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Genetic Mapping in a Natural Population of Collared Flycatchers (*Ficedula albicollis*): Conserved Synteny but Gene Order Rearrangements on the Avian Z Chromosome

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ABSTRACT

Data from completely sequenced genomes are likely to open the way for novel studies of the genetics of nonmodel organisms, in particular when it comes to the identification and analysis of genes responsible for traits that are under selection in natural populations. Here we use the draft sequence of the chicken genome as a starting point for linkage mapping in a wild bird species, the collared flycatcher—one of the most well-studied avian species in ecological and evolutionary research. A pedigree of 365 flycatchers was established and genotyped for single nucleotide polymorphisms in 23 genes selected from (and spread over most of) the chicken Z chromosome. All genes were also found to be located on the Z chromosome in the collared flycatcher, confirming conserved synteny at the level of gene content across distantly related avian lineages. This high degree of conservation mimics the situation seen for the mammalian X chromosome and may thus be a general feature in sex chromosome evolution, irrespective of whether there is male or female heterogamety. Alternatively, such unprecedented chromosomal conservation may be characteristic of most chromosomes in avian genome evolution. However, several internal rearrangements were observed, meaning that the transfer of map information from chicken to nonmodel bird species cannot always assume conserved gene orders. Interestingly, the rate of recombination on the Z chromosome of collared flycatchers was only ~50% that of chicken, challenging the widely held view that birds generally have high recombination rates.

THE possibility of identifying genes coding for traits that are under selection in natural populations and, given the availability of such knowledge, subsequently studying key aspects of evolutionary biology has only recently become a realistic goal (Feder and Mitchell-Olds 2003; Slate 2005; Vasemagi and Primmer 2005). Examples of questions that should be possible to address are how local adaptation relates to genotypic variation and how genetic variation can be maintained for fitness-related traits by, for instance, genotype–environment interaction (Stearns 1992). Various approaches may be taken to attack the missing link between genotypes and phenotypes in wild populations of nonmodel organisms, including candidate gene approaches (Tabor *et al.* 2002), transcriptome profiling through EST sequencing (Le Quere *et al.* 2004) or microarray-based hybridization (Drnevich *et al.* 2004), genome scans for regions subject to selective sweeps (Storz 2005), and linkage/quantitative trait loci (QTL) mapping (Slate 2005). To various extents, all these approaches require some prior knowledge of the genetics of the species under study, which until recently has been a major limiting factor in the case of most natural populations. This is particularly true for genetic mapping approaches in which a large number of polymorphic markers have to be developed and placed on a primary linkage map.

However, an increasing number of linkage maps are now being reported from natural populations of organisms, such as butterflies (Jiggins *et al.* 2004), fishes (Chistiakov *et al.* 2005), fungi (Marra *et al.* 2004), insects (Lorenzen *et al.* 2005), molluscs (Hubert and Hedgecock 2004), and amphibians (Smith *et al.* 2005). They are so far generally limited to species that can easily be bred in captivity or where sufficiently large litter sizes are being produced in natural settings and are accessible to sampling to allow the establishment of pedigrees necessary for linkage analysis. Unfortunately, this is not the case for most species of birds. In addition to the well-established linkage map of the chicken (Groenen *et al.* 2001), mapping is underway in some domestic galliforms of agricultural interest, including quail (Kikuchi

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et al. 2005) and turkey (Reed *et al.* 2005), but there are only preliminary attempts at linkage mapping in wild species, notably by the work of Hansson *et al.* (2005). Given the role birds play in evolutionary research, improved genetic resources, such as the development of detailed linkage maps, are likely to open the way for advancing our understanding of the role of natural selection in wild bird populations (Edwards *et al.* 2005).

In this study we focus on one of the most well-studied avian species in evolutionary research, the collared flycatcher (*Ficedula albicollis*). By the standards of the field, it has become a "role model" organism for studies of, for example, life history evolution, mate choice, sexual selection, and speciation (Gustafsson and Sutherland 1988; Gustafsson and Pärt 1990; Gustafsson *et al.* 1995; Ellegren *et al.* 1996; Saetre *et al.* 1997; Qvarnström *et al.* 2000, 2006, Merilä *et al.* 2001; Veen *et al.* 2001; Michl *et al.* 2002), and this is also valid for its closely related sister species, the pied flycatcher (*F. hypoleuca*) (Alatalo *et al.* 1986; Merino *et al.* 1996; Tapio and Lehikoinen 2000; Both and Visser 2001). For the former species, this work mainly originates from a long-term study of a Baltic Sea island population. This population, showing high philopatry, has been closely followed and monitored for fitness-related traits, including lifetime reproductive success, for >25 years. It forms an ideal setting for searching for genotype–phenotype–function connections, provided that at least some basic knowledge of the species' genome can be obtained. In addition, natural hybridization between the two flycatcher species makes this system highly suitable for studying the genetic basis of reproductive isolation barriers.

