either the East Lansing or Compton reference mapping populations. Other mapping approaches have been used, including screening of brain and embryonic cDNA libraries with microsatellite probes (Ruyter-Spira et al., 1996, 1998a).

Currently there are several chicken EST projects at University of Delaware (Tirunagaru et al., 2000), University of Hamburg (Abdrakhamanov et al., in preparation), Institute of Animal Health and Roslin Institute (EC ChickMap project) and a combined database has been established at Roslin (http://www.ri.bbsrc.ac.uk/) with 21,713 (September, 2000) chicken EST sequences. In most cases, random clones have been sequenced and consequently, some ESTs represent abundantly expressed genes or sequences (mitochondrial gene cluster, translation factors, etc). At Roslin, cDNA libraries are now routinely pre-screened with probes for abundant sequences to reduce this redundancy (10% of ESTs isolated from a 5-day-old embryo cDNA library were mitochondrial DNA and other genes represented less than 1% of total, e.g. rRNA, transthyretin 1, nuclear antigen 1, splicing factor, alpha tubulin and haemoglobin). Searches of sequences homology between the chicken ESTs and other genomes, currently has a significant hit rate of about two-thirds and may represent over 12,000 unique genes (Table 11). These results suggest that the cDNA libraries used so far are heterogenous and a rich source of ESTs. These estimates are based on preliminary examination of the EST dataset and require a more thorough analysis. Chicken ESTs can be analysed as part of the EST web sites (see below) either by sequence homology (BLAST) or by looking for keywords in annotations of the ESTs. All sequences will be/are submitted to the EMBL/GenBank nucleotide sequence databases and cDNA clones are available from all sites (see below). Together with information on the pattern of expression of ESTs (e.g. from microarray experiments), data on EST sequence homologies, map location and genetic variation will provide useful information for candidate gene and trait-association studies. Our goal will be to develop a complete gene catalogue for the chicken genome to make this a realistic strategy.

Chicken EST WWW sites:
http://www.ri.bbsrc.ac.uk/cgi-bin/est-blast/blast.pl/
http://genetics.hpi.uni-hamburg.de/dt40.html/
http://www/rzpd.de/
http://www.chickest.udel.edu/

Single nucleotide polymorphisms in the chicken genome

(Prepared by S. A'Hara and D.W. Burt)

Bi-allelic single nucleotide polymorphisms (SNPs), single base changes in a DNA sequence, are the most common class of genetic polymorphism and are currently being presented as the DNA marker of choice for many branches of biology. The intense level of interest shown by researchers is fueled by the perceived two-fold benefits of SNP markers, viz; their high abundance (1 SNP per 300–500 bases in humans, Nielsen, 2000) – enabling markers to be readily identified and placed in, or near, genes or regions of particular interest, and the bi-allelic (digital "yes/no") nature of SNPs – allowing greater ease of analysis than the majority of existing analogous marker systems (whilst an SNP can theoretically be tri- or tetra-allelic, the very low mutation rate of SNPs means that in practise the overwhelming majority of SNP loci are simply bi-allelic).

Ultimately, the utility of SNPs in poultry for genetic map development and trait improvement relies on the "holy trinity" of factors required for a useful genetic marker: ease of discovery, frequency of occurrence and the ease of genotyping.

Direct sequencing of PCR amplified DNA is perhaps the quickest and easiest avenue to SNP discovery. Sequence traces can be examined manually for polymorphisms or with the aid of programs designed to automate base calling (e.g. the Phred, Phrap, Consed and PolyPhred suite of programs; Nickerson et al., 1997). As submissions to poultry EST databases increase, it may also be possible to identify SNPs in silico by aligning multiple accessions of the same sequence. Notably however, there are problems associated with this practice (see Kwok and Gu, 1999). Pooled DNA samples can also be PCR amplified and sequenced to enable allele frequencies to be assessed, thereby facilitating population-based studies.

A direct sequencing approach of PCR amplified DNA has been applied to the Roslin broiler/layer cross and has enabled identification of 139 SNPs in the 31,000 bases analysed, generating a frequency of approximately one SNP per 225 bp. (Table 12). This frequency corresponds closely with the work of Smith et al. (2000), who recorded 19 SNPs in a survey of some 5,000 bp of chicken sequence amplified using PCR primers initially generated from turkey ESTs (1 SNP per 260 bases). Both studies found the most frequent polymorphism was a C/T transition, and this C/T polymorphism bias is consistent with the situation in the human genome (Cooper et al., 1985).

This high frequency of SNPs is in contrast to the relatively low frequency of microsatellites in the avian genome (Primmer et al., 1997). Therefore, whilst there is a requirement for a greater number of bi-allelic SNPs markers to be scored to generate the same level of information as multi-allelic microsatellites (Krugylak, 1997), their availability and ease of typing makes them a viable alternative.

With a multitude of new SNP assay systems, such as the DASH (Hybaid), Invader (Third Wave, AgBio) and microsphere-based methods (Chen et al., 2000), becoming readily available and cost effective, SNP markers will become an integral part of poultry mapping projects in the near future.

Specialised regions of the chicken genome: telomeres

(Prepared by P.A. Thomson and T. Burke)

Chicken telomeres have been shown, by in situ hybridisation, to terminate in the conserved vertebrate repeat, $(TTAGGG)_n$ on metaphase chromosomes (Meyne et al., 1989), and with greater resolution on the expanded, lampbrush, chromosomes found in the diplotene stage of growing oocytes (Solovei et al., 1994). The sequence and location of these repeats have been confirmed by the detection on Southern blots of *Bal*31-exonuclease sensitive heterogeneous fragments of $(TTAGGG)_n$ of 8–20 kb (Muyldermans et al., 1994; Venkatesan and Price, 1998). Interstitial sites containing telomere repeats have been identified on several of the macrochromosomes and the microchromosomes have been shown to vary in their pattern of hybridisation (Nanda and Schmid, 1994).

