DOI: 10.1534/genetics.108.088195

A Gene-Based Genetic Linkage Map of the Collared Flycatcher (*Ficedula albicollis*) Reveals Extensive Synteny and Gene-Order Conservation During 100 Million Years of Avian Evolution

Niclas Backström,* Nikoletta Karaiskou,†,[1] Erica H. Leder,† Lars Gustafsson,‡ Craig R. Primmer,† Anna Qvarnström‡ and Hans Ellegren*,[2]

**Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, SE-752 36 Uppsala, Sweden, †Division of Genetics and Physiology, Department of Biology, University of Turku, 20014 Turku, Finland and ‡Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, SE-752 36 Uppsala, Sweden*

Manuscript received February 17, 2008
Accepted for publication May 1, 2008

ABSTRACT

By taking advantage of a recently developed reference marker set for avian genome analysis we have constructed a gene-based genetic map of the collared flycatcher, an important "ecological model" for studies of life-history evolution, sexual selection, speciation, and quantitative genetics. A pedigree of 322 birds from a natural population was genotyped for 384 single nucleotide polymorphisms (SNPs) from 170 protein-coding genes and 71 microsatellites. Altogether, 147 gene markers and 64 microsatellites form 33 linkage groups with a total genetic distance of 1787 cM. Male recombination rates are, on average, 22% higher than female rates (total distance 1982 *vs.* 1627 cM). The ability to anchor the collared flycatcher map with the chicken genome via the gene-based SNPs revealed an extraordinary degree of both synteny and gene-order conservation during avian evolution. The great majority of chicken chromosomes correspond to a single linkage group in collared flycatchers, with only a few cases of inter- and intrachromosomal rearrangements. The rate of chromosomal diversification, fissions/fusions, and inversions combined is thus considerably lower in birds (0.05/MY) than in mammals (0.6–2.0/MY). A dearth of repeat elements, known to promote chromosomal breakage, in avian genomes may contribute to their stability. The degree of genome stability is likely to have important consequences for general evolutionary patterns and may explain, for example, the comparatively slow rate by which genetic incompatibility among lineages of birds evolves.

GENOMICS is in a phase where new technology allows genome characterization beyond that of traditional model organisms and species of medical or agricultural interest. For example, genomic analyses of nonmodel species holds great promise for dissecting the genetic background to fitness traits in natural populations, to adaptive population divergence, to speciation, and to other key aspects of evolutionary biology (Ellegren and Sheldon 2008). Genomic characterization of new and phylogenetically divergent lineages has the additional benefit that it provides the necessary comparative perspective for addressing the evolution of genome organization. Specifically, with genetic maps or genome sequence information available across taxa, the broad-scale pattern of genome and chromosomal evolution can be investigated. This, in turn, opens the possibility of investigating to what extent evolution at the chromosomal level sets the stage for the evolutionary processes, which occur on the level of the phenotype.

Reshuffling of chromosomal segments, through translocations and inversions, is an integral part of genome evolution. However, it is clear that the rate of rearrangement differs radically among lineages as well as on a temporal scale (Kohn *et al.* 2006; Ferguson-Smith and Trifonov 2007). From comparative mapping of chicken and different mammals it was suggested that the rate of chromosomal rearrangement in the avian lineage is very low (Burt *et al.* 1999). This has subsequently been confirmed through analyses of vertebrate genome sequence data, including chicken (Bourque *et al.* 2005), the only bird that has had its genome sequenced to date (International Chicken Genome Sequencing Consortium 2004). Moreover, evidence for an unusually stable avian karyotype with few interchromosomal rearrangements has been obtained by cross-species chromosome painting or the use of other types of *in situ* hybridization probes (Shetty *et al.* 1999; Shibusawa *et al.* 2001, 2004a,b; Raudsepp *et al.* 2002; Guttenbach *et al.* 2003; Kasai *et al.* 2003; Derjusheva *et al.* 2004; Schmid *et al.* 2005; Itoh *et al.*

[1]*Present address:* Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, P.O. Box 54, 124 Thessaloniki, Macedonia, Greece.

[2]*Corresponding author:* Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden. E-mail: hans.ellegren@ebc.uu.se

2006; Fillon *et al.* 2007; Griffin *et al.* 2007; Nishida-Umehara *et al.* 2007). However, these experiments rarely have the resolution for detecting intrachromosomal or small-scale interchromosomal rearrangements.

Genetic maps are available for turkey (Reed *et al.* 2005) and quail (Kayang *et al.* 2006), two agricultural species that are closely related to chicken as members of the order Galliformes. However, a lack of genetic markers, in particular those informative for comparative mapping, has been a major obstacle to linkage analyses of bird species belonging to other orders. As a consequence, linkage mapping in natural bird populations is still in its infancy. Hansson *et al.* (2005) developed a partial microsatellite-based linkage map (58 markers) in the great reed warbler (*Acrocephalus arundinaceus*), a species from the order Passeriformes, the largest and ecologically most well-studied group of birds. This study also made the unexpected observation that the recombination rate was twice as high in females as in males, which is in contrast to the prevailing trend of recombination usually being lower in the heterogametic sex (in birds, males are ZZ and females ZW). Backström *et al.* (2006) reported on a gene-based linkage map of the Z chromosome of another passerine species, the collared flycatcher (*Ficedula albicollis*). The Z chromosome was found to be completely syntenic between collared flycatcher and chicken. Subsequently, Dawson *et al.* (2007) developed an extended great reed warbler map and found a high degree of chromosomal conservation when compared to chicken (see also Åkesson *et al.* 2007).

We have recently adopted the comparative anchor-tagged sequences approach (Lyons *et al.* 1997) to develop a genomewide, gene-based marker resource for avian comparative mapping (Backström *et al.* 2008). This set of 200+ markers target conserved exonic sequences in genes spread over all chromosomes currently covered in the chicken genome assembly, with a mean marker interval of 4 Mb. The uniform distribution of these markers across the chicken genome means that, if they are used for comparative mapping in other birds, the degree of synteny and gene-order conservation across a significant part of the avian genome can be revealed. Here we present a genetic linkage map of the collared flycatcher based on the new marker set. This species has long been in focus for studies of sexual selection, life-history evolution, and speciation (Gustafsson and Sutherland 1988; Gustafsson and Pärt 1990; Gustafsson *et al.* 1995; Ellegren *et al.* 1996; Qvarnström *et al.* 2000, 2006; Veen *et al.* 2001; Saether *et al.* 2007) and hence is a well-established "ecological model organism." Importantly, songbirds (passeriforms) and galliforms diverged at the time of the major radiation of avian lineages ≈100 million years (MY) ago (Van Tuinen *et al.* 2000). With genetic map data for the collared flycatcher we can thus address genome evolution at the level of gene order across two highly divergent lineages of the avian phylogenetic tree.

MATERIALS AND METHODS

Species samples and DNA extraction: Blood samples were collected from collared flycatcher (*F. albicollis*) families breeding on the Baltic islands Öland and Gotland and DNA was extracted by a standard proteinase K digestion/phenol–chlorophorm purification protocol. The mapping pedigree consisted of 24 half-sib families with a few interconnections and 11 F_2's, in total 322 birds (supplemental Table 1) after excluding all recognized extra-pair offspring (see below).

Marker genotyping: In a previous resequencing effort, we surveyed 200 collared flycatcher genes for intronic diversity among 10 unrelated individuals from the same population as the mapping pedigree, which uncovered 904 segregating sites (Backström *et al.* 2008). From this, 341 single nucleotide polymorphisms (SNPs) with a minor allele frequency of >0.1 and representing the majority of all genes screened were selected for genotyping in the pedigree; for many genes, more than one SNP from the same intron were included. An additional 43 SNPs were obtained from 21 different genes previously screened for variability in collared flycatchers (Borge *et al.* 2005) (Table 1). The total of 384 SNPs were genotyped using the Golden Gate Assay (Fan *et al.* 2003) from Illumina (San Diego) at the SNP Technology Platform, Uppsala University (http://www.medsci.uu.se/molmed/snpgenotyping/index.htm). The overall genotype call rate was 95.7% and the reproducibility was 100% according to duplicate analysis of 5.4% (7218/132,848) of the genotypes. The quality of the genotype data was further assessed by testing for Hardy–Weinberg equilibrium (HWE) using the chi-square distribution for each assay. All SNPs conformed to HWE.

Seventy microsatellites were isolated from the closely related pied flycatcher (*Ficedula hypoleuca*: Leder *et al.* 2008). Sixty-three of these markers, as well as five EST-linked microsatellites and nine microsatellites from other passerines (Karaiskou *et al.* 2008), were PCR multiplexed in sets of six to nine loci using 30 ng of DNA per reaction. Each PCR multiplex could be analyzed on a single run of an ABI3130xl (Applied Biosystems). Detailed PCR multiplex protocols and electrophoresis details can be found in Karaiskou and Primmer (2007).

Data analysis: Microsatellites were scored using the GeneMapper software (Applied Biosystems). SNPs from the same intron were combined into haplotypes using the available pedigree information. For both types of markers, missing data points of parents were inferred from the haplotypes of offspring and mates when possible. Offspring showing deviations from the expected parental genotypes/haplotypes were not included in further analysis. These are likely to represent extra-pair offspring since previous work in this population has revealed that ~15% of all offspring result from extra-pair copulations (Sheldon *et al.* 1997; Sheldon and Ellegren 1999).

Linkage analyses were performed with CRI-MAP (Green *et al.* 1990). Initially, all markers were tested against each other with the two-point option and markers that clustered together with significant lod score support (>3.0) were treated as linkage groups. Framework maps were constructed with the *build* option and the best position of all markers within an ordered linkage group was then estimated with recurrent runs of the option *flips4* until no better order could be found (best order map).