The recent report of a draft sequence of the chicken genome (International Chicken Genome Sequencing Consortium 2004) offers a most promising resource for the transfer of genetic information among bird species and for the study of avian evolutionary genomics (Ellegren 2005). Here, we make use of chicken genomic resources to make a targeted attempt at linkage mapping in collared flycatchers. We have generated a pedigree of >350 birds with parentage confirmed by genetic profiling; the pedigree consists of 27 half-sib families where each male has mated with several females. In this study we specifically focus on the Z sex chromosome, according to the following. First, we evaluate the usefulness of the chicken genome sequence for developing conserved sex-linked gene markers in a distantly related bird species. Chicken belongs to the order Galliformes and collared flycatcher to the order Passeriformes; these lineages split early in the radiation of neognath (non-ratite) birds, probably ~100 million years ago (van Tuinen and Hedges 2001). Second, we use these gene markers for the identification of single nucleotide polymorphisms (SNPs) in the collared flycatcher. Third, by collecting data for some 20,000 SNP genotypes in the pedigree (53 SNPs typed in 365 individuals) we establish a high-density, gene-based linkage map of the collared flycatcher Z chromosome. Finally, we use the flycatcher Z chromosome linkage map to address the questions of conserved synteny in avian sex chromosome evolution and the evolution of avian recombination rates.

MATERIALS AND METHODS

Sampling and DNA extraction: Blood samples were collected from collared flycatchers breeding in nest boxes on the islands Öland and Gotland in the Baltic Sea in 2001–2004. Approximately 10 μl blood was collected from adults and offspring by venipuncture of the cutaneus ulnar wing vein with a sterile syringe. Blood was stored frozen in EDTA buffer until usage. DNA was extracted by cell digestion with proteinase K at 37° overnight, followed by three rounds of phenol-chlorophorm DNA purification. Purified DNA was precipitated with NaAc and 95% EtOH, washed once in 70% EtOH, and the dried pellet was dissolved in water.

The structure of the pedigree with 365 birds is described in supplemental Table 1 (http://www.genetics.org/supplemental/). The final linkage analysis was made for 62 litters with a total of 280 offspring, fathered by 27 males, each of which had mated with 2–4 females; the pedigree thus had the two-generation half-sib design.

Identification of extra-pair offspring: Extra-pair paternity is known to occur in collared flycatchers (Sheldon and Ellegren 1999) and illegitimate offspring must obviously be excluded from linkage analysis, at least for the study of male recombination fractions. Although extra-pair offspring (EPO) are most likely to be identified from non-Mendelian inheritance in large-scale SNP data sets, we sought to minimize the number of EPO being subjected to SNP typing by first genotyping all families for five microsatellite markers (FhU2, FhU4, Mcyμ4, Pdoμ5, and Pca3) (Ellegren 1992; Primmer *et al.* 1996; Double *et al.* 1997; Griffith *et al.* 1999; Dawson *et al.* 2000). These markers were analyzed essentially following the conditions described in the original reports, using fluorescently labeled primers. Fragment length analysis was performed on a MegaBACE 96 capillary instrument using ROX size standard (Applied Biosystems), and genotypes were scored using the software Genetic Profiler (Amersham Biosciences).

Offspring whose genotypes deviated from Mendelian expectations on the basis of the presumed parental genotypes at two or more microsatellite loci were excluded from further analyses. Although retaining offspring with an incompatible genotype combination at a single locus meant that some EPO were likely to remain after this first selection, it allowed for including offspring in the final analysis in which a microsatellite genotyping error or a microsatellite mutation had potentially occurred. Of the 344 offspring initially tested, 44 (12.8%) were identified as EPO according to microsatellite fingerprinting. From the subsequent SNP genotyping another 20 offspring were also classified as EPO, giving a total frequency of 18.6%. After removal of these 64 offspring, the final data set used in linkage analysis included 280 offspring.