Chicken telomeres have been studied in attempts both to investigate the nucleosomal organisation of higher eukaryotic telomeres and to isolate telomere-binding proteins. As a result, chicken telomeres are known to be arranged in nucleosomes that differ from those found in the bulk of the chromatin only by their preferential binding of histone H1 (Muyldermans et al., 1994). However, the identification of a protein that specifically binds single-stranded DNA with G-G base-pairing capabilities suggests that the extreme ends of chicken telomeres may terminate in a tertiary structure bound by a specific protein (Gualberto et al., 1992, 1995). Recent investigations into the expression of telomerase in chicken cells suggest that this ribonucleoprotein, important in the maintenance of telomere length, is constituitively expressed in somatic cells (Venkatesan and Price, 1998).

Genetic maps are expected to contain gaps corresponding to regions of high recombination or to small microchromosomes, some of which may contain little non-heterochromatic DNA. Two approaches can be used to integrate telomeres into the chicken genetic maps. The first method may be thought of as an end-in approach, achieved by the identification of BAC clones containing telomeric repeats, followed by their genetic and physical mapping. This approach has resulted in the identification of terminal markers in seven linkage groups, and markers for five linkage groups in which only one marker was in a more distal position (Thomson et al., in preparation). Only two characterised markers were unlinked to other markers within the current map, suggesting that the coverage at the ends of chicken chromosomes is reasonably complete, despite a reduction in the numbers of microsatellites in these regions (Primmer et al., 1997). The recently compiled genetic consensus map suggests that about 1.6% of mapped markers (excluding AFLPs) are as yet unlinked and that some microchromosomes have no associated linkage markers (Groenen et al., 2000).

The second approach is to add terminal markers from each linkage group to the physical map. This would result in both the localisation of these groups to chromosomes and the possible identification of small linkage groups that form the telomeric regions of chromosomes, but are apparently unlinked either due to a high recombination rate (Rodionov et al., 1992c) or a scarcity of markers. Caution must be taken, however, in assuming that terminal markers on linkage groups equate to physi-

Table 12. SNPs identified in the Roslin broiler/layer cross by direct sequencing of PCR products.