Microsatellite clone sequences were used in cross-species MEGABLAST searches against the chicken genome sequence (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9031), first with default settings and then with relaxed settings according to Dawson *et al.* (2006). Both search methods generated the same set of significant (arbi-

TABLE 1

Markers included in the collared flycatcher linkage map

Marker	Linkage group	Gene description[a]	Ensembl ID[b]	Chicken chromosome	Chicken genome start position (bp)
		Gene-based SNPs			
00548	Unlinked	Hypothetical protein	00548	22	1,221,445
02079	Unlinked	Hypothetical protein	02079	10	1,854,152
02419	*Fal9*	No longer in the Ensembl database, not mapped to new identifiers			
04550	*Fal27*	No longer in the Ensembl database, not mapped to new identifiers			
05087	*Fal13*	Hypothetical protein	05087	19	5,590,343
07726	*Fal16*	Hypothetical protein	07726	11	10,700,742
08235	*Fal6*	Hypothetical protein	08235	Un	42,824,332
08544	*Fal10*	No description	08544	6	17,299,861
12630	*Fal28*	Magmas-like protein	12630	14	13,386,833
15691	*Fal6*	Uncharacterized protein C15orf24 precursor	15691	5	32,353,219
15738	*Fal5*	Similar to CG1218-PA	15738	4	26,412,511
17140	*Fal5*	No longer in the Ensembl database, not mapped to new identifiers			
18798	*Fal6*	Kinesin light chain	18798	5	52,944,389
20352	*Fal1*	No description	20352	2	56,437,562
20904	*Fal1*	Hypothetical protein	20904	2	67,141,279
21277	*Fal1*	No description	21277	2	80,448,704
22644	*Fal2*	No description	22644	3	58,129,917
25613	*Fal8*	Hypothetical protein	25613	1	108,461,452
25924	Unlinked	Hypothetical protein	25924	4	93,260,081
27425	*Fal4*	Hypothetical protein	27425	1	171,909,504
27623	*Fal4*	No longer in the Ensembl database, not mapped to new identifiers			
ABHD10	*Fal8*	Abhydrolase domain-containing mitochondrial precursor	24813	1	91,842,859
ACADL	*Fal7*	Acyl-coenzyme A dehydrogenase, long chain	04557	7	2,734,809
ACADSB	*Fal10*	Acyl-coenzyme A dehydrogenase, short/branched chain	15724	6	33,024,247
ACHA9	*Fal5*	Neuronal acetylcholine receptor subunit α-9 precursor	23080	4	70,872,474
ACLY[c]	*Fal1*	ATP citrate lyase	05502	27	4,344,212
ACOT8	*Fal15*	Acyl-coenzyme A thioesterase 8	11074	20	10,473,488
ACTB[c]	*Fal32*	Actin, cytoplasmic type 5	39969	10	1,891,946
ADAL	*Fal23*	Adenosine deaminase-like	06419	10	7,196,900
ADH5	*Fal5*	Alcohol dehydrogenase 5 (class III), χ polypeptide	19994	4	61,539,229
ADIPOR1	*Fal27*	Adiponectin receptor 1	00132	26	1,090,425
ALAS1[c]	*Fal21*	5-Aminolevulinate synthase, nonspecific, mitochondrial precursor	06295	12	2,762,886
AN32B	*Fal26*	Acidic leucine-rich nuclear phosphoprotein 32 family member B	02401	28	1,324,041
ANAPC5	*Fal9*	Anaphase-promoting complex subunit 5	06640	15	5,498,421
ANKRD49	*Fal4*	Ankyrin repeat domain-containing protein 49	27818	1	189,909,241
ARF1	Unlinked	ADP-ribosylation factor 1	08661	2	2,259,733
ARHGEF9	Unlinked	Rho guanine nucleotide exchange factor 9	12303	4	11,866,519
ARHL2	Unlinked	Poly(ADP-ribose) glycohydrolase ARH3	03624	23	4,457,042
ARP6	*Fal3*	Actin-related protein 6	18851	1	49,106,063
ASB6	*Fal25*	Ankyrin repeat and SOCS box-containing 6	06983	17	6,201,193
ATG4B	*Fal14*	Cysteine protease ATG4B	10179	9	5,828,498
ATP6AP2	*Fal4*	ATPase, H+ transporting, lysosomal accessory protein 2	26187	1	115,861,428
ATP6V1E1	Unlinked	Vacuolar H+ ATPase E1	21281	1	63,935,883
BZW1	*Fal7*	Basic leucine zipper and W2 domain-containing protein 1	13380	7	12,346,390
C12orf29	*Fal3*	Hypothetical protein	18208	1	44,668,513
C7orf27	*Fal32*	HEAT repeat domain-containing protein C7orf27 precursor	06938	14	3,350,565
C8orf53	*Fal1*	Uncharacterized protein C8orf53	25969	2	140,964,418
CACYBP	*Fal19*	Calcyclin-binding protein	07248	8	7,413,974

(*continued*)

TABLE 1

(Continued)

Marker	Linkage group	Gene description[a]	Ensembl ID[b]	Chicken chromosome	Chicken genome start position (bp)
CATB	*Fal2*	Cathepsin B precursor	26896	3	110,173,920
CBPZ	*Fal5*	Carboxypeptidase Z precursor	25149	4	84,149,787
CCDC104	Unlinked	Coiled-coil domain-containing protein 104	13093	3	129,733
CCDC132	*Fal1*	Coiled-coil domain containing 132	15463	2	22,899,725
CCDC137	Unlinked	MGC16597 protein	07177	18	9,164,974
CCNG1	*Fal12*	Cyclin-G_1	02636	13	6,483,608
CCT2	*Fal3*	Chaperonin-containing TCP1, subunit 2	16215	1	37,378,125
CDH9	*Fal1*	Cadherin-9 precursor	21079	2	72,557,369
CEPU1[c]	*Fal24*	Protein CEPU-1 precursor	29072	24	1,827,157
CGI-62	*Fal1*	UPF0418 protein C8orf70	25374	2	125,087,485
CHC[c]	*Fal13*	Clathrin heavy chain	39267	19	7,239,507
CHD1L	*Fal8*	Chromodomain helicase DNA-binding protein 1-like	24254	1	83,862,026
CHM1B	*Fal18*	Charged multivesicular body protein 1b	06500	4	1,509,856
CHMP5	*Fal1*	Charged multivesicular body protein 5	21491	2	87,941,041
CNTN1	*Fal3*	Contactin-1 precursor	15506	1	30,637,524
COEA1	*Fal1*	Collagen α-1(XIV) chain precursor	26472	2	142,375,665
CRIPT	*Fal2*	Postsynaptic protein CRIPT	16264	3	27,956,254
CT030	Unlinked	UPF0414 transmembrane protein C20orf30	00227	22	346,000
DC1L1	*Fal1*	Cytoplasmic dynein 1 light intermediate chain 1	18728	2	40,471,155
DDAH1	*Fal11*	Dimethylarginine dimethylaminohydrolase 1	14108	8	16,977,584
DECR1	*Fal1*	2,4-dienoyl-CoA reductase, mitochondrial precursor	25647	2	129,135,915
DLD	*Fal3*	Dihydrolipoamide dehydrogenase	12884	1	15,844,353
DPYSL3	Unlinked	Dihydropyrimidinase-like 3	12260	13	18,592,762
DST	*Fal2*	Bullous pemphigoid antigen 1	26267	3	89,753,995
EDF1	Unlinked	Endothelial differentiation-related factor 1 homolog	14657	17	932,044
EF1A1	*Fal2*	Elongation factor 1-α 1	25653	3	84,252,820
EF1A	*Fal15*	Elongation factor 1-α	09385	20	8,992,662
EIF3S1	Unlinked	Eukaryotic translation initiation factor 3, subunit 1-α	13336	10	21,935,894
ENO1[c]	*Fal20*	α-Enolase	03745	21	3,197,152
ETNK1	*Fal3*	Ethanolamine kinase 1	21571	1	68,619,958
FAK1	*Fal1*	Focal adhesion kinase 1	26060	2	151,344,071
FN[c]	*Fal7*	Fibronectin	05663	7	4,362,118
FTH[c]	*Fal6*	Ferritin H-subunit	11687	5	8,042,629
GAS7	Unlinked	Growth-arrest-specific protein 7	00500	18	181,941
GH1[c]	*Fal8*	Growth hormone factor 1	24989	1	96,197,113
GNB1	*Fal20*	Guanine nucleotide-binding protein (G protein) β polypeptide 1	02040	21	1,907,993
GTF2B	*Fal11*	Transcription initiation factor IIB	10015	8	15,855,345
HARS	*Fal12*	Histidyl-tRNA synthetase	01152	13	825,160
HEPACAM	*Fal24*	Hepatocyte cell adhesion molecule	00574	24	244,611
HMGB2	*Fal5*	High-mobility group protein B2	17483	4	44,739,576
HMGN2	*Fal29*	Nonhistone chromosomal protein HMG-17	00504	23	132,497
IGF2R	*Fal2*	Insulin-like growth factor 2 receptor	18986	3	47,356,791
IGFBP7	*Fal5*	Insulin-like growth factor-binding protein 7 precursor	18503	4	50,637,829
KCNIP4	Unlinked	Kv channel-interacting protein 4	23272	4	77,264,411
KCRB[c]	*Fal6*	Creatine kinase B-type	18765	5	52,833,368
KIAA1706	*Fal1*	CDNA FLJ14480 fis, clone MAMMA1002215	19789	2	46,847,660
LARP1	*Fal12*	La-related protein 1	06374	13	12,129,582
LDHA	*Fal6*	L-lactate dehydrogenase A chain	10181	5	13,644,404
LHCGR[c]	*Fal22*	Luteinizing hormone/choriogonadotropin receptor	14806	3	7,517,756
MAGOH	*Fal11*	Mago-nashi homolog, proliferation-associated	17388	8	25,398,748
MBP	*Fal1*	Myelin basic protein	22187	2	92,901,749
METRNL	Unlinked	Meteorin-like protein precursor	02154	18	3,058,036
MIC1	*Fal1*	Colon cancer-associated protein Mic1	24206	2	106,186,277
MITD1	*Fal4*	MIT domain-containing protein 1	27060	1	136,635,855

(*continued*)

TABLE 1

(Continued)