Marker development and screening for SNP markers: The protocol for identification of polymorphic markers to be used in flycatcher linkage analysis was to design PCR primers from exonic sequences flanking introns of Z-linked chicken genes, amplification of the orthologous loci in flycatchers, followed by SNP screening using resequencing of eight unrelated male birds. We initially selected 135 candidate markers, which in each case flanked a single intron of an equal number of Z-linked genes. The markers were chosen on basis of (i) being

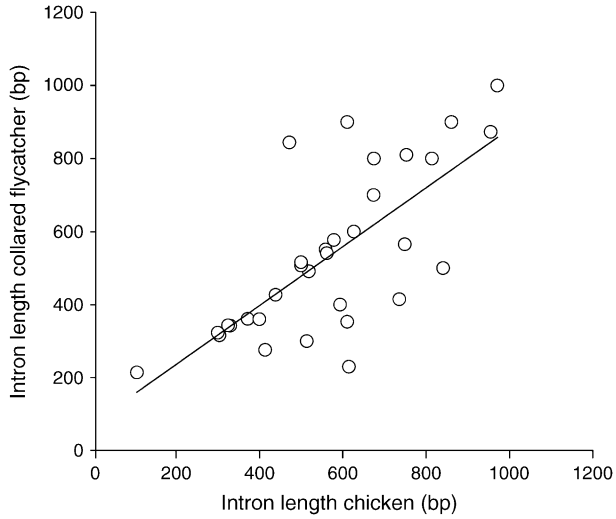

FIGURE 1.—Correlation between intron size (in base pairs) in chicken and collared flycatcher ($y = 0.81x + 74.7$).

evenly distributed along the chicken Z chromosome in the draft assembly (http://www.ensembl.org/Gallus_gallus/mapview?chr=Z), (ii) flanking introns preferably in the size range of 200–1000 bp in chicken (which, assuming that intron length in chicken and flycatcher would be correlated, was expected to facilitate amplification and direct sequencing in flycatcher; the assumed correlation was subsequently confirmed within the range of intron sizes analyzed: $r^2 = 0.52$, $P < 0.01$, Figure 1), and (iii) showing some degree of exonic sequence conservation when BLASTed (http://www.ncbi.nih.gov/BLAST/) to other vertebrate genome sequences (mainly human, mouse, rat, and zebrafish). Importantly, particularly conserved regions were targeted for primer design. Most of the markers come from Ensembl predicted genes (http://www.ensembl.org/info/data/docs/genome_annotation.html). In addition, we specifically searched for chicken introns containing microsatellite sequences using the default setting of the Sputnik program (http://espressosoftware.com/pages/sputnik.jsp); 42 such introns were identified, included in the total of 135. The position of genes on the chicken Z chromosome as indicated in Table 1 is according to build 1.1 of the chicken genome; these positions are likely to change when new builds are constructed and given that errors in the draft assembly seem to occur (see below).

PCR reactions were performed in 20-μl reactions in a PTC-100 thermal cycler (MJ Research) with 0.5 pmol of each primer, 50 μM of each dNTP, 2.5 mM $MgCl_2$, 0.025 units AmpliTaq Gold (Applied Biosystems), and 50 ng of template DNA. The general temperature profile was 5 min at 95°, 30–40 cycles of 30 sec at 95°, 30 sec at annealing temperature (45–60°) and 30–60 sec at 72°, followed by a final extension step at 72° for 5–10 min. Primer sequences are provided in supplemental Table 2 (http://www.genetics.org/supplemental/). Amplification success was initially examined using three male samples. For markers revealing a specific amplification product, a total of eight male samples (16 chromosomes) were amplified and these products were purified with Exo-SAP (USB). Four μl of purified product was subject to sequencing using 1 pmol of forward or reverse primer and 5 μl of DYEnamic ET Terminator cycle sequencing mix (Amersham Biosciences) for 29 cycles of 20 sec at 95°, 15 sec at 50°, and 30 sec at 72°. Sequencing reactions were purified with the 96-well Autoseq system (Amersham Biosciences) and run on a MegaBACE 96 capillary instrument.

Sequences were edited and aligned in Sequencher (Gene Codes Corporation) and SNPs, typically identified from overlapping forward and reverse sequencing, were scored by hand. The obtained collared flycatcher sequences were used in BLASTN searches at Ensembl (http://www.ensembl.org/Gallus_gallus) to confirm orthology to a single region on chicken Z chromosome. SNPs subsequently used for pedigree genotyping were selected with preference for high frequency alleles (>20% minor allele frequency, MAF).

SNP genotyping: The 365 individuals were genotyped at each of 53 selected SNP loci using the 12-plex GenomeLab SNPStream system (Beckman Coulter; BELL *et al.* 2002). The primers for PCR and minisequencing were designed using the Autoprimer software (http://www.autoprimer.com, Beckman Coulter) and are available upon request; note that these were internal primers and thus different from the exonic, chicken-derived primers (supplemental Table 2 at http://www.genetics.org/supplemental/) initially used for obtaining sequence information from the collared flycatcher. The overall genotype call rate was 96%, and the accuracy was 99.94% according to duplicate analysis of 22% of the total number of genotypes (4694/21,479). When possible, missing data were inferred from the genotypes of parents/offspring.