Chr.	Gene/STS	Accession Number	Bases Screened	No. of SNPs	SNP substitutions	PCR Primers Forward 5' - 3'	Reverse 3' - 5'
1	RAIDD gene, Cosmid 42	AJ246055	800	3	A/G, A/G, A/G	F:TCACAATCACCACACTCCGT	R:CTAATTTGAGCAGGGGTGGA
1	LAMP1	M59364	570	3	A/G, C/T, A/G	F:GATCACCTTCCACTTTGT	R:TGTCATTCGATGCTTCAA
1	Progesterone receptor binding protein PCR 1	U95088	990	1	G/C	F:TGGGAAAGGGAGTTGAGTTG	R:CTGTGAAGAAAGGCTGAGGG
1	Progesterone receptor binding protein PCR 2		1100	7	A/G, C/T, C/T, C/T, A/C, A/G, A/G	F:GCAGTGCCATCACTAGCGTA	R:TTCGCGGGATCAAAGATTAG
2	Ovalbumin Y PCR 1	J00922	400	0	Nil	F:TTGTTTGTTGCCCATTCTTTT	R:AGCACAGCCGCAAATACTGTGG
2	Ovalbumin Y PCR 2		750	1	C/T	F:CTGCAAGGCTGTACCACGTA	R:TTCCCATTTTGTCACAAGCA
2	Ovalbumin Y PCR 3		850	5	G/T, A/C, C/G, A/G, C/T	F:CAAGCCTAACCAATCACTGCC	R:TCAGTGTTACCTCTTGCCCC
2	STS, Cosmid 35	AJ232119	730	Nil	Nil	F:TTCACAGAGGTGGAATGCAA	R:TTTTCAGCTGGACCCATTTC
3	STS, Cosmid 8, 3q11, Flpter 0.15	N/A	440	7	C/T, T/G, C/T, T/A, A/C, A/C, A/G	F:AACACCTGGTTCCTCCACAG	R:TATTTCAATGCATTTGGGCA
3	Estrogen flanking DNA PCR1	U60211	570	Nil	Nil	F:ACTAAGGCCTCTTTCCTGCC	R:CATCTGCGGAGACTCACAAA
3	Estrogen flanking DNA PCR2	U60211	600	1	A/G	F:GGGGAGGGTGAGGAAATCTA	R:GCAGGCTCCAATCTATCTGC
4	STS, Cosmid 16, 4p13-p12, Flpter 0.09, PCR 1	AJ237187	840	6	C/G, C/T, C/T, A/G, C/T, C/T	F:TGCAGATGCACAGACATTCA	R:ATCAAGCAAATGCAGCACAG
4	STS, Cosmid 16, 4p13-p12, Flpter 0.09, PCR 2	AJ237188	700	0	Nil	F:CCTTCCTAAAGCGGGATTTC	R:GAGTGAACTGGAGAGGGCTG
5	Tyrosine Hydroxylase PCR 1	X59515	960	9	C/T, C/T, A/G, A/C, C/T, A/G, T/G, C/T, G/C	F:ATGGAGATCCCTCCAGCTCT	R:CCCATGTCTGTCTGGTGATG
5	Tyrosine Hydroxylase PCR 2		530	3	A/G, G/T, A/G	F:GAAGGCTGGCTGACTTGTTC	R:AAAGTAGCACCAATTCCCCC
5	Tyrosine Hydroxylase PCR 3		1010	12	C/G, T/G, A/G, C/T, A/G, A/C, C/T, C/T C/T, A/G, A/G, C/T	F:AATCCTTTCATGCAACACCC	R:CCTACAGCCTACCCAATGGA
5	Insulin PCR 1	J00872	420	2	C/T, A/G	F:TGACTTTTAAAGCCTGATGAATAAAA	R:ATTCCCCACCAAGGACATTC
5	Insulin PCR 2	J00874	360	2	C/T, A/G	F:TGAGAGCATTAGCTTTGGGAA	R:TGGACACATAAAATGGCACAA
5	Insulin PCR3	J00873	280	0	Nil	F:TCCTCCTTCATGGGTGATTT	R:CCAAGGGACACAATGAAAGC
5	IGFII PCR 1	S02962	750	1	C/T	F:GGTGTTCCAGCTTGCTAATAA	R:CTAGTGTTGGCACTGGGGAT
5	IGFII PCR 2		600	5	A/G, C/T, A/G, A/C, C/T	F:GGACCACTCTCTGCTCCTTCAG	R:GATCTTGCTTCCCCATTGAA
5	Cyclin D PCR 1	N/A	580	1	A/G	F:GTGCTGACAAGGAGCAAA	R:ATGGAAGGGAGACAGAGCAA
5	Cyclin D PCR 2	N/A	850	1	C/T	F:TGCGGTAACATAAATGGCAA	R:CTCTGGTGTTCCTGAGAGGC
5	Cyclin D PCR 3	N/A	950	1	C/T	F:GCTACAGGTATGAGCCCTGC	R:CACATCCCACCAAGGTATCC
5	STS, Cosmid 27	AJ231880	520	3	A/G, C/T, C/T	F:CTCTGCAGCTCCAAGTTCAG	R:AGCTGTGGAGAGCAGAGGAG
5	B-Creatine Kinase	M33713	360	1	A/G	F:ACTCGCTTCTGTACAGGGCT	R:ATGGGCAGGTCAGGATGTAG
6	STS, Cosmid 56	AJ246128	555	2	A/C, C/T	F:CTTGTGTGCCTGCAATGAACAG	R:AGCCTGTCTGTATTTCCCC
7	STS, Cosmid 3	AJ246045	750	12	A/C, C/T, C/T, A/G, A/G, C/T, A/G, A/G, A/G, A/G, A/C, G/T	F:AGAATGATGCAGATTTGGGC	R:GTGTGAAGCTGTACCTGGCA
8	STS, Cosmid 32	AJ232024	710	3	A/G, C/T, G/T	F:CTGGGAGCATACAATCCAGC	R:CTTGCTTTTCCTTCAGTGCC
8	VIT2	X13607	575	2	A/G, G/C	F:TCTACGAATTACAAGAGGCT	R:CTTCCTGAATGACATACCTT
15	beta 1 crystalline	U09951	880	3	C/T, A/G, A/G	F:GCGATGTGTGAGACCACAAA	R:AGGGTATGGCACATCAGGAG
23	STS, cosmid 14	AJ231767	620	2	C/T, G/C	F:AGCTCACAAGCGTCTTTGGT	R:CAAAGGGGCTGATGGAGTTA
10	Aromatase PCR 1	D50335	930	0	Nil	F:AGCCCTGATCTCGTTCACTG	R:AATCCAGTTCCGTTCACTGAG
10	Aromatase PCR 2		800	5	A/T, T/G, A/G, G/T, C/T	F:CGGGAGGAACAAAGAAAACA	R:GGGAGAGAGAGAAGGGGA
11	STS, Cosmid 1	AJ231695	250	1	A/C	F:AGCTATTTGGGGCTGGTTTT	R:CGTGGAGATGGAGTGGTTTT
18	Fatty Acid Synthetase	JO2839	800	3	A/G, A/G, A/G	F:AAGAAGGTCTGGGTGGAGACTA	R:GGACTGATACTTTTCCATCGCT
14	STS, GCT0903 microsatellite	AJ001457	460	7	C/T, A/G, C/T, A/G, A/G, A/G, C/T	F:ACTCCCAAGTCAGTGCAAG	R:CATGGTTTGAGCAGGAAGG
17	Adenylate Kinase	D00251	680	2	A/G, A/G	F:CTGAAGCACCACAAGATCCATCT	R:AGGTGTCTGCCTTAGCCAACAT
13	STS, GCT901 microsatellite	AJ001455	400	3	A/G, C/T, A/T	F:CCATTTTCCCATTTCTCGTG	R:GGATACCCAAAACGCTGAAA
E49	STS, cosmid 20, ROS0113	AJ231834	800	5	A/G, A/G, C/T, A/C, G/C	F:TCTCAGCATGCAGGAAATTG	R:GAGGCAGAACAGTGATGCAA
E49	POU2 transcription factor	U77715	680	2	A/T, A/G	F:AGGGTGAGGAAATCTA	R:GCAGGCTCCAATCTATCTGC
19	anonomous DNA thought to be leptin	AF01272 7	320	1	C/T	F:GGGGATGGAGGAAGAAAGAG	R:TGTTGTGCAACAAGTGACCA
28	Anti-Mullerian Hormone	U61754	800	1	A/C	F:AGGTGTGGGTGAAGAGTTGG	R:CCAGCCCAAGGTATGGAGTA
27	Growth Hormone	D10484	970	9	A/G, A/C, A/T, C/T, A/C, A/G, G/T, C/T, C/G	F:CTAAAGGACCTGGAAGAAGGG	R:AACTTGTCGTAGGTGGGTCTG
Z	Interferon beta	X92479	750	1	C/T	F:AACACCCCAAACACCATCAT	R:GTCATGCCAGTCTCCCTGTT
Z	Prolactin receptor	AJ011128	680	0	Nil	F:GGGCAACTAATGAAATGGGA	R:CCCCAGTTCACAGGTAGAGG
		Total=	30920	139			

Sequence context of SNPs
All SNPs are in non-coding regions, with the exception of the IGFII SNP highlighted in bold. It is a silent mutation.

GCTCAGCTCC(A/G)AGATGGCCCA AGAGGGTGTC(G/A)CAGAAGGGGT ACACCATTGT(G/A)TGCTTGTATG
AAGTGGTCAC(A/G)AAAGTACACTT GTAGTTTTCA(T/C)TGAAAATAAC AGATTAATAA(A/G)CCCACATCAA
AGGGAGACTG(G/C)CATTCAACAC

ATAGCATGCA(A/G)TGAGTTGAAT AAAAGACTAG(C/T)GTTGAACAAA AAAAAACTTG(C/T)TT(C/T)TACCCTACAG
ATTACAAAAA(A/C)CAGCTAGAAAGAAATGGCAT(A/G)CTTAAAGCTA AGTAGTCCTA(A/G)CTATGTTTGT

N/A
TTCATTGATG(C/T)CCTTTGTTTC
GAACAATTTT(G/T)GAAAAGTGAGGTAT(A/C)ATTAAGTCAA CCATTTACAT(C/G)AAGGTGGAGT ACTTTATATT(-/G)TCA(A/G)GTAACTGTAGT
TGTGTTTTCT(C/T)TCCTGCACAA
N/A
CTGTGCTACGC(C/T)GAGACTTGAA CTGAACAACC(G/T)AAATTAGGTGCTTGGTCCT(C/T)CCTGCAGCCA TGGAACCTCA(A/T)GTGCCCACAG
TTAGTGTCAT(A/C)ATCACTGTGACAACACCAG(C/A)A(G/A)TGCTTTACCT