Marker	Linkage group	Gene description[a]	Ensembl ID[b]	Chicken chromosome	Chicken genome start position (bp)
MMAA	*Fal5*	Methylmalonic aciduria type A protein, mitochondrial precursor	16214	4	32,303,140
MOSPD2	*Fal4*	Motile sperm domain-containing protein 2	26743	1	125,737,284
MPP1[c]	*Fal18*	Myelin proteolipid protein	NA	NA	NA
MPP6	*Fal1*	MAGUK p55 subfamily member 6	17898	2	31,506,073
MRPS18A	*Fal2*	28S ribosomal protein S18a, mitochondrial precursor	16751	3	32,021,644
NAT5	*Fal33*	*N*-acetyltransferase 5	20554	7	38,349,143
NDST3	*Fal12*	Bifunctional heparan sulfate *N*-deacetylase/*N*-sulfotransferase 3	19599	4	56,617,452
NDUFA7	*Fal33*	NADH dehydrogenase 1 α subcomplex subunit 7	00895	28	871,010
NSMAF	*Fal1*	Protein FAN (factor associated with N-SMase activation)	24908	2	115,869,042
NY-SAR-48	*Fal26*	Sarcoma antigen NY-SAR-48 isoform a	05915	28	3,757,065
OAZ	*Fal26*	Ornithine decarboxylase antizyme	01183	28	1,446,253
ODC11[c]	*Fal2*	Ornithine decarboxylase	26527	3	99,660,031
PARK7	*Fal20*	Protein DJ-1 Parkinson disease protein 7 homolog	00742	21	235,467
PDCD11	*Fal10*	RRP5 protein homolog programmed cell death protein 11	13446	6	25,047,124
PDHL1	*Fal4*	Phosphoglycerate dehydrogenase-like 1	27270	1	148,754,342
PES1	*Fal9*	Pescadillo homolog 1	12619	15	11,118,356
PKHB2	*Fal14*	Pleckstrin homology domain-containing family B member 2	03399	9	3,184,527
PNN	*Fal6*	Pinin	16532	5	40,003,168
POLR2C	*Fal16*	DNA-directed RNA polymerase II subunit RPB3	01768	11	552,555
POLR2H	*Fal14*	DNA-directed RNA polymerases I, II, and III subunit RPABC3	13907	9	17,035,879
PPIL4	*Fal2*	Peptidyl-prolyl *cis-trans* isomerase-like 4	20195	3	50,008,973
PRS4	*Fal6*	26S protease regulatory subunit 4	17367	5	46,255,522
PSMB1	*Fal2*	Proteasome (prosome, macropain) subunit, β-type, 1	18217	3	42,604,002
PSMC2	*Fal3*	Proteasome (prosome, macropain) 26S subunit, ATPase, 2	13403	1	13,964,547
PSMC3	*Fal6*	Proteasome 26S ATPase subunit 3	13163	5	25,021,822
PSMC5	*Fal17*	26S protease regulatory subunit 8	00469	27	1,607,679
PSMD14	*Fal7*	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	18142	7	22,963,970
PSMD6	*Fal21*	26S proteasome non-ATPase regulatory subunit 6	11836	12	13,910,990
PTMS	*Fal8*	Parathymosin	23363	1	80,288,088
RAB12	*Fal1*	*Gallus gallus* similar to Rab12 protein	22528	2	101,592,940
RAB3GAP1	*Fal7*	Rab3 GTPase-activating protein catalytic subunit	19948	7	32,114,339
RABL4	*Fal3*	Putative GTP-binding protein RAY-like	20454	1	53,623,713
RASGEF1A	*Fal10*	RasGEF domain family, member 1A	03978	6	5,681,912
RBBP7	*Fal4*	Histone-binding protein RBBP7	26698	1	124,845,745
RBM18	Unlinked	Probable RNA-binding protein 18	02045	17	9,445,180
RBM26	*Fal4*	RNA-binding protein 26	27331	1	157,186,094
RHO[c]	*Fal31*	Rhodopsin (opsin-2)	33236	12	20,163,795
RL13	*Fal30*	60S ribosomal protein L13	09974	11	20,692,991
RNASEH1	*Fal2*	Ribonuclease H1	26438	3	96,571,133
ROBO1	*Fal9*	Roundabout1 protein	25008	1	99,689,366
RPL5[c]	*Fal11*	60S ribosomal protein L5	09525	8	14,745,903
RPL7A[c]	*Fal25*	60S ribosomal protein L7a	21881	17	7,532,190
RPL11	*Fal29*	Ribosomal protein L11	06305	23	5,837,007
RPL23	*Fal17*	60S ribosomal protein L23	02496	27	3,957,106
RPL30[c]	*Fal1*	60S ribosomal protein L30	36621	2	132,392,442
RPL37A[c]	*Fal7*	60S ribosomal protein L37a	18702	7	24,873,612
SBDS	*Fal13*	Shwachman–Bodian–Diamond syndrome	01658	19	782,450
SERINC1	*Fal2*	Tumor differentially expressed 2	23989	3	63,939,040
SPCS1	*Fal21*	Signal peptidase complex subunit 1	02526	12	749,082
STYX	Unlinked	Serine/threonine/tyrosine-interacting protein	20280	5	60,813,050
TGFB2[c]	*Fal2*	Transforming growth factor β-2 precursor	15664	3	20,477,096
THUMPD3	*Fal31*	THUMP domain-containing protein 3	13674	12	20,067,036
TIMM17A	*Fal27*	Translocase of inner mitochondrial membrane 17 homolog A	00123	26	1,096,567
TM[c]	*Fal23*	Tropomyosin α-1 chain	05572	10	5,107,950

(*continued*)

TABLE 1

(Continued)

Marker	Linkage group	Gene description[a]	Ensembl ID[b]	Chicken chromosome	Chicken genome start position (bp)
TMEM32	*Fal18*	Transmembrane protein 32 precursor	09949	4	4,186,140
TUBGCP3	*Fal4*	Gamma-tubulin complex component 3	27189	1	142,174,289
TXNDC14	Unlinked	Thioredoxin domain containing 14	11887	5	18,159,850
UBE2J1	*Fal2*	Ubiquitin-conjugating enzyme E2, J1	25442	3	78,396,237
UCHL3	*Fal4*	Ubiquitin carboxyl-terminal esterase L3	27356	1	159,089,890
UCHL5	*Fal11*	Ubiquitin carboxyl-terminal hydrolase L5	03977	8	3,533,310
UQCRC1	*Fal21*	Ubiquinol-cytochrome-c reductase complex core protein 1	09300	12	9,301,215
VIPR2	*Fal1*	Vasoactive intestinal peptide receptor 2	10623	2	9,569,988
VISL1	Unlinked	Visinin-like protein 1	26565	3	102,957,139
VPS26A	Unlinked	Vacuolar protein sorting-associated protein 26A	06635	6	11,919,165
WDR24	*Fal28*	WD repeat protein 24	03862	14	13,908,481
YME1L1	*Fal1*	YME1-like 1	12112	2	15,827,680
YPEL5	Unlinked	Yippee-like 5	14726	3	8,031,083
		Microsatellites			
EST9	*Fal10*			6	24,700,943
EST10	*Fal10*			6	24,725,801
EST16	*Fal1*	Adenylate cyclase-activating polypeptide 1		2	104,980,406
EST31	*Fal19*	Peroxiredoxin-6		8	4,336,642
EST46	*Fal30*	α-Fetoprotein enhancer-binding protein		11	21,546,200
Fhy215	*Fal1*				
Fhy216	*Fal3*				
Fhy217	*Fal3*				
Fhy220	*Fal4*				
Fhy221	*Fal16*				
Fhy223	*Fal4*	NW_001471545.1 Gga1 WGA43_2		1	140,906,192
Fhy224	*Fal9*	NW_001471459.1 Gga15 WGA207_2		15	1,723,096
Fhy225	*Fal2*				
Fhy226	*Fal2*				
Fhy227	*Fal8*				
Fhy228	*Fal22*				
Fhy230	*Fal1*	NW_001471633.1 Gga2 WGA60_2		2	49,531,823
Fhy231	Unlinked				
Fhy234	*Fal1*				
Fhy235	*Fal22*	NW_001471676.1 Gga3 WGA95_2		3	5,902,333
Fhy236	*Fal15*	NW_001471568.1 Gga20 WGA258_2		20	12,551,523
Fhy237	*Fal6*				
Fhy301	*Fal16*	NW_001471434.1 Gga11 WGA182_2		11	4,504,384
Fhy304	*Fal5*				
Fhy306	*Fal5*				
Fhy310	*Fal17*				
Fhy321	*Fal10*				
Fhy326	*Fal6*	NW_001471698.1 Gga5 WGA124_2		5	16,318,616
Fhy328	*Fal5*				
Fhy329	*Fal2*	NW_001471669.1 Gga3 WGA102_2		3	48,212,536
Fhy336	*Fal1*				
Fhy339	*Fal8*				
Fhy341	*Fal3*				
Fhy342	Unlinked				
Fhy344	*Fal5*	NW_001471687.1 Gga4 WGA113_2		4	84,661,433
Fhy350	*Fal9*	NW_001471461.1 Gga15 WGA209_2		15	9,498,662
Fhy356	*Fal3*	NW_001471552.1 Gga1 WGA4_2		1	7,812,491
Fhy361	*Fal1*	NW_001471633 Gga2 WGA60_2		2	45,075,848
Fhy370	*Fal8*	NW_001471529.1 Gga1 WGA29_2		1	92,668,432
Fhy401	*Fal5*				

(*continued*)

TABLE 1

(Continued)

Marker	Linkage group	Gene description[a]	Ensembl ID[b]	Chicken chromosome	Chicken genome start position (bp)
Fhy403	*Fal1*				
Fhy404	Unlinked				
Fhy405	*Fal9*				
Fhy407	*Fal4*	NW_001471554.1 Gga1 WGA51_2		1	177,252,431
Fhy408	*Fal3*				
Fhy413	*Fal1*				
Fhy415	*Fal1*	NW_001471639.1 Gga2 WGA66_2		2	79,942,990
Fhy427	*Fal3*	NW_001471510.1 Gga1 WGA11_2		1	21,414,296
Fhy428	*Fal1*				
Fhy429	Unlinked				
Fhy431	*Fal10*				
Fhy432	Unlinked				
Fhy444	*Fal4*				
Fhy448	*Fal2*				
Fhy450	*Fal7*				
Fhy452	*Fal4*				
Fhy453	*Fal10*	NW_001471713.1 Gga6 WGA139_2		6	3,534,436
Fhy454	Unlinked				
Fhy458	*Fal1*	NW_001471651.1 Gga2 WGA78_2		2	129,424,241
Fhy464	*Fal2*				
Fhy465	*Fal4*	NW_001471554.1 Gga1 WGA51_2		1	176,719,296
Fhy466	*Fal7*	NW_001471729.1 Gga7 WGA155_2		7	13,437,385
Fhy467	*Fal1*				
FhU3	*Fal1*				
FhU4	*Fal3*				
FhU5	*Fal1*				
GG-C25	*Fal6*			5	31,720,224
SS12	*Fal3*				
ZF-C59	*Fal1*	Adenylate cyclase-activating polypeptide 1		2	104,980,320
ZF-S8	Unlinked	Hypothetical protein		18	4,041,446
ZF-S9	*Fal12*			13	8,811,918

[a] The contig number from the chicken genome sequence build 2.1 is described for microsatellites.
[b] ENSEMBL ID for the orthologous chicken gene (ENSGALG000000xxxxx).
[c] Loci from Borge *et al.* (2005).

trarily set at $<E^{-5}$) hits, although the relaxed settings generated somewhat lower *E*-values. Because of this procedure 20 of the microsatellites could be anchored to a single location in the chicken genome and therefore be used for comparative studies. Chicken genomic locations were taken from Build 2.1 of the chicken genome, obtained from http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?taxid=9031. Data on chicken recombination rates were from the same source. The particular markers that we used for collared flycatcher mapping were not always included in the chicken genetic map. To obtain recombination rate estimates for orthologous regions of the collared flycatcher and chicken genomes, we therefore used data from genetic markers located preferably within 1 Mb of our markers in the chicken genome. Graphical presentations of genetic maps were created in MapChart (Voorrips 2002).

RESULTS

Marker analysis: We genotyped 384 previously identified SNPs in a pedigree of 322 collared flycatchers. After excluding SNPs found to be monomorphic in the pedigree (36 sites) or with a sample call rate <60% (23), segregation data for 321 SNPs from 170 genes became available. These genes are from 26 of the 28 chromosomes contained within the most recent (May 2006) version of the chicken genome assembly; it was not possible to develop conserved markers for *Gga16* and *Gga25* due to a lack of assigned genes to these small microchromosomes. SNPs from the same intron were combined into haplotypes to increase the informativeness of each gene in pedigree analysis. In addition, 71 polymorphic microsatellites were scored in the pedigree. We thereby had a total of 241 loci ("markers") available for mapping. The average number of informative meioses for SNP haplotypes and microsatellites was 184 and 240, respectively (supplemental Table 2).