Linkage analysis: Genotype data were used to infer intra-intronic haplotypes of all individuals assuming no recombination between SNPs within introns in the single generation scored. All linkage analyses were done in CRIMAP (GREEN *et al.* 1990) using an LOD score of 3.0 as threshold for significant linkage. Pairwise linkage analyses between all marker pairs were done using the option two point and a framework marker map was created with the option build. To include all linked loci in an extended build, unordered loci from the first build were tested with recurrent runs of the option flips4 until no better order could be obtained. For comparison of gene orders and recombination rates of collared flycatcher and chicken, data from chicken were primarily taken from http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?taxid=9031&chr=Z. However, since the draft assembly contains errors in order of contigs on the Z, we used information from the most recent version (H. CHENG, unpublished data) of the East Lansing chicken linkage map (available at http://poultry.mph.msu.edu/) to get as accurate data as possible on gene order and recombination rates in chicken. The East Lansing genetic map was developed by multipoint analysis using 88 backcross progeny and >2500 markers, including 1207 SNPs based on the chicken draft genome assembly (WASHUC1). Throughout the article we use the term synteny to refer to conserved gene content of orthologous chromosomes, irrespective of gene order.

RESULTS

Development of polymorphic gene markers in collared flycatchers: Of 135 primer pairs (corresponding to 135 different genes) designed from chicken exon sequence, 75 (55%) amplified a specific product in flycatchers and 39 (29%) of these could be readily sequenced. We used the retrieved flycatcher sequences in BLAST searches against the chicken genome. In the great majority of cases there was a single best hit to the expected gene on the chicken Z chromosome, indicating orthology (Table 1). All cases of weakly supported orthology concerned loci for which there was no or only limited exon sequence in collared flycatcher retrieved.

TABLE 1

All gene markers placed on the collared flycatcher linkage map

Gene	Gene symbol[a]	Gene ID[b]	Position on chicken Z[c]	Length[d] Exon	Length[d] Intron	BLASTN Best hit	BLASTN Score
Vasculin	*GPBP1*	23746	1005436	120	844	Z 1005351	428E-31
Importin-11	*IPO11*	23770	2866719	0	744	Z 1784654	0,082
ADAMTS6 variant 2	*ADAMTS6*	23786	3783173	56	491	Z 3783135	150E-122
Peptidylprolyl isomerase domain	*PPWD1*	23799	3862227	107	577	Z 3862780	630E-43
Zinc transporter 5	*SLC30A5*	23871	5128095	93	361	Z 5128443	130E-33
Growth hormone receptor precursor	*GHR*	23973	6621490	13	507	Z 6622618	0,016
NAD(P) transhydrogenase	*NNT*	23990	6824865	0	540	—	—
Poly (ADP-ribose) polymerase, member 8	*PARP8*	24009	7878620	35	895	Z 7878950	0,00011
Similar to TGF β-inducible protein 1	*24105*	24105	9914755	166	360	Z 9915877	120E-61
Ras GTPase-activating-like protein	*IQGAP2*	24162	10609097	150	343	Z 10609027	500E-57
Glycine dehydrogenase	*GLDC*	24274	12149860	99	452	Z 12150318	140E-43
N-acylsphingosine amidohydrolase 3 like	*ASAH3L*	24328	13516515	0	573	Z 13516956	0,0043
Amyloid β A4 precursor	*APBA1*	24379	15556988	0	632	Z 15557080	0,0051
Transient receptor cation channel 3	*TRPM3*	24411	15902290	108	541	Z 15902836	330E-81
Transient receptor cation channel 6	*TRPM6*	24457	17282846	77	733	Z 17283040	120E-18
Hypothetical protein	*24555*	24555	19651339	75	427	Z 19651751	230E-09
Hypothetical protein	*24638*	24638	22459475	83	332	Z 22454175	130E-26
Phosphodiesterase 6 β subunit	*GAF*	24804	25383953	83	487	Z 25384564	660E-33
Neuregulin-1	*NRG1*	24876	25804434	59	506	Z 25803889	270E-25
ATP-binding cassette A member 1	*ABCA1*	24891	26519192	164	230	Z 26519097	160E-62
Fructose-bisphosphate aldolase B	*ALDOB*	25056	27566818	62	460	Z 27541710	640E-13
Zinc finger FYVE domain 16	*ZFYVE16*	25117	28483373	85	379	Z 28483805	260E-31
Hypothetical protein	*25189*	25189	29865246	31	878	—	—

[a] For hypothetical proteins, gene symbols were given according to the ENSEMBL transcript ID.
[b] Gene IDs are given as the last five numbers of the ENSEMBL transcript ID (ENSGALT000000*xxxxx*).
[c] Position of the first nucleotide in the exon immediately upstream of the intron amplified in the collared flycatcher on the chicken Z chromosome, according to the draft genome assembly (build 1.1).
[d] Length of the obtained sequence in collared flycatchers.