N/A

TGCTTTTTTA(A/G)GCAGAAGTTC

TCACTTACAA(G/C)CCTGATGGCT GGATTCTATG(C/T)AAGAAAACTG TCAGCCAACC(C/T)GTGCAGCTGTGGCCCCACC(A/G)TGTGCCACAG
TTACAGTCAC(C/T)GCTCCATTTC AGCTTCAGCA(C/T)GGGGATGGTG
N/A

AAGTGCAATG(C/T)CCAACAATGTTTGCTGTT(C/T)CTGCTTTATG CACGCAGCCT(A/G)GTTACACATA CTGAGAAACA(A/C)AGCACTAATG
CCACTCTTTC(C/T)GAGAAAAAAACTTCCCAATACC(A/G)GAATCTGAAC TATTTGCTAG(T/G)TATGTGGGAG GACCAATCCA(C/T)ACTTCACCAC
GAAGTTGTGC(G/C)TGATATCAGC

AGTGGAATTC(G/A)CTTTGATGGCTGA(G/T)TTTTAGGTTC TTAAAGGGTA(G/A)CATAATTCAG

GTGGTGATGG(C/G)GGGGTTGCG(G/T)CAGGAGGGGAGAAGCTTT(A/G)AAAGAATCTT GACAACTCTA(C/T)CCCACTTCTT
GATAGGCAGT(A/G)GCAGACAGGC GCAACACTG(A/C)TTC(C/T)AGGGGAG(C/T)TCTCCAGGCC
GCATTTAATG(C/T)AATTTGAAAAACTTAAT(A/G)CTATTCTTCA CCCTCTGTAC(A/G)CCCTTCCCAC GCTGCAGATA(T/C)AAGGGAAAGG

TCAGTTTGAC(C/T)ATGACTACATTCAT(A/G)TGCTATATGA
TATGACTTAC(C/T)TTCTTTTCCC CCAAGAAGCC(A/G)GAAGCGGGCA
N/A
GTAAAATGGG(C/T)GAGCAGCAAT
CCCCTTAATT(A/G)CCTGAGCAGTGCCTTCTG(C/T)GTGCATGGGA TCAGCTGCGGC(A/G)TATGGCACGG TACATACTTA(A/C)AGGATGCTCA
CAATGCTGTG(C/T)CAAGATGATG
AGAGGAGCAC(A/G)TGAGGAGCTG
AAAGATTCTG(C/T)CCTAACCACA
GGGACTCCGG(C/T)CTGAGCGCAC
GTTACTGCCC(A/G)(C/T)ACATTTCATT ACAAAGGAAG(C/T)CAAGAGACCA
TGAGCACTTA(A/G)TGGATGACCT
TGCTCGCAGA(A/C)CTGTGTTTCT CACCATCACC(C/T)AGGGCTGTGC
ATGCTGCAGG(A/C)CATCATTCAC ACCACCGTGC(C/T)ATCCTAACACAAGCA(C/T)GACCCACCAG CATATACAGC(A/G)GAGGAACTGA
GATCCTCCTC(A/G)CATGTTAAC TGCAACAAGC(C/T)TTTTCCTTCC
TCCTTATGGACCTGGCACA(A/G)ATGC(A/G)TGTGAC(A/G)AGAAAGAGGA GCTGGGAGTT(A/G)CACGTCCCATC(A/C)T(G/T)TTTATTGGTC

AAGTAACTGC(A/G)TGGTTGCTTTTT(C/T)TTCTGGGAGATTCTTGGTG(T/G)TTTTTTGTCT
AAGGTATTGC(A/G)TTTTAAACAAA(G/C)CAATTTAACA
TCGTCAGCTC(C/T)GGACCGTGAG CGTGAGCACC(A/G)AGCCCTGAGC GGGAACAAAC(A/G)CACTGGGAGT
CTGGGGCAC(C/T)CT(G/C)TGTGCCTCCC
N/A
TTGTTACAGT(A/T)GAAACTAATT TCACCATAAT(T/G)AAATGCTATA TATATATACATAT(A/G)TTCCTTCAAA
ATTCAATGCT(G/T)CCATGTGTGG(C/T)GTGCTGAGAG
ACATGCATGG(C/A)TGCAGCATCC
AGATACCTAC(G/A)TGCCCTTAAG CCCTCCATCT(A/G)TCTTCCTCCC GGGGCATTAT(A/G)AAACTTTTCT
TTTCTAGGAC(C/T)GTGCTCAGAG GGATCAAAGC(A/G)TGTCTGACAG CTGACTTGGG(C/T)TCACGCAGTG
GTTAGTTGCC(A/G)TTTCTCTTTTCCA(A/G)TATTGCTTTTCCC(A/G)CTTCTTAGAA
TCACAATCCA(C/T)CCATATCCAT

CTGTGTTCAT(A/G)ACCACAAACT GTGCCATTG(A/G)CACCTGGGCA
GGTGGCAGCC(A/G)GAAATCTCCC TGGACTCACA(C/T)GACGCTGATCTATGTC(A/T)TTTCAAGGAC

ATGATTCAAC(A/G)GTTAAAAAAT GGTATTGCA(A/G)AAGTGAGAAG CAGGAAGCT(C/T)GGGAGCTTGT GTTTTCAGAG(A/C)GCTGCCTCTG
AGGAAGCTCT(G/C)TTGTTTGCTT
GCTTCCACTA(A/T)CTCTCACAAT GGGAATGAAA(A/G)TACCCTTGCC

AAAACAGTGA(C/T)GGCACTCTGT

GACAGAATAG(C/A)CTCAAACTTC
TTTCTAGTGG(A/G)CAGAGCTCTG CTGCTCACCC(A/C)GCGTGGCTCGGAAG(A/T)GTTTGGGGTA TCAGCACCTG(C/T)GCTCGTATCA
GCACATGGAG(A/C)TGCAGCTGAA
GTGTCATGGAATCAC(A/G)GCGTGGTTGGGT(G/T)GGAAGGGGCC ATCGTGGCAC(C/T)GTTGGGTTGG TGCCCCCCAC(C/G)CTCAAACTGC