Linkage mapping: Linkage analysis detected significant two-point linkage (lod score >3.0) to at least one

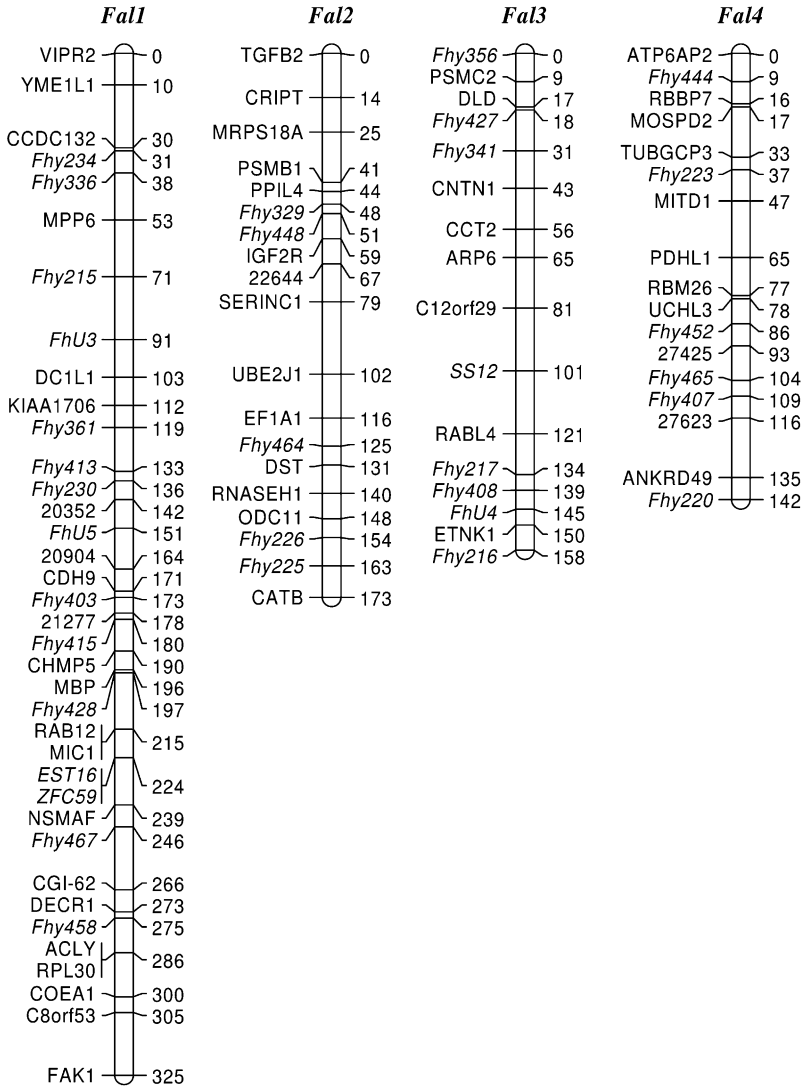

Figure 1.—A genetic linkage map of the collared flycatcher genome. Cumulative genetic distances in centimorgans are given to the right of each chromosome. Gene markers (genotyped by SNPs) are in regular text while anonymous microsatellites are in italics.

other marker for 211 of the 241 loci. These 211 markers form a best-order genetic map consisting of 33 linkage groups (Figure 1), ranging in size from a genetic length of 325 cM (with 37 markers) to 0 cM (two groups with two markers showing no recombination). We refer to these linkage groups as *Fal1-33* (*F. albicollis* linkage groups 1–33). The mean genetic distance between adjacent markers in the map is 10.0 cM (±6.7 SD). A framework map based on markers showing a multi-point lod score >3 is presented in supplemental Figure 1; generally, the framework map differs from the best-order map only by the inability to confidently place some markers on either side of adjacent loci.

The total sex-average length of the best-order map is 1787 cM, with significantly more recombination in males ($P = 0.01$, Wilcoxon's test for paired data). Overall, recombination is 22% higher in males than in females (total distance 1982 *vs.* 1627 cM), although for individual intervals the female point estimate is sometimes higher than the male estimate. Sex-specific linkage maps are presented in supplemental Figure 2.

Thirty markers (22 genes and eight microsatellites) remain unlinked. It is clear that this is at least in part due to a lack of power to detect linkage, since unlinked markers are highly overrepresented among markers with a low number of informative meioses (supplemental Table 2). However, nine unlinked genic SNP haplotypes and six unlinked microsatellites appear as singletons despite >70 informative meioses. Because the microsatellites were isolated by random library screening (Leder *et al.* 2008), it is possible that they correspond to regions of the chicken genome, which is not yet contained within the chicken genome assembly. For the gene-based SNPs, a possible explanation of the absence of linkage is that they are located in regions of high recombination within conserved linkage groups. Given the observation that recombination rates tend to be elevated toward telomeric ends of avian chromosomes (International Chicken Genome Sequencing Consortium 2004; Schmid *et al.* 2005; Wahlberg *et al.* 2007), this explanation is supported by the fact that 6 of 9 unlinked genes are the most distal marker on

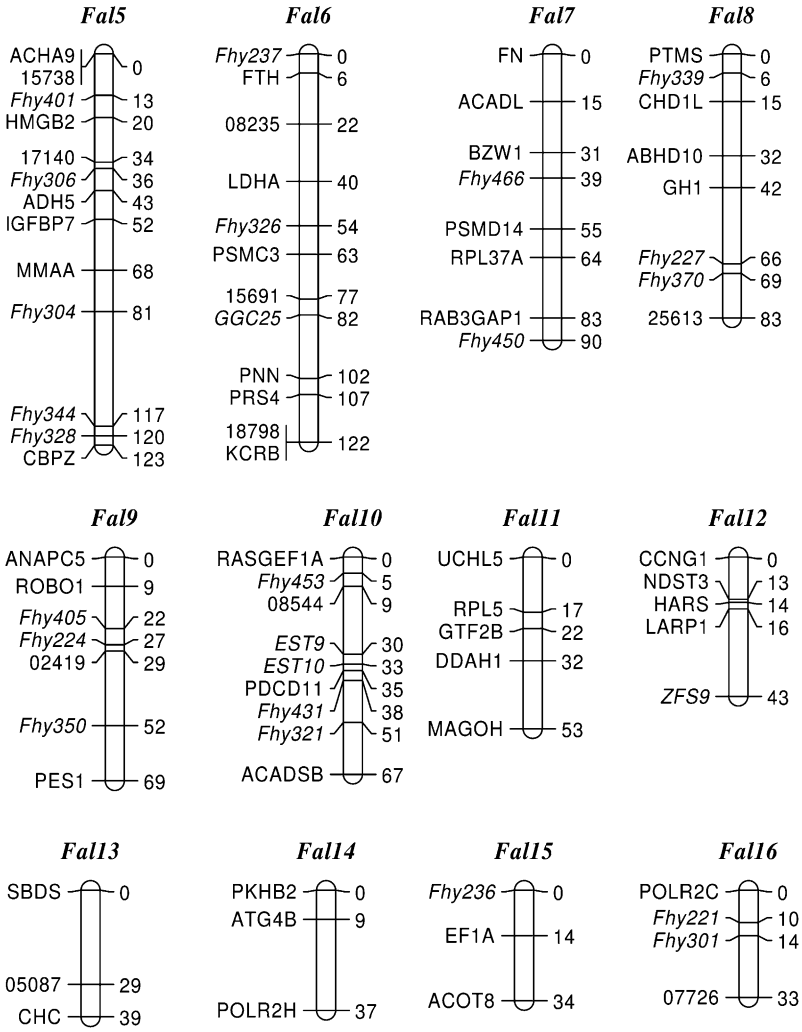

FIGURE 1.—*Continued.*

chicken chromosomes in our marker set (at 2p, 3p, 4q, 5q, 10p, and 10q, respectively). Alternatively, they may indicate chromosomal rearrangements (see supplemental data).

Comparative mapping: A major advantage of this map is that genic markers allow the identification of homologous regions in the genomes of other species; this is particularly important for the ability to transfer genomic information from model organisms to nonmodel ones. The 33 collared flycatcher linkage groups correspond to 24 different chromosomes in chicken, with the 2 remaining chicken chromosomes from which we had markers (*Gga18* and *Gga22*) each being represented by two to three unlinked singletons. By comparing the location of gene sequences in the chicken and collared flycatcher genomes (as well as of 20 flycatcher microsatellites for which a homologous sequence could be identified in chicken), we find a remarkable degree of chromosomal conservation, both at the level of shared synteny and at the level of conserved gene order (Figure 2). No fewer than 18 chicken chromosomes correspond to a single linkage group in the collared flycatcher, indicating completely conserved synteny.

Seven chicken chromosomes are orthologous to two to three collared flycatcher linkage groups, suggestive of fusion/fission events. However, as discussed in detail in the supplemental data, several of these cases are likely to represent a lack of power to connect linkage groups that originate from the same collared flycatcher chromosome. The interval between SNP-based gene markers is generally <10 Mb in the chicken genome. As recombination rates are known to vary across the genome, particularly due to the presence of recombination hot spots, physical intervals of this size may correspond to genetic distances longer than that possible for detecting linkage. Of course, length expansions in the collared flycatcher genome would accentuate such problems. In the end, only two rearrangements that distinguish the karyotypes of the chicken and the collared flycatcher are strongly supported by our data (supplemental data) and are also independently confirmed in other species. These include a fission of the ancestral chromosome 1 in Passeriformes to yield the collared flycatcher linkage groups *Fal3* and *Fal8* (supported by data in GUTTENBACH *et al.* 2003; DERJUSHEVA *et al.* 2004; ITOH and ARNOLD 2005) and the fusion of ancestral

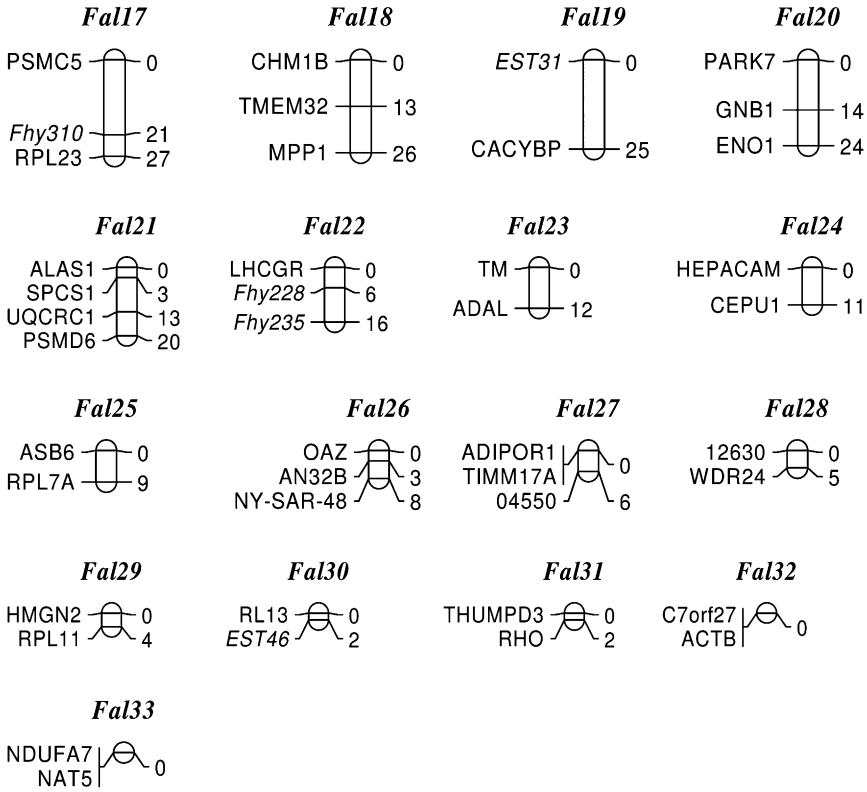

FIGURE 1.—*Continued.*

chromosomes 4 (corresponding to *Fal5*) and 10 (*Fal18*) in the chicken lineage to yield *Gga4* (supported by REED *et al.* 2005). One or a few more rearrangements could be indicated by our data but would need a denser map for confirmation.