From resequencing of 18,450-bp intronic sequence in each of eight unrelated males, a total of 126 SNPs were identified, yielding an average SNP density of 1 every 146 bp of Z-chromosome sequence. Thirty-one introns showed at least one SNP with an MAF of >20% in the 16 chromosomes screened. Fifty-three such high-frequency SNPs from the 31 introns were subsequently genotyped in the pedigree. For 22 introns initially identified to contain microsatellite sequences in chicken, none showed length polymorphism in collared flycatcher. Generally, these introns showed little sign of remaining simple repetitive sequence in the collared flycatcher.

Linkage mapping: We collected ~20,000 SNP genotypes (53 sites × 365 individuals) in a mini-sequencing platform for SNP typing. Seven SNP markers turned out to be monomorphic in the pedigree and for another three markers only one to two families were informative for segregation analysis; these markers were excluded from further analyses. The remaining 43 SNPs were from 23 different introns (Table 1) and since no recombination event could be detected between SNPs within introns in an initial two-point analysis, genotype data from markers with >1 SNP were converted to haplotype data, which were used in subsequent analysis. The 23 genes are rather evenly spread from position 0.1–29.9 Mb in the draft assembly of the chicken Z chromosome sequence (which has a total length, excluding gaps, of 31 Mb).

Two-point analysis revealed that all gene markers were significantly (LOD score >3.0) linked to at least one other locus in a way consistent with these 23 genes being part of a single linkage group. Since all parent and offspring females showed just one allele at all marker loci, we concluded that this linkage group is located on the collared flycatcher Z chromosome. The build procedure of CRIMAP resulted in a 44.4-cM linkage group with 11 loci significantly ordered (LOD score >3.0) in a framework map (Figure 2). Most of the remaining loci can be placed with significant support in either of two alternative intervals. A best-order linkage group for all 23 loci was subsequently obtained by evaluation using the flips4 option; this group spanned 62.7 cM (Figure 3).

Gene order rearrangements during avian sex chromosome evolution: A comparison of the order of loci on the flycatcher framework map to the order of orthologous loci on the chicken physical map reveals two major rearrangements close to the respective ends of the

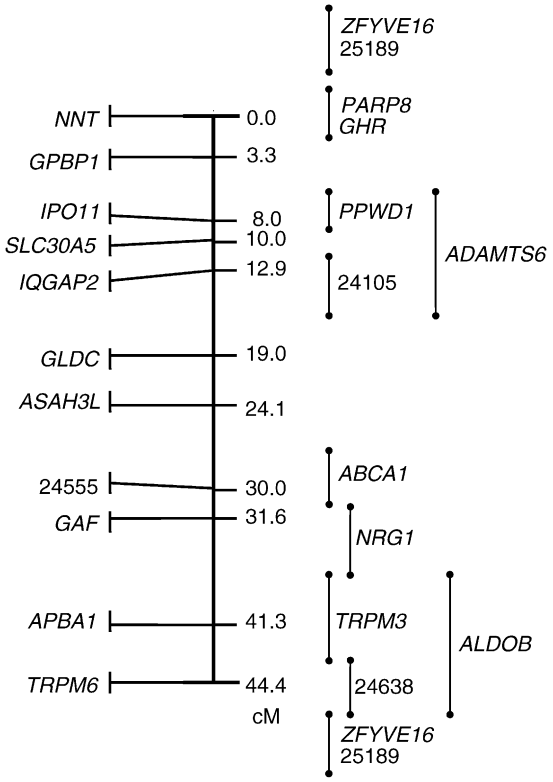

FIGURE 2.—A framework linkage map of the collared flycatcher Z chromosome. Positions are given as the cumulative genetic position of gene markers in the linkage map. Unordered markers are shown to the right with alternative locations indicated by vertical bars. Note that most unordered markers can be placed with statistical support in either of two locations.

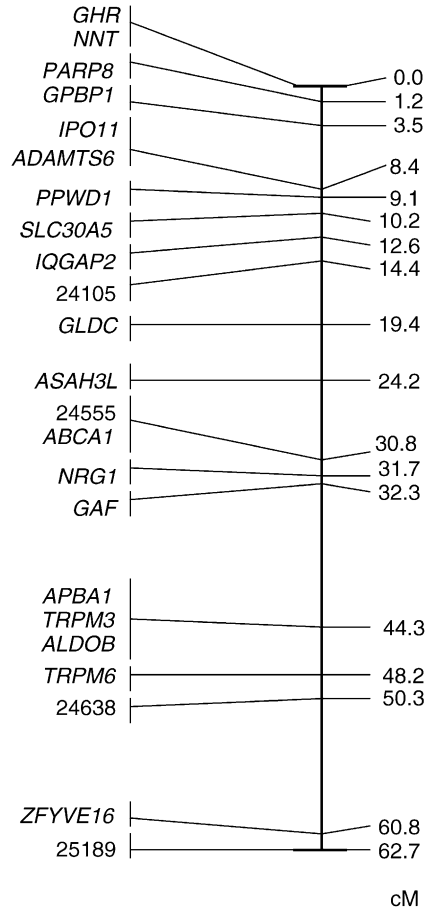

FIGURE 3.—A best-order linkage map of the collared flycatcher chromosome based on all gene markers genotyped in the pedigree. Positions are given as the cumulative genetic position of gene markers in the linkage map.