CTAGGCTGAC(C/T)CAGCCCAAGC
N/A

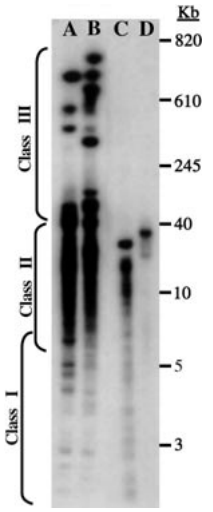

Fig. 14. Avian telomere terminal restriction fragment (TRF) profiles illustrating the different size classes of telomere sequence arrays. Equivalent amounts of DNA were digested with *Hae*III and fragments were separated by CHEF-PFGE, Southern blotted and hybridized with a telomere probe, $(5\text{-TTAGGG-}3')_7$. Profiles shown are from two chickens (lanes A and B), an American bald eagle (lane C), and a Northern goshawk (lane D). The chicken profiles exhibit the three size categories of telomere arrays, similar to that of many avian species (Delany et al., 2000). Whereas, the eagle and goshawk do not exhibit the Class III arrays, the karyotypes of these species are notable for their reduced number of microchromosomes.

cally terminal markers in the genome. Terminal linkage markers are only positioned relative to mapping data on one side, and therefore the ascribed terminal regions of linkage groups have a tendency to accumulate "problem" loci. This, combined with the low resolution of physical mapping on metaphase chromosomes, means that we cannot identify the most distal marker conclusively. This again underlines the need for further specific targeting of the telomeric regions in the production of markers, rather than the reliance on the identification of markers through standard mapping techniques.

Avian telomere biology

(Prepared by M.E. Delany)

The experiments of Muller (1938) and McClintock (1941), in Drosophila and maize, respectively, established that the cytogenetic function of the telomere is to maintain normal chromosome architecture. The molecular organization of the telomere was established 40 years later, wherein the sequence of the telomere was discovered in Tetrahymena (Blackburn and Gall, 1978) and the role of the specialized enzyme telomerase in maintaining telomeres was established (Greider and Blackburn, 1985). The relationship between down-regulation of telomerase, telomere erosion and genome stability to proliferation and senescence profiles of cells in vitro rapidly became a focal point for research on in vivo aging and oncogenesis in human and other model systems (Harley, 1995).

Cytogenetic localization of telomere arrays by FISH provided evidence that the ends of avian chromosomes, similar to other organisms, possess the highly conserved telomere repeat sequence, 5′-TTAGGG-3′ (Meyne et al., 1989). Nanda and Schmid (1994) localized the telomere repeat to not only the ends of chicken chromosomes, but also to interstitial regions of several macrochromosomes (e.g. 1q) and centromere locations. Further, three types of telomere sequence hybridization patterns were identified on the microchromosomes: (a) terminal plus centromere, (b) terminal only, and (c) complete chromosome coverage.

Avian models

Some bird species (e.g. birds-of-prey) possess a variant karyotype with far fewer microchromosomes as compared to the prototypical arrangement of 30 pairs of microchromosomes and 10 pairs of macrochromosomes (DeBoer, 1976; Bloom et al., 1993). In addition, birds exhibit a remarkable diversity of natural life spans having maximum longevity expectations that range from 5 to 80 yr, depending on the species (Holmes and Austad, 1995a). And finally, it is notable that avian (chicken) cells in vitro share in common with human cells the characteristic of resistance to immortalization (Lima and Macieira-Coelho, 1972). Combined, these features are useful and key for establishing the conservation of various aspects of telomere biology within and among avian species and in comparison to other higher vertebrates.

Recently, my laboratory initiated studies to investigate the molecular organization of the telomere arrays of the chicken genome, as well as other poultry and non-poultry species (Delany et al., 2000). The overall objective of this research is to understand telomere biology in the context of avian chromosome evolution and genome organization, and establish the role that telomere stability plays in avian cell senescence in vitro and in vivo.

Telomere array quantity and organization

Although the avian genome is only one-third of the size of the human genome, the total amount of telomere sequence was found to be 5 to 10 times more abundant (e.g., 4% of the chicken genome consists of telomere sequence). Three distinct categories of telomere arrays were identified, designated Class I, II, and III (see Fig. 14, lanes A and B). These classes of arrays vary for molecular weight, fragment pattern (discrete versus overlapping) as identified by telomere terminal restriction fragment (TRF) analysis, chromosomal location (terminal versus interstitial) and shortening-profile.

Class I arrays are in the size range of 0.5 to 10 kb, exhibit discrete and genotype-specific banding patterns, are interstitially located, and show no evidence of telomere shortening. The Class II arrays are in the size range of 10 to 40 kb, resolve as an overlapping smear of TRFs, show evidence for terminal location based on digestion by *Bal*31, and exhibit shortening in somatic tissues. The third category of telomere arrays, Class III, is hundreds of kilobases in size and in some birds, e.g. poultry, arrays of 1 to 2 Mb were found. These results confirm initial reports of telomere array size in the chicken (Lejnene et al., 1995, Bloom et al., 1993), but conflict with a more recent report (Venkatasen and Price, 1998) which concluded such extreme-size arrays were the outcome of undigested interstitial arrays. Although ultra-long arrays as large as 150 kb have been described for the laboratory mouse (Kipling and Cooke, 1990), the mega-arrays found in chicken and other avian genomes represent the largest telomere arrays described for any organism to date.

Telomere arrays shorten and telomerase activity is down regulated in somatic tissues of adult chickens

The average size of the Class II telomeres shortens by 3 to 5 kb in a wide-variety of somatic tissues as compared to (1) the germ lineage tissues of adults spanning 2 to 5 yr of age (intra-individual comparisons) and (2) corresponding tissues from E10 embryos (inter-age comparisons) (Delany et al., 2000; Taylor and Delany, 2000). Correspondingly, telomerase activity in vivo down-regulates in a tissue-specific manner in somatic tissues during embryonic development (e.g., brain, heart, muscle, liver) or postnatally (e.g., pancreas, lung, kidney). Constitutively high levels of somatic telomerase activity were observed only in tissues with high rates of proliferation and resident stem cell populations, such as intestine, spleen and gonad (Taylor and Delany, 2000). Similarly, chicken embryo fibroblasts were observed to down-regulate telomerase activity in vitro and exhibited telomere shortening in arrays of similar size to the Class II arrays (Venkatasen and Price, 1998). Thus, Class II telomere shortening and down-regulation of telomerase activity in chicken somatic tissues more closely resembles the profiles seen in human somatic cells than that of murine wherein constitutive telomerase activity is found in many postnatal somatic tissues and telomere erosion is immaterial under normal circumstances.