Generally, gene order within syntenic groups is completely conserved in the chicken–collared flycatcher comparison. There are 18 cases of intrachromosomal rearrangements in the best-order map. However, only 7 of these remain in the framework map [corresponding to *Gga1-Fal4* (two cases), *Gga1-Fal8*, *Gga3-Fal2*, *Gga4-Fal5*, *Gga15-Fal9*, and *Gga20-Fal15*; supplemental Figure 1]; the other 11 cases are in the form of inversed order of markers that is not statistically supported (multi-point lod score <3). Six of the 7 well-supported cases are consistent with rather short inversions of ~1.5–6 Mb according to the chicken genome sequence, while the seventh represents a large proportion of *Gga4-Fal5* that spans at least 38 Mb.

DISCUSSION

This work reveals an evolutionary stasis of gene order and chromosome organization in a comparison of two highly diverged avian lineages, estimated to have separated 100 million years ago (MYA) ago. Our results confirm the stability of avian genomes previously indicated by results from comparative mapping in chicken and mammals (BURT *et al.* 1999; BOURQUE *et al.* 2005) and from cytogenetic (*e.g.*, GRIFFIN *et al.* 2007) and genetic (*e.g.*, DAWSON *et al.* 2007) analyses within birds. However, as previous work has been largely insensitive to intrachromosomal structures, and to fine-scale interchromosomal organization, our findings extend the observation of the genomic stability of birds to be valid also at the level of gene order and organization and across distantly related taxa.

Stasis of avian genome organization: The karyotype of the collared flycatcher has not been characterized, but the great majority of passerine birds so far analyzed have a chromosome number between 78 and 80, *i.e.*, quite similar to the $2n = 78$ of chicken (GREGORY *et al.* 2006). The structure of the karyotype is also well conserved with a few large macrochromosomes and a large number of very small microchromosomes. Moreover, available data from measurements of DNA content indicate that genome size is more or less identical in chicken and birds from the order Passeriformes, at just ≈1 Gb (GREGORY *et al.* 2006). Assembled genome sequence has been assigned to 28 of the 36 chicken autosomes (*Gga*29-36 are not covered in the assembly and represent microchromosomes with <5–10 Mb DNA). Most of these chromosomes correspond to a single linkage group in the collared flycatcher map. We find strong support for two interchromosomal rearrangements that have occurred since the divergence of the lineages leading to the chicken and the collared flycatcher, rearrangements which are also supported by previous observations (INTERNATIONAL CHICKEN GENOME SEQUENCING CONSORTIUM 2004; GRIFFIN *et al.* 2007).

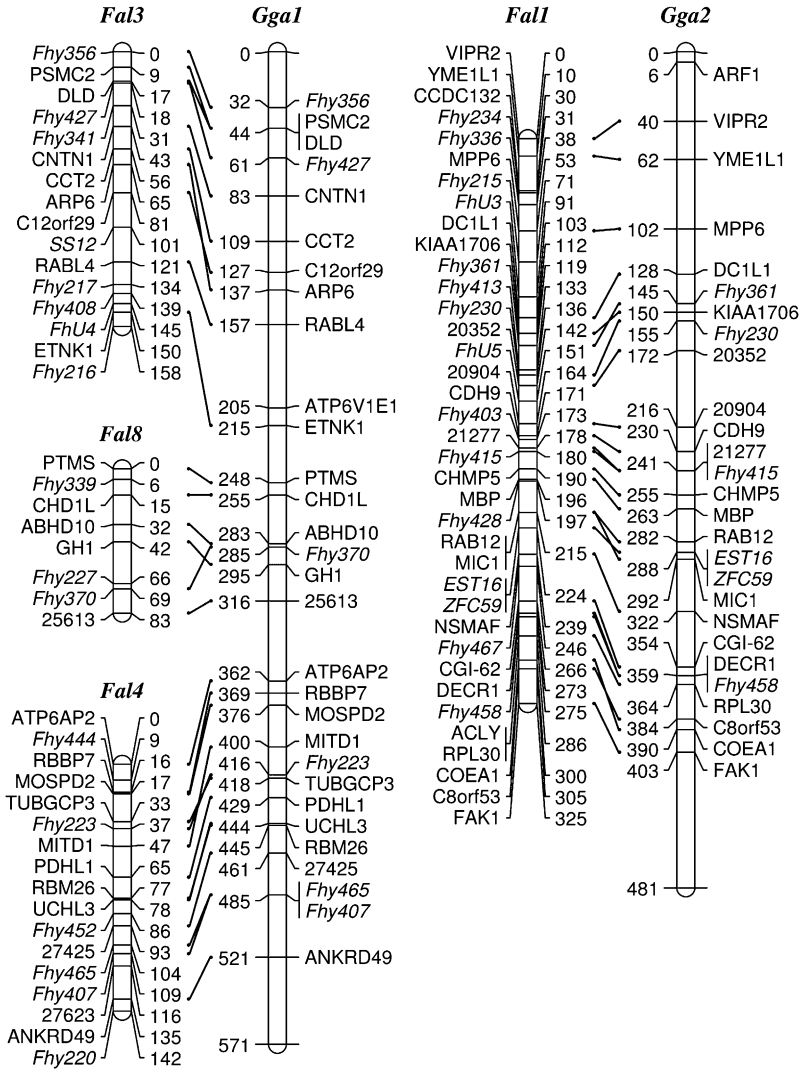

FIGURE 2.—Genome conservation revealed by the alignment of orthologous collared flycatcher linkage groups and chicken chromosomes. Chromosomes/linkage groups are drawn proportional to their genetic length (the same for both species) with the full length of chicken chromosomes displayed.

There are other gaps in the collared flycatcher linkage map aligned to chicken that might indicate the presence of one to three additional fissions or fusions (corresponding to *Gga1*, *Gga3*, and *Gga12*). However, it is possible that we lack the power to detect linkage in these cases because markers flanking the gaps might be too far apart, contain too little information, or are separated by a region of high recombination rate (see supplemental data).

There is also evidence for at least seven intrachromosomal rearrangements in the chicken–collared flycatcher comparison. Four of these have occurred in the vicinity of the sites for interchromosomal rearrangement, indicating that there are regions of avian chromosomes that are particularly fragile and prone to different types of rearrangement. The number of observed intrachromosomal rearrangements in the collared flycatcher–chicken comparison is roughly two to three times the number of interchromosomal rearrangements. A similar ratio is also found in mammals (PEVZNER and TESLER 2003; PONTIUS *et al.* 2007). This indicates that it is not just the inter- or the intrachromosomal rate of rearrangement that is low in birds; rather, it is the overall rate of rearrangements that is uniformly low.

The incidence of inter- and intrachromosomal rearrangements seen in this study can be used to roughly estimate the minimum rate of chromosomal rearrangement in birds. We have to take into account that our map does not cover the entire genome and that the resolution may be insufficient for detecting small rearrangements within mapped regions. With this caveat in mind and assuming a 66% genomic coverage of the map (see below) and 100 MY since the most recent common ancestor of Galliformes and Passeriformes (200 MY of evolution in total), there is an overall minimum rate of chromosomal exchange during avian evolution of approximately one event/15 ($200/9 \times 0.66$) MY (0.045/MY). Separate estimates for inter- and intrachromosomal rearrangement give one fission/fusion event/66 MY (0.015/MY) and one large-scale inversion event/19 MY (0.05/MY). This is considerably lower than what has been estimated for mammalian lineages (≈0.6–2.0; PONTIUS *et al.* 2007) and gives a quantitative idea of the relative stability of avian genomes.