flycatcher linkage group. However, one of these (Figure 4), *NNT*, being distal to *GPBP1*, *IPO11*, and *SLC30A5* in the flycatcher map but proximal in the chicken physical map, is likely to represent an error in the draft assembly of the chicken genome since the same order as we find in flycatcher has recently also been observed in chicken linkage analysis (H. CHENG, unpublished data). The other rearrangement (for which chicken physical and genetic data are concordant) concerns a 14-cM flycatcher/10-Mb chicken interval tagged by four loci, *APBA1*, *TRPM6*, *24555*, and *GAF* (Figure 4; note while two of these loci had relatively low BLAST scores, two were highly supported). The order of loci in the two species cannot straightforwardly be accounted for by a single inversion event and hence point at more complex structural changes. Moreover, the best-order map indicates additional rearrangements of complex nature in the vicinity of *APBA1-TRPM6-24555-GAF* (Figure 5). An inversion is indicated for a segment harboring *24105* and *IQGAP2* but here the distance between loci is small, within map error. It can be concluded that while Z-chromosome synteny is perfectly conserved among the 23 genes investigated in the chicken–flycatcher comparison, a number of internal rearrangements have taken place during avian sex chromosome evolution.

Comparison of recombination rates: Given that the most distal markers in the best-order flycatcher map do not seem to be involved in intrachromosomal rearrangements, we can tentatively compare chicken and flycatcher recombination rates by comparing the length of our linkage group with the length of the part of the chicken Z chromosome genetic map flanked by the corresponding markers. This syntenic segment has a genetic length of 63 and 135 cM in collared flycatcher and chicken, respectively, indicating that the recombination rate in flycatchers is only ~50% of that in chicken. Analyses of shorter segments within the maps give similar differences (data not shown).

DISCUSSION

Although cytogenetic approaches, mainly cross-species fluorescence *in situ* hybridization with chromosome-specific probes (ZOO-FISH), have been used to study the patterns of synteny conservation in birds (SHETTY *et al.* 1999; GUTTENBACH *et al.* 2003; SHIBUSAWA *et al.* 2004a,b; ITOH and ARNOLD 2005), genome evolution at

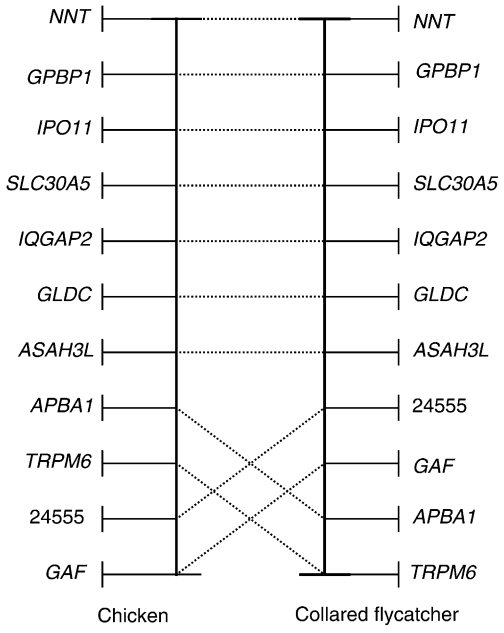

FIGURE 4.—A comparison of the relative location of orthologous genes on the chicken and collared flycatcher Z chromosome maps. Gene order in collared flycatcher is from the framework linkage map presented in Figure 2. In chicken, data are from the draft genome assembly updated with information from linkage mapping.