It has not been possible to definitively establish either the presence or absence of shortening within the megabase-size arrays in somatic tissues. Largely, this is because of the inability to resolve, with any degree of certainty, changes on the order of several thousand bases in the context of arrays that are hundreds or millions of kilobases. For example, shortening of 3 kb (as detected in Class II arrays) requires reproducible resolution and accurate quantitation of the difference between 800 and 757 kb molecular weight species; this level of discretion is not attainable by pulse-field gel electrophoresis, the method used to separate high molecular weight DNA.

Consideration for the function of megabase telomere arrays

In a survey of 18 bird species the preponderance of avian genomes exhibited each of the three size classes of telomere arrays (Delany et al., 2000). The exceptions were two bird-of-prey species, the American bald eagle and Northern goshawk, whose telomere profiles did not include the Class III megabase-sized arrays (Fig. 14, lanes C and D). Notably, these raptor species possess a karyotype with a very low number of microchromosomes, 4 pairs rather than 30. A hypothesis currently under study is that the Class III arrays map to the microchromosomes functioning to protect these very small genetic elements from any potentially negative effects of telomere erosion over the long-lived natural life span of many birds (Holmes and Austad, 1995a).

Many interesting questions remain. How does the abundance of the tandemly repeated telomere sequence fit with the emerging model suggesting reduced levels of repetitive DNA and increased gene density on the microchromosomes? Are there microchromosomes consisting predominately of telomere sequence? Are the interstitial telomere arrays of the macrochromosomes reflective of microchromosome fusions? How did the diversity of avian genomes evolve? Continued studies of the interstitial and terminal telomere arrays should provide new and relevant information to the development of a cohesive model of the avian genome.

Aging and lifespan in birds

(Prepared by H. Hoehn)

Despite metabolic rates 2- to 2.5-fold higher than mammals of similar size, 2–4 times higher blood sugar levels, and 3–4 °C higher body temperatures, most avian species are longer-lived than their mammalian counterparts of equal body size (Calder, 1990). While it was previously held that birds in nature vanish due to predation, accidents, and other exogenous factors unrelated to aging, more recent studies show evidence for aging (increases in mortality rates over the life span) in a considerable proportion of avian species (Holmes and Austad, 1995a; Finch and Pike, 1996). In domestic fowl, a variety of aging effects are well documented (e.g. Ledur et al., 2000). Both wild and captive birds live 1.7–3 times longer than corresponding mammals (Holmes and Austad, 1995a, b), even though the lifetime energy expenditures of avian species exceed those of mammals by 1.9–3.7 fold (Jurgens and Prothero, 1991). The average genome size of birds is only 1/3 that of mammals such that a more compact genome could account for greater lifespans. However amongst the birds themselves, genome size and relative longevity appear to be positively correlated (Monaghan and Metcalf, 2000).

Comparative studies between bats and similar sized non-flying mammals have clearly shown that the ability to fly by itself promotes longevity (Austad and Fischer, 1991). In addition, the rate of free radical production (and thereby oxidative damage) appears to be substantially lower in birds than in comparable mammals. For example, as a marker of oxidative DNA-lesions, 8-oxodG levels were lower in brain mtDNA of pigeons than of rats, in brain nuclear DNA of canaries than of mice, and in heart nuclear DNA of parakeets compared to mice (Herrero and Barja, 1999). Most importantly, the tissues of birds may be better protected against free radical mediated lipid peroxidation due to their low degree of fatty acid unsaturation, which may be a general characteristic of long-lived homeothermic vertebrates (Pamplona et al., 1999). Paradoxically, the levels of tissue antioxidants, with the possible exception of selenium (Goede, 1993), were found to be negatively correlated with the maximum lifespan potential in most vertebrate species. The substantially lower generation of reactive oxygen species is therefore thought to be the major factor accounting for the disproportionate longevity of birds (Ku and Sohal, 1993; Perez-Campo et al., 1998). The overall lower generation of reactive oxidant species could also explain why higher blood sugar levels and higher body temperatures are less detrimental in birds (Iqbal et al., 1999), since there should be less opportunity for harmful synergism between free radicals and advanced glycosylated (Maillard) end products that may be instrumental in age-related tissue degeneration (Kristal and Yu, 1992; Martin et al., 1996).

As in many other species including our own (Westendorp and Kirkwood, 1998), delayed reproduction seems to be an