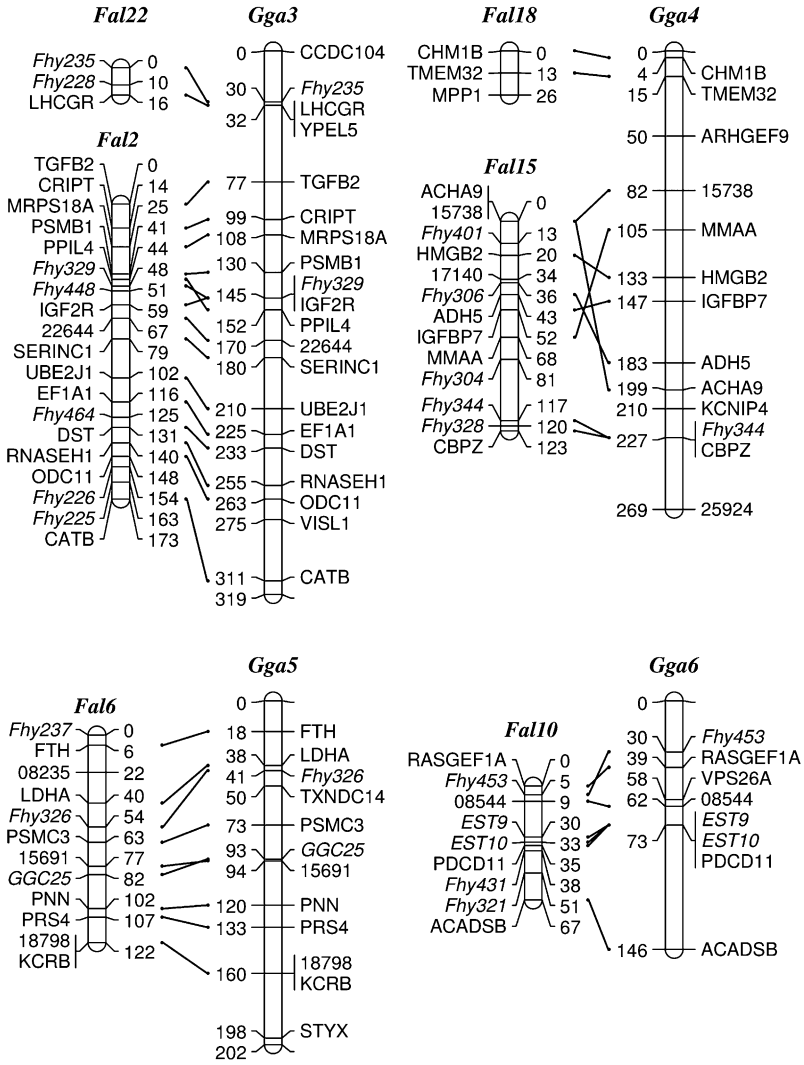

FIGURE 2.—*Continued.*

The genomic integrity shown by the comparison of two distantly related avian lineages provides insight into vertebrate karyotype evolution. It has been shown that the rate of chromosomal diversification varies considerably on a temporal scale (FERGUSON-SMITH and TRIFONOV 2007). For example, while the rate of chromosomal diversification is thought to have been comparatively high in the lineage leading from an early vertebrate ancestor to the eutherian ancestor, by contrast it was considered to be low in the lineage from the eutherian ancestor to the primate ancestor (and continued to be so in the human lineage) (KOHN *et al.* 2006). However, no such temporal heterogeneity is indicated in either the chicken or the collared flycatcher lineage. In fact, the ancestral vertebrate karyotype is highly conserved in the chicken so genomic stability must have been prevalent all along the lineage leading to modern birds since the split of synapsids and diapsids 310 MYA. The only clear exception is provided by birds of prey that have an atypical bird karyotype that lacks distinct macrochromosomes and microchromosomes (DEOLIVEIRA *et al.* 2005).

Why does the degree of genomic integrity differ extensively between birds and mammals? One possible explanation relates to the role of repeat elements in governing chromosome fragility and the dearth of active families of interspersed repeat elements in avian genomes (INTERNATIONAL CHICKEN GENOME SEQUENCING CONSORTIUM 2004). Breakpoints for chromosome rearrangements tend to be enriched with interspersed repetitive elements, low-copy repeats, and segmental duplications (BAILEY *et al.* 2004; FREUDENREICH 2007; KEHRER-SAWATZKI and COOPER 2007), including in chicken (GORDON *et al.* 2007). Mechanistically, unequal crossing over between repeated sequences within or between chromosomes is likely to contribute to this pattern (LUPSKI and STANKIEWICZ 2005). The bird genome has remained compact and repeat poor during avian evolution (ORGAN *et al.* 2007); there is only one abundant class of interspersed repeats in the chicken genome: the long interspersed *CR1* element, with a vanishingly small proportion representing anything other than short and heavily truncated copies (INTERNATIONAL CHICKEN GENOME SEQUENCING CONSORTIUM

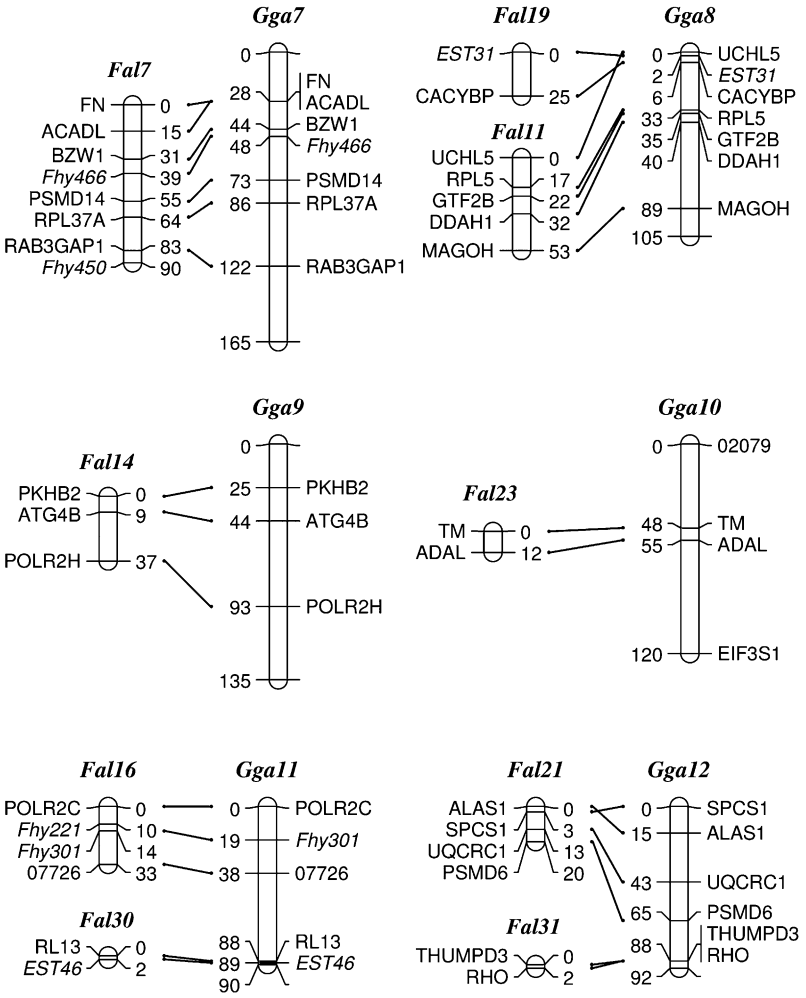

FIGURE 2.—*Continued.*

2004). In light of the potential link between a low-repeat content and chromosomal integrity, it is tempting to invoke a nonadaptive explanation to genomic stability of birds.

One interesting aspect of genomic stability of birds is the role of chromosomal rearrangements in speciation (NOOR *et al.* 2001; RIESEBERG 2001; NAVARRO and BARTON 2003). It is known that crosses between chromosomal variants can result in hybrid inviability or impaired fitness (CAPANNA and CASTIGLIA 2004). Speciation rates differ considerably between birds and mammals with the evolution of post-zygotic incompatibility being ~10 times slower in birds than in mammals (PRICE and BOUVIER 2002; FITZPATRICK 2004). It is possible that the formation of post-zygotic barriers in birds is delayed due to the slow rate of chromosomal rearrangement.

A partial genetic map, based mainly on microsatellites and AFLP markers, has been developed for another passerine bird, the great reed warbler (HANSSON *et al.* 2005; ÅKESSON *et al.* 2007; DAWSON *et al.* 2007). The seven collard flycatcher–chicken gene-order differences that we detected in this study are not evident in the comparison between the great reed warbler and the chicken (DAWSON *et al.* 2007). This could suggest that these inversions arose in the collared flycatcher lineage subsequent to the split of Muscicapoidea–Sylvoidea. However, the physical marker density and chromosomal coverage in the great reed warbler map is relatively low, especially for markers informative in comparative mapping, so it may be that the resolution is not sufficient for detection of some intrachromosomal rearrangements. Neither of these inversions is seen in the fairly dense map of the turkey genome (REED *et al.* 2005) so they may have arisen either early in the evolution of Galliformes or somewhere along the passerine lineage leading to the collared flycatcher. The great reed warbler map revealed two clear cases of inversions when compared to chicken chromosomes 1 and 2, respectively (DAWSON *et al.* 2007). However, they are not seen in the collared flycatcher–chicken comparison despite dense marker coverage in these regions. They are therefore likely to have arisen in the lineage leading to the great reed warbler subsequent to the split of Muscicapoidea–Sylvoidea.

Sex-specific recombination rates: There is evidence for heterochiasmy in collared flycatchers and the direction (22% more recombination in males) is in agreement with the Haldane–Huxley rule, which states that,

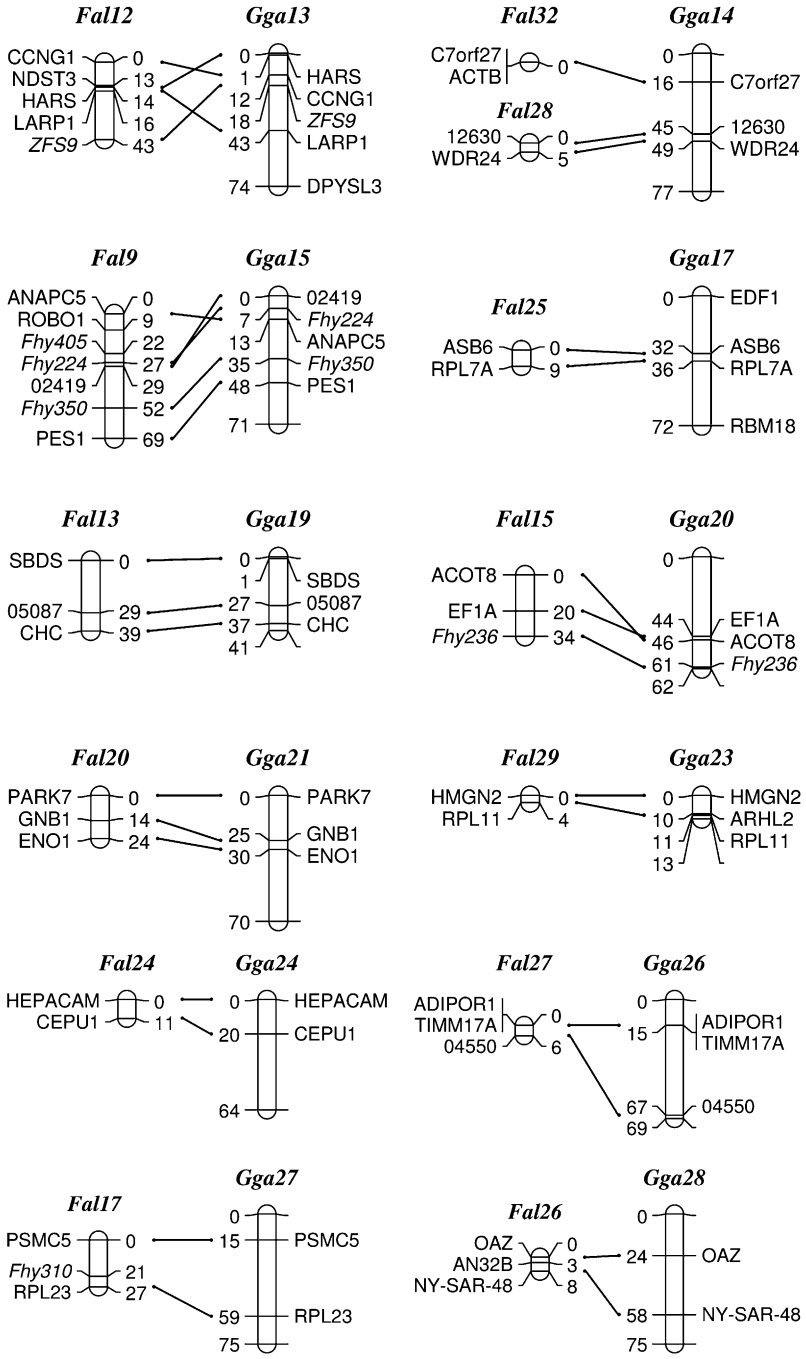

Figure 2.—*Continued.*

in species where the autosomal recombination rate differs quantitatively between sexes, it is usually the heterogametic sex that has a reduced recombination rate. However, as in several galliform birds (Groenen *et al.* 2000; Kayang *et al.* 2004; Reed *et al.* 2005), the difference is not as pronounced as that seen in most mammals [in humans, for example, the female rate is ~1.6 times higher than the male rate (Broman *et al.* 1998)]. The great reed warbler shows a quite contrasting pattern, with a more than twofold excess of recombination in females (Hansson *et al.* 2005; Dawson *et al.* 2007), *i.e.*, against the expectations of the Haldane–Huxley rule. This difference between two passerine birds is unexpected and not easily conceived in light of alternative hypotheses for the evolution of heterochiasmy (Lenormand 2003). For example, there is some support for the idea that haploid selection plays a role (Lenormand and Dutheil 2005). According to this model, and assuming epistasis, the sex experiencing the largest variance in reproductive success should recombine less to keep favorable gene combinations together. However, both collared flycatchers (Sheldon *et al.* 1997; Sheldon and Ellegren 1999) and great reed warblers (Hasselquist *et al.* 1998) are polygynous species and there is no obvious reason to believe that the intensity of gametic selection would differ between