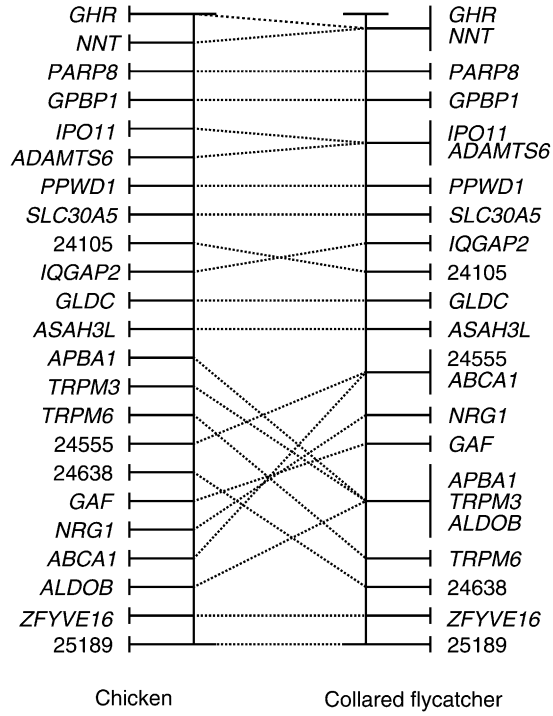

FIGURE 5.—A comparison of the relative location of orthologous genes on the chicken and collared flycatcher Z chromosome maps. Gene order in collared flycatcher is from the best-order linkage map presented in Figure 3. In chicken, data are from the draft genome assembly updated with information from linkage mapping.

the level of gene content and gene order across phylogenetically divergent bird lineages has not been investigated so far. A main goal of this study was therefore to analyze chicken and collared flycatcher gene maps in a comparative genomic context. An evaluation of the extent to which information on the organization of the chicken genome can be transferred to nonmodel species is of significance both for future genomic studies of natural populations and for our general understanding of avian genome evolution. Moreover, by focusing on the organization of the Z chromosome, we can address the degree of sex chromosome conservation in a system of female heterogamety. The latter is motivated by the fact that while there have been numerous interchromosomal rearrangements among autosomes during mammalian evolution (MURPHY *et al.* 2005), the gene content of the X chromosome is largely conserved across all eutherian lineages (MURPHY *et al.* 1999; RAUDSEPP *et al.* 2004). Is this a consequence of the mode of dosage compensation in mammals or is it a general feature of sex chromosome evolution?

Conserved synteny on the avian Z chromosome: We found that 23 of 23 genes more or less evenly distributed along the Z chromosome of chicken also map to the Z chromosome of the collared flycatcher. This result shows that there is a very high degree of sex chromosome conservation at the level of gene content across distantly related bird lineages, which mimics the situation seen for the mammalian X chromosome. A possible explanation is that a particularly high degree of conservation is inherently associated with heteromorphic sex chromosomes. OHNO (1973) first hypothesized that there is selection to maintain the gene content of the X chromosome, because if dosage compensation occurs on a gene-by-gene basis, the transfer of a gene from an autosome to X would initially imply an imbalance in expression levels between the two sexes that cause problems in development and other life processes. Also, the translocation of an X-linked and dosage-compensated gene to an autosome may be associated with deleterious effects on expression levels. Potentially, the observed high degree of conserved synteny on the avian Z chromosome might be a consequence of similar selective constraints.

However, the case for, and the mode of, dosage compensation in birds is still a matter of uncertainty (ELLEGREN 2002). It seems clear that Z chromosome inactivation does not occur (SCHMID *et al.* 1989). There are reports of equalized expression levels in males and females for a limited number of Z-linked genes (KURODA *et al.* 2001; MCQUEEN *et al.* 2001), possibly regulated at the level of transcription since both Z chromosomes of male chicken are transcribed (KURODA *et al.* 2001; KUROIWA *et al.* 2002). The observations of a heterogeneous, noncoding RNA that accumulates on the Z chromosome in the female nucleus but is heavily

methylated and silent in males (Teranishi *et al.* 2001) and Z chromatin being enriched with hyperacetylated histones in females but not in males (Bisoni *et al.* 2005) point to epigenetic mechanisms similar to that suggested to be involved with dosage compensation in other organisms. While more work is clearly needed for avian dosage compensation to be understood, the absence of Z chromosome inactivation and the difference in genetic basis for sex determination between birds and mammals indicate that conserved synteny of sex chromosomes may be a general feature of heterogametic organisms, irrespective of whether there is male or female heterogamety or how dosage compensation is mediated.

An alternative explanation for our findings is that the high degree of conservation at the level of gene content is typical for most avian chromosomes, *i.e.*, not being restricted to the Z chromosome. There are several lines of evidence indicating that, overall, genomic stability is higher in birds than in mammals. Comparative map data suggest that chromosomal rearrangements have occurred at a significantly lower rate in the avian than in the mammalian lineage (Burt *et al.* 1999; Bourque *et al.* 2005). Moreover, studies of karyotype evolution in birds using cross-species chromosome painting probes (ZOO-FISH) have revealed an extensive degree of chromosomal conservation across phylogenetically divergent lineages (Shetty *et al.* 1999; Guttenbach *et al.* 2003; Shibusawa *et al.* 2004a,b; Itoh and Arnold 2005), although there are exceptions, as in birds of prey (Bed'Hom *et al.* 2003; de Oliveira *et al.* 2005). In most cases, chicken-derived probes of macrochromosomes and of the Z chromosome each hybridize to a single chromosome in paleognathous as well as in other neognathous birds. Furthermore, a karyotype of $2n = 78$ is widely conserved across birds (Burt 2002). Although none of these approaches has the resolution of addressing genome conservation at the level of gene content, together they show that avian genomes are unusually stable at a gross cytogenetic level. Additional gene mapping data, or genome sequence information, from avian lineages distant from the chicken will be needed for unveiling whether a similarly high conservation of gene content that we observed for the avian Z chromosome is also found in autosomes.