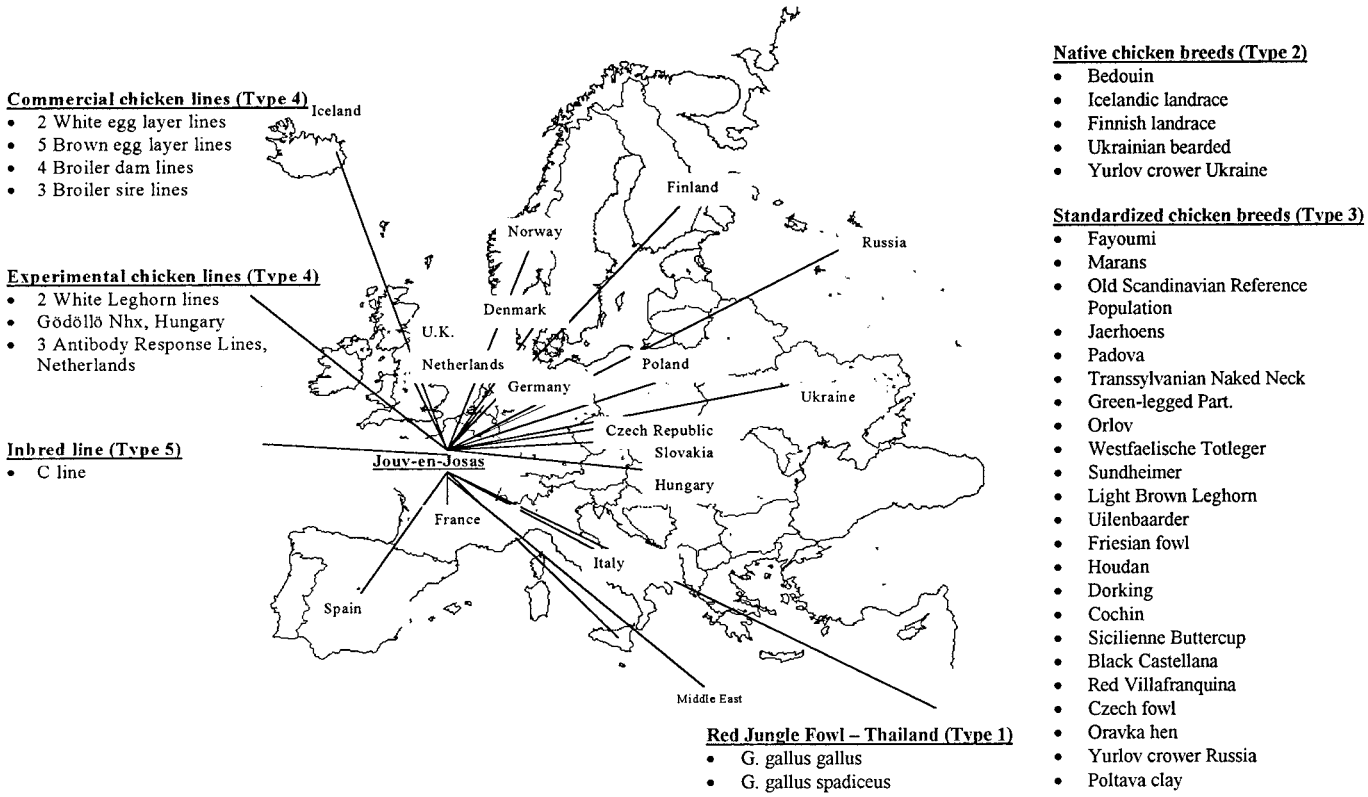

Fig. 15. 51 Chicken populations sampled in the AVIANDIV project.

additional factor that promotes longevity in birds, as predicted by a leading theory of aging (Lithgow and Kirkwood, 1996). For example, the maximum lifespan of passerine birds and chickens (including most galliform species) rarely exceeds 10–15 years, and these species start reproduction early. On the other hand, condors, ravens, sea gulls, parakeets, scarlet macaws, and other non-passerine species can reach maximum lifespans beyond 70 years (e.g. Klimkiewicz and Futcher, 1989). These species start reproduction late (albatrosses as late as 10 years of age) and have smaller clutch sizes, supporting the idea that less investment in reproduction may benefit somatic maintenance.

Altogether, the puzzle of apparently slower aging and disproportionate longevities of avian species is far from being solved and remains a challenge to biogerontology. The potential of "birds as animal models for the comparative biology of aging" (Holmes and Austad, 1995b) undoubtedly deserves further exploration.

Assessment of biodiversity in chickens

(Prepared by S. Weigend, M. Tixier-Boichard, J. Hillel, A. Vignal, M.A.M. Groenen, K. Wimmers, T. Burke, and A. Mäki-Tanila)

Chicken genetic resources comprise a great variety of breeds and populations ranging from Red Jungle Fowl, the assumed progenitor of all domestic breeds, to highly selected commercial and specialized lines, including native and local chicken breeds. Genetic diversity can be described at several levels, from phenotypic observations to molecular data. In an EC funded project (AVIANDIV) eight laboratories have collaborated to assess the genetic variation among and within populations sampled from a wide range of chicken breeds (Fig. 15). Populations were classified a priori into five "types", according to selection history and selection pressure. "Type 1" included two populations of Red Jungle Fowl, which originated from birds caught recently (1997) in several regions of Thailand. "Type 2" included five populations that have not been selected for any particular trait and that exhibited considerable morphological variation. Their geographical origin was the Middle-East, and eastern and northern Europe. "Type 3" included 23 standardised breeds that have experienced selection on morphological traits. Their geographical origin was the Mediterranean region, eastern-central and north-western Europe. "Type 4" included 20 lines selected on a quantitative trait or an economic index, with six experimental lines, two white-egg layers, five brown-egg layers, four broiler dam lines, and three broiler sire lines. "Type 5" included one highly inbred line, composed of four sub-lines. Principal component analysis indicated that the most meaningful descriptors were "type", year of creation, overall frequency of phenotypic mutants, geographic location, founder population and current size (Tixier-Boichard et al., unpublished). To date, DNA pools from 43 populations have been typed at 21 microsatellite loci (Table 13). Using an automated sequencer (Perkin Elmer, Applied Biosystems Division) the allele frequencies were calculated based on peak areas for all

alleles of each of the loci. The mean expected heterozygosity across all genotyped loci was 47%. This estimate is lower than the estimates of heterozygosity reported for broilers (53% – pooled blood), but higher than for layers (27%) (Crooijmans et al., 1996). Across breeds, locus *MCW0098* displayed the lowest mean heterozygosity (26%), whilst *MCW0034* showed the highest (66%). The number of alleles per locus across breeds ranged between 3 and 23 with a mean of 9.62. The mean number of alleles per locus per breed was 3.67 (range 1.7–6.2). The wide range of diversity in the sampled breeds and the high level of microsatellite polymorphism is reflected by the fact that the least polymorphic locus, *MCW0098* was polymorphic in 64% of the breeds, while 8 of the 21 markers were polymorphic in all 43 breeds (Hillel et al., unpublished). To evaluate the potential utility of point mutations (SNPs) as a new class of marker for assessing biodiversity in chickens, random sequences were selected for direct sequencing of PCR products from 100 individuals originating from the 10 most diverse populations collected. The sequencing results available to date revealed, on average, a frequency of 1.69 SNPs per 100 bp sequenced (Table 14). However, between fragments there were considerable differences ranging from no SNP (fragment 28A) or as low as 0.15 SNP per 100 bp (fragment 32P) up to 3.69 SNPs per 100 bp (P18). Furthermore, it appears that at least some of the SNPs are clustered within the fragment.