them. As it stands now, further work in additional bird species is needed to obtain a more broad-scale picture of avian recombination rates.

Genome coverage: How large a proportion of the collared flycatcher genome is covered by this map? One way to approach this question is to make use of physical information from the chicken genome. The amount of chicken sequence contained within linkage groups shared between the chicken and the collared flycatcher is 663 Mb, or 66% of the total ≈1-Gb autosomal genome (the sequence assigned to chromosomes in the chicken genome assembly covers ~90% of the genome). Obviously, additional sequence is covered in the flycatcher map if the 15 highly polymorphic unlinked singletons are included and when considering the fact that each end marker in all linkage groups covers some flanking distance. Assuming a similar genome size as in chicken (Gregory *et al.* 2006), it may be estimated that our map covers 75–80% of the collared flycatcher genome. It is more difficult to assess the proportion of the total genetic length of the flycatcher genome covered by the map. First, we probably lack markers from a number of microchromosomes to which assembled sequence has not yet been assigned in the chicken. However small a microchromosome is, it contributes 50 cM to the total map length since there is an obligate crossing over per chromosome (Jones and Franklin 2006). Second, recombination rates tend to increase toward telomeres, so it can therefore be difficult to assess the amount of recombination outside linkage groups by extrapolation. When it comes to genetic coverage, we thus conclude that it must be less than the physical coverage estimated above.

Genomics of natural populations: There is a slow but steady progress in applying genetic maps for quantitative trait locus (QTL) mapping in natural animal populations (Slate 2005), either directly in the wild or by using wild-caught individuals raised in the laboratory. Notable examples from ecologically important animal models include the analysis of the genetic basis of body armour in the stickleback *Gasterosteus aculeatus* (Peichel *et al.* 2001; Colosimo *et al.* 2005) and of body weight and morphology in the free-living Soay sheep *Ovis aries* (Beraldi *et al.* 2007) and the red deer *Cervus elaphus* (Slate *et al.* 2002). In the two latter systems, microsatellite markers developed in closely related domestic animals could be used across species. In sticklebacks, anonymous, species-specific microsatellites were used and random approaches for map development have also included the use of microsatellites, RAPD, or AFLP markers in a number of other species (Reid *et al.* 2007; Troggio *et al.* 2007).The development of the collared flycatcher map represents an application of a new approach to genetic mapping in natural populations. For several reasons, it benefited from an earlier systematic effort to develop a set of conserved reference markers evenly spread across the avian genome (Backström *et al.* 2008).

First, through identification of particularly conserved sites for primer design, these markers had a high amplification success rate in collared flycatchers. Second, the design of intronic amplicons 500–1000 bp in size uncovered >900 polymorphic sites with just a moderate amount of screening (10 unrelated individuals; Backström *et al.* 2008), from which we could choose the most informative polymorphisms for genotyping. SNP-based mapping is particularly attractive since large-scale analysis can be conducted using automatic procedures; this study generated 150,000 genotypes. Third, and importantly, since the reference set of markers was developed to represent a uniform coverage of all assembled chicken chromosomes, the markers also covered a significant part of the collared flycatcher genome. Clearly, using the same number of randomly selected markers would not have given the same degree of genome coverage. Indeed, the high degree of synteny and gene-order conservation in birds added to this benefit, although, in principle, the use of markers evenly distributed in a related species should always give better coverage than using random markers. Fourth, as the first map of a natural population based on protein-coding gene markers, it is straightforward to transfer genetic information from model species by means of anchoring of orthologous loci. These features should apply equally to any bird species and we therefore envision that the marker set will become important for future studies of the genetics of natural bird populations. The use of the same set of markers should also aid comparison of maps across many different species. More generally, the approach of designing evenly distributed, conserved gene markers from one or more model species could potentially be applied to many groups of organisms.

While SNPs offer a nearly inexhaustible source of genetic markers that are amenable to large-scale analysis, they come with the price of typically showing lower levels of polymorphism compared to microsatellites. For this reason, we also included a set of microsatellites in this study, as this was expected to facilitate anchoring of linkage groups. However, the difference in average information content between microsatellites and SNPs was not very large (mean number of informative meioses of 240 and 184, respectively). Moreover, we could not detect any significant difference in the percentage of unlinked markers from the different categories (8 of 71 for microsatellites *vs.* 22 of 170 for SNP haplotypes). This is likely due to the fact that for most genes we combined SNPs into haplotypes, thereby increasing their informativeness.

To conclude, we report here a gene-based genetic map of the collared flycatcher that, when compared to the chicken map, demonstrates extensive synteny and gene-order conservation during 100 MY of evolution. This high degree of genome stability of birds is likely to have important consequences for their general evolutionary patterns, including processes of speciation.

Financial support was obtained from the Swedish Research Council. SNP genotyping was performed at the SNP Technology Platform at Uppsala University, which is gratefully acknowledged.

LITERATURE CITED

Åkesson, M., B. Hansson, D. Hasselquist and S. Bensch, 2007 Linkage mapping of AFLP markers in a wild population of great reed warblers: importance of heterozygosity and number of genotyped individuals. Mol. Ecol. **16:** 2189–2202.

Backström, N., M. Brandstrom, L. Gustafsson, A. Qvarnstrom, H. Cheng *et al.*, 2006 Genetic mapping in a natural population of collared flycatchers (*Ficedula albicollis*): conserved synteny but gene order rearrangements on the avian Z chromosome. Genetics **174:** 377–386.

Backström, N., S. Fagerberg and H. Ellegren, 2008 Genomics of natural bird populations: a gene-based set of reference markers evenly spread across the avian genome. Mol. Ecol. **17:** 964–980.

Bailey, J. A., R. Baertsch, W. J. Kent, D. Haussler and E. E. Eichler, 2004 Hotspots of mammalian chromosomal evolution. Genome Biol. **5:** R23.

Beraldi, D., A. F. McRae, J. Gratten, J. Slate, P. M. Visscher *et al.*, 2007 Mapping quantitative trait loci underlying fitness-related traits in a free-living sheep population. Evolution **61:** 1403–1416.

Borge, T., M. T. Webster, G. Andersson and G. P. Saetre, 2005 Contrasting patterns of polymorphism and divergence on the Z chromosome and autosomes in two Ficedula flycatcher species. Genetics **171:** 1861–1873.

Bourque, G., E. M. Zdobnov, P. Bork, P. A. Pevzner and G. Tesler, 2005 Comparative architectures of mammalian and chicken genomes reveal highly variable rates of genomic rearrangements across different lineages. Genome Res. **15:** 98–110.

Broman, K. W., J. C. Murray, V. C Sheffield, R. L. White and J. L. Weber, 1998 Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am. J. Hum. Genet. **63:** 861–869.

Burt, D. W., C. Bruley, I. C. Dunn, C. T. Jones, A. Ramage *et al.*, 1999 The dynamics of chromosome evolution in birds and mammals. Nature **402:** 411–413.

Capanna, E., and R. Castiglia, 2004 Chromosomes and speciation in Mus musculus domesticus. Cytogenet. Genome Res. **105:** 375–384.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal, Jr., M. Dickson, *et al.*, 2005 Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. Science **307:** 1928–1933.

Dawson, D. A., T. Burke, B. Hansson, J. Pandhal, M. C. Hale *et al.*, 2006 A predicted microsatellite map of the passerine genome based on chicken-passerine sequence similarity. Mol. Ecol. **15:** 1299–1320.

Dawson, D. A., M. Akesson, T. Burke, J. M. Pemberton, J. Slate *et al.*, 2007 Gene order and recombination rate in homologous chromosome regions of the chicken and a passerine bird. Mol. Biol. Evol. **24:** 1537–1552.

de Oliveira, E. H., F. A. Habermann, O. Lacerda, I. J. Sbalqueiro, J. Wienberg *et al.*, 2005 Chromosome reshuffling in birds of prey: the karyotype of the world's largest eagle (Harpy eagle, *Harpia harpyja*) compared to that of the chicken (Gallus gallus). Chromosoma **114:** 338–343.

Derjusheva, S., A. Kurganova, F. Habermann and E. Gaginskaya, 2004 High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds. Chromosome Res. **12:** 715–723.

Ellegren, H., and B. C. Sheldon, 2008 Genetic basis of fitness differences in natural populations. Nature **452:** 169–175.

Ellegren, H., L. Gustafsson and B. C. Sheldon, 1996 Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. Proc. Natl. Acad. Sci. USA **93:** 11723–11728.

Fan, J. B., A. Oliphant, R. Shen, B. G. Kermani, F. Garcia *et al.*, 2003 Highly parallel SNP genotyping. Cold Spring Harbor Symp. Quant. Biol. **68:** 69–78.

Ferguson-Smith, M. A., and V. Trifonov, 2007 Mammalian karyotype evolution. Nat. Rev. Genet. **8:** 950–962.

Fillon, V., M. Vignoles, R. P. Crooijmans, M. A. Groenen, R. Zoorob *et al.*, 2007 FISH mapping of 57 BAC clones reveals strong conservation of synteny between Galliformes and Anseriformes. Anim. Genet. **38:** 303–307.

Fitzpatrick, B. M., 2004 Rates of evolution of hybrid inviability in birds and mammals. Evolution **58:** 1865–1870.

Freudenreich, C. H., 2007 Chromosome fragility: molecular mechanisms and cellular consequences. Front. Biosci. **12:** 4911–4924.

Gordon, L., S. Yang, M. Tran-Gyamfi, D. Baggott, M. Christensen *et al.*, 2007 Comparative analysis of chicken chromosome 28 provides new clues to the evolutionary fragility of gene-rich vertebrate regions. Genome Res. **17:** 1603–1613.

Green, P., K. Falls and S. Crook, 1990 *Documentation for CRIMAP*, Version 2.4. Washington University School of Medicine, St. Louis.

Gregory, T. R., J. A. Nicol, H. Tamm, B. Kullman, K. Kullman *et al.*, 2006 Eukaryotic genome size databases. Nucleic Acids Res. **35:** D332–D338.

Griffin, D. K., L. B. Robertson, H. G. Tempest and B. M. Skinner, 2007 The evolution of the avian genome as revealed by comparative molecular cytogenetics. Cytogenet. Genome Res. **117:** 64–77.

Groenen, M. A., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles *et al.*, 2000 A consensus linkage map of the chicken genome. Genome Res. **10:** 137–147.

Gustafsson, L., and T. Pärt, 1990 Acceleration of senescence in the collared flycatcher Ficedula albicollis by reproductive costs. Nature **347:** 279–281.

Gustafsson, L., and W. Sutherland, 1988 The costs of reproduction in the collared flycatcher Ficedula albicolis. Nature **335:** 813–815.

Gustafsson, L., A. Qvarnström and B. C. Sheldon, 1995 Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature **375:** 311–313.

Guttenbach, M., I. Nanda, W. Feichtinger, J. S. Masabanda, D. K. Griffin *et al.*, 2003 Comparative chromosome painting of chicken autosomal paints 1–9 in nine different bird species. Cytogenet. Genome Res. **103:** 173–184.

Hansson, B., M. Akesson, J. Slate and J. M. Pemberton, 2005 Linkage mapping reveals sex-dimorphic map distances in a passerine bird. Proc. R. Soc. B Biol. Sci. **272:** 2289–2298.

Hasselquist, D., S. Bensch and T. von Schantz, 1998 Low frequency of extrapair paternity and offspring survival in the great reed warbler. Behav. Ecol. **6:** 27–38.

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

Itoh, Y., and A. P. Arnold, 2005 Chromosomal polymorphism and comparative painting analysis in the zebra finch. Chromosome Res. **13:** 47–56.

Itoh, Y., K. Kampf and A.P. Arnold, 2006 Comparison of the chicken and zebra finch Z chromosomes shows evolutionary rearrangements. Chromosome Res. **14:** 805–815.

Jones, G. H., and C. Franklin, 2006 Meiotic crossing-over: obligation and interference. Cell **126:** 246–248.

Karaiskou, N., and C. R. Primmer, 2007 PCR-multiplexing for maximising genetic analyses with limited DNA samples: an example in the collared flycatcher, *Ficedula albicollis*. Ann. Zool. Fenn. (in press).

Karaiskou, N., L. Buggiotti, E. Leder and C. R. Primmer, 2008 High degree of transferability of 86 newly developed zebra finch EST-linked microsatellite markers in 8 bird species. J. Hered. (in press).

Kasai, F., C. Garcia, M. V. Arruga and M. A. Ferguson-Smith, 2003 Chromosome homology between chicken (*Gallus gallus domesticus*) and the red-legged partridge (*Alectoris rufa*): evidence of the occurrence of a neocentromere during evolution. Cytogenet. Genome Res. **102:** 326–330.

Kayang, B. B., A. Vignal, M. Inoue-Murayama, M. Miwa, J. L. Monvoisin *et al.*, 2004 A first-generation microsatellite linkage map of the Japanese quail. Anim. Genet. **35:** 195–200.

Kayang, B. B., V. Fillon, M. Inoue-Murayama, M. Miwa, S. Leroux *et al.*, 2006 Integrated maps in quail (Coturnix japonica) confirm the high degree of synteny conservation with chicken (Gallus gallus) despite 35 million years of divergence. BMC Genomics **7:** 101.

Kehrer-Sawatzki, H., and D. N. Cooper, 2007 Structural divergence between the human and chimpanzee genomes. Hum. Genet. **120:** 759–778.

Kohn, M., J. Hogel, W. Vogel, P. Minich, H. Kehrer-Sawatzki *et al.*, 2006 Reconstruction of a 450-My-old ancestral vertebrate protokaryotype. Trends Genet. **22:** 203–210.

Leder, E., N. Karaiskou and C. R. Primmer, 2008 70 new microsatellites for the pied flycatcher and amplification in other passerine birds. Mol. Ecol. Res. (in press).

Lenormand, T., 2003 The evolution of sex dimorphism in recombination. Genetics **163:** 811–822.

Lenormand, T., and J. Dutheil, 2005 Recombination difference between sexes: a role for haploid selection. PLoS Biol. **3:** e63.

Lupski, J. R., and P. Stankiewicz, 2005 Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet. **1:** e49.

Lyons, L. A., T. F. Laughlin, N. G. Copeland, N. A. Jenkins, J. E. Womack *et al.*, 1997 Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet. **15:** 47–56.

Navarro, A., and N. H. Barton, 2003 Chromosomal speciation and molecular divergence: accelerated evolution in rearranged chromosomes. Science **300:** 321–324.

Nishida-Umehara, C., Y. Tsuda, J. Ishijima, J. Ando, A. Fujiwara *et al.*, 2007 The molecular basis of chromosome orthologies and sex chromosomal differentiation in palaeognathous birds. Chromosome Res. **15:** 721–734.

Noor, M. A., K. L. Grams, L. A. Bertucci and J. Reiland, 2001 Chromosomal inversions and the reproductive isolation of species. Proc. Natl. Acad. Sci. USA **98:** 12084–12088.

Organ, C. L., A. M. Shedlock, A. Meade, M. Pagel and S. V. Edwards, 2007 Origin of avian genome size and structure in non-avian dinosaurs. Nature **446:** 180–184.

Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. Cole, P. F. Colosimo *et al.*, 2001 The genetic architecture of divergence between threespine stickleback species. Nature **414:** 901–905.

Pevzner, P., and G. Tesler, 2003 Genome rearrangements in mammalian evolution: lessons from human and mouse genomes. Genome Res. **13:** 37–45.

Pontius, J. U., J. C. Mullikin, D. R. Smith, K. Lindblad-Toh, S. Gnerre *et al.*, 2007 Initial sequence and comparative analysis of the cat genome. Genome Res. **17:** 1675–1689.

Price, T. D., and M. M. Bouvier, 2002 The evolution of F1 postzygotic incompatibilities in birds. Evolution **56:** 2083–2089.

Qvarnström, A., T. Pärt and B.C. Sheldon, 2000 Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature **405:** 344–347.

Qvarnström, A., J. E. Brommer and L. Gustafsson, 2006 Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. Nature **441:** 84–86.

Raudsepp, T., M. L. Houck, P. C. O'Brien, M. A. Ferguson-Smith, O. A. Ryder *et al.*, 2002 Cytogenetic analysis of California condor (*Gymnogyps californianus*) chromosomes: comparison with chicken (*Gallus gallus*) macrochromosomes. Cytogenet. Genome Res. **98:** 54–60.

Reed, K. M., L. D. Chaves, M. K. Hall, T. P. Knutson and D. E. Harry, 2005 A comparative genetic map of the turkey genome. Cytogenet. Genome Res. **111:** 118–127.

Reid, D. P., C. A. Smith, M. Rommens, B. Blanchard, D. Martin-Robichaud *et al.*, 2007 A genetic linkage map of Atlantic halibut (*Hippoglossus hippoglossus* L.). Genetics **177:** 1193–1205.

Rieseberg, L., 2001 Chromosomal rearrangements and speciation. Trends Ecol. Evol. **16:** 351–358.

Saether, S. A., G.-P. Saetre, T. Borge, C. Wiley, N. Svedin *et al.*, 2007 Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. Science **318:** 95–97.

Schmid, M., I. Nanda, H. Hoehn, M. Schartl, T. Haaf *et al.*, 2005 Second report on chicken genes and chromosomes 2005. Cytogenet. Genome Res. **109:** 415–479.

Sheldon, B. C., and H. Ellegren, 1999 Sexual selection resulting form extrapair paternity in collared flycatchers. Anim. Behav. **57:** 285–298.

Sheldon, B. C., J. Merilä, A. Qvarnström and H. Ellegren, 1997 Female genetic benefit from extra-pair copulation predicted by relative size of male secondary sexual character. Proc. R. Soc. Lond. Ser. B Biol. Sci. **264:** 297–302.

Shetty, S., D. K. Griffin and J. A. Graves, 1999 Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Res. **7:** 289–295.

Shibusawa, M., S. Minai, C. Nishida-Umehara, T. Suzuki, T. Mano, *et al.*, 2001 A comparative cytogenetic study of chromosome homology between chicken and Japanese quail. Cytogenet. Cell Genet. **95:** 103–109.

Shibusawa, M., M. Nishibori, C. Nishida-Umehara, M. Tsudzuki, J. Masabanda *et al.*, 2004a Karyotypic evolution in the Galliformes: an examination of the process of karyotypic evolution by comparison of the molecular cytogenetic findings with the molecular phylogeny. Cytogenet. Genome Res. **106:** 111–119.

Shibusawa, M., C. Nishida-Umehara, M. Tsudzuki, J. Masabanda, D. K. Griffin *et al.*, 2004b A comparative karyological study of the blue-breasted quail (*Coturnix chinensis*, Phasianidae) and California quail (*Callipepla californica*, Odontophoridae). Cytogenet. Genome Res. **106:** 82–90.

Slate, J., 2005 Quantitative trait locus mapping in natural populations: progress, caveats and future directions. Mol. Ecol. **14:** 363–379.

Slate, J., P. M. Visscher, S. MacGregor, D. Stevens, M. L. Tate *et al.*, 2002 A genome scan for quantitative trait loci in a wild population of red deer (*Cervus elaphus*). Genetics **162:** 1863–1873.

Troggio, M., G. Malacarne, G. Coppola, C. Segala, D. A. Cartwright *et al.*, 2007 A dense single-nucleotide polymorphism-based genetic linkage map of grapevine (*Vitis vinifera* L.) anchoring Pinot Noir bacterial artificial chromosome contigs. Genetics **176:** 2637–2650.

van Tuinen, M., C. G. Sibley and S. B. Hedges, 2000 The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. Mol. Biol. Evol. **17:** 451–457.

Veen, T., T. Borge, S. C. Griffith, G. P. Saetre, S. Bures *et al.*, 2001 Hybridization and adaptive mate choice in flycatchers. Nature **411:** 45–50.

Voorrips, R. E., 2002 MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. **93:** 77–78.

Wahlberg, P., L. Stromstedt, X. Tordoir, M. Foglio, S. Heath *et al.*, 2007 A high-resolution linkage map for the Z chromosome in chicken reveals hot spots for recombination. Cytogenet. Genome Res. **117:** 22–29.

Communicating editor: N. A. Jenkins