Intrachromosomal rearrangements: In contrast to the observation of conserved synteny, several changes in gene order during avian sex chromosome evolution were evident from the collared flycatcher linkage map. Intrachromosomal rearrangements of the Z chromosome have also been indicated by chromosome painting among Galliform species (Shibusawa *et al.* 2004a,b), *i.e.*, among species that are much more closely related than in the Galliformes–Passeriformes comparison. The finding of internal rearrangements has important practical consequences for the exploitation of data from the chicken genome in studies of less well-characterized bird species. If such rearrangements tend to occur on a broad scale across the genome, then map information will often not be directly transferable between species. This caveat is important to point out because a high genomic stability at the level of conserved synteny may incorrectly be taken to suggest that gene orders are also conserved. Moreover, the complex nature of the rearrangements inferred in Figures 4 and 5 means that it can be difficult to predict the location of homologous genes unless detailed comparative map information is available. We finally note that conserved synteny but frequent internal rearrangements are also a hallmark of mammalian X chromosome evolution (Blair *et al.* 1994; Millwood *et al.* 1997; Bourque *et al.* 2004).

Recombination rate evolution: An important observation in this study was that the recombination frequency on the collared flycatcher Z chromosome seems lower than that in chicken. On the basis of early linkage mapping studies in chicken, subsequently confirmed by genome analysis, it has generally been thought that birds have high recombination rates, at least when compared to most mammals. To some extent this can be explained by the effect of the number of chromosomes; with an obligate chiasma per chromosome or chromosome armper meiosis needed for proper segregation, the total genetic distance of a genome should be expected to correlate with the number of chromosomes. However, recombination rates (in centimorgans per megabase) of individual chicken chromosomes are also high when compared to the rate in similarly sized mammalian chromosomes (International Chicken Genome Sequencing Consortium 2004). Our data now suggest that high recombination rates may not be a ubiquitous feature of bird genomes, at least not the sex chromosomes. Preliminary data from microsatellite-based linkage groups in the great reed warbler (*Acrocephalus arundinaceus*) point in a similar direction (Hansson *et al.* 2005).

There is a body of literature on the evolution of recombination rates (see, *e.g.*, reviews by Otto and Lenormand 2002; Rice 2002). It would be premature at this point to speculate on how, for example, life history differences between chicken and flycatcher could relate to differences in recombination rates between the two lineages. However, one more principal characteristic is worth mentioning. First, strong selection for particular traits is likely to be coupled with selection for high recombination rate, since the highest selective response is expected when beneficial alleles are inherited together and unfavorable combinations removed (Rodell *et al.* 2004). In other words, the avoidance of Hill–Robertson interference is dependent on the rate of recombination. Empirical data from plants indicate that strong artificial selection during domestication has selected for elevated recombination rates (Ross-Ibarra 2004) and there are also observations in the same direction among domestic animals (Burt and Bell 1987). Thus, it is possible that

chicken domestication may have led to the evolution of a higher rate of recombination compared to other birds.

Conserved gene markers: The idea of systematic use of evolutionarily conserved exonic primers spanning introns (Lyons *et al.* 1997) has proven useful for polymorphism ascertainment in a variety of organisms (Friesen *et al.* 1999; Hellborg and Ellegren 2003; Aitken *et al.* 2004), including birds (Primmer *et al.* 2002). The availability of the recently obtained chicken genome sequence (International Chicken Genome Sequencing Consortium 2004) now offers a huge resource of potential targets for conserved anchors in the avian genome. Since Galliformes, together with Anseriformes, likely form the most basal clade (Galloanserae) among neognath (non-ratite) birds (Edwards *et al.* 2005), the use of chicken-derived primer sequences for amplification of orthologous sequence in a passerine bird constitutes a critical test of the applicability of chicken gene sequences in distantly related birds. The observed success ratio of 29% (39/135; to the point of obtaining flycatcher intron sequence data) is similar to that reported by Primmer *et al.* (2002) (20%; 8/41). Clearly, however, this ratio can be increased substantially by including sequence information from another bird species in primer design.

Linkage maps are most often constructed using anonymous markers like microsatellites or, to some extent, amplified length polymorphisms