Data collected during the lifetime of the project are accessible via the Poultry Biodiversity Database (http://w3.tzv.fal.de/aviandiv/index.html). A DNA bank for the sampled chicken populations has been established at INRA in Jouy-en-Josas, France.

Table 13. Variation at 21 microsatellite loci typed in DNA pools of 43 diverse chicken populations

Marker loci	Chr.	Number of alleles		Average Heterozygote frequencies	Frequency of polymorphic breeds per locus
		Across breed	Per breed		
MCW0098	4	3	1.7	0.26	0.64
MCW0294	Z	12	2.5	0.29	0.75
MCW0248	1	4	2.0	0.30	0.66
MCW0103	3	7	2.8	0.35	0.89
MCW0216	13	6	2.2	0.37	0.86
MCW0067	10	5	2.4	0.39	0.95
MCW0037	3	4	2.3	0.41	0.89
MCW0222	3	6	2.6	0.42	0.91
MCW0081	5	12	4.9	0.43	0.95
MCW0330	17	11	3.2	0.48	0.89
MCW0014	6	13	4.3	0.49	1.00
MCW0078	5	7	4.0	0.51	1.00
MCW0295	4	9	3.6	0.52	0.93
MCW0206	2	14	4.2	0.53	1.00
MCW0111	1	8	3.6	0.53	1.00
ADL0112	10	7	4.0	0.55	1.00
MCW0183	7	16	5.1	0.57	0.93
MCW0069	24	12	5.3	0.60	1.00
ADL0268	1	7	4.4	0.61	0.95
LEI0192	6	23	5.7	0.61	1.00
MCW0034	2	16	6.2	0.66	1.00
Total		9.62	3.67	0.47	0.91

Table 14. Single Nucleotide Polymorphisms (SNPs) detected by sequencing ten random DNA fragments in 100 individuals of ten diverse chicken populations

Fragment	Length sequenced (bp)	Number of SNPs	Frequency/100 bp
7G1	520	11	2.11
69P	470	9	1.91
28A	510	0	0.00
32P	650	1	0.15
32N	420	6	1.42
P18	460	17	3.69
P56[a]	570	16[b]	2.8
P49	645	8	1.24
Total	4245	72	1.69

[a] One of the polymorphic sites is a 5-bp deletion.
[b] One population is missing in the analysis. The number of SNPs may be higher

Chicken genome databases

(Prepared by A. Law)

In order to keep abreast of the rapid pace of genome mapping in any species, it is vital to have flexible and simple access to the data underlying that work. In chicken, this need is largely addressed using tools developed by the Roslin Institute bioinformatics group (http://www.roslin.ac.uk/bioinformatics).

Details of all published work on chicken genome mapping are stored in the chicken version of the ARKdb database (http://www.roslin.ac.uk/arkdb/about_ARK.html). This database system is built on top of an industrial-strength relational database management system (RDBMS), INGRES and was designed from the ground up to handle the varied types of data associated with genome mapping in farm animals. It stores details of markers, PCR primers, 2-point linkage data, full linkage maps as well as cytogenetic maps that tie linkage groups to specific chromosomes and links to relevant entries in sequence and literature databases. Brief abstracts are recorded from each paper and the experimental conditions underpinning each publication are entered. This data is actively curated by editors. The entire ARKdb system is publicly accessible via a web interface. Of particular note is the map viewer, Anubis (http://www.roslin.ac.uk/anubis; Mungall, 1996). The Anubis map viewer was the first genome browser to be implemented as a fully interactive graphical user interface. Map displays are built "on the fly" in response to user requests. Single or multiple maps can be viewed from one or more species, aligned with each other and with homologous genes indicated. Although there are other sites that publish chicken genetic linkage maps, they are restricted to static images. Anubis is currently being redeveloped using the Java™ programming language to provide higher levels of interactivity and customisation in the map displays.

Although the ARKdb model provides structures in which to record published data, it does not handle the raw experimental data from which that published information was derived. A second database design, also developed at the Roslin Institute serves this function for many chicken mapping projects. Called

resSpecies, it stores details of pedigrees, genotypes and (optionally) traits and provides export routines to reformat the data into the forms required by several of the most popular mapping programs. New export routines can be added as required. Again, this system is fully accessible via the web. Currently access is limited to project collaborators, but work is under way to provide public access to published data. This public access will be integrated with the ARKdb mapping database system to allow users to "drill down" from map displays to the level of individual genotypes in the data set.

In much the same way that resSpecies provides an underpinning of the genetic linkage maps, work is also underway to develop systems to underpin physical maps. These will store clone details and overlaps with links to sequence databases and published markers. The access to this data will, like resSpecies, be restricted to project collaborators with published information being freely accessible.

A step further down the progression from genome-scale to base-pair mapping is the determination of the sequences of cloned DNA fragments. In chicken, like other farm animal species, the majority of this work has involved the sequencing of cDNA clones to derive ESTs. There are several projects underway in this field, notably that of Joan Burnside (Delaware), Jean-Marie Buerstedde (Hamburg), Dave Burt (Roslin) and Nat Bumstead (Compton). Each group has sequenced clones from a specific library and makes those sequences available. The German group has several web-based forms available for searching their sequences in a variety of ways. All the currently available chicken EST sequences have been collected together at Roslin, where a web-based BLAST search form can be used to identify homologues to genes from other species.

URLs:	
ARKdb	http://www.roslin.ac.uk/arkdb_about_ARK.html http://www.roslin.ac.uk/bioinformatics/databases.html
Chick Ace	http://www.zod.wau.nL/research/chicken/frame_chicken.html (Groenen and Cheng, in this report) Chicken Genome Mapping site of Wageningen University http://www.zod.wau.nl/vf/research/chicken/frame_chicken.html/ US Poultry Gene Mapping http://poultry.mph.msu.edu/
ResSpecies	http://www.roslin.ac.uk/bioinformatics/databases.html http://www.roslin.ac.uk/cgi-bin/resSpecies/resSpecies.sh
ESTs:	
Hamburg	http://genetics.hpi.uni-hamburg.de/dt40est.html
Delaware	http://udgenome.ags.udel.edu/chickest/chick.htm
Roslin	http://www.roslin.ac.uk/cgi-bin/est-blast.pl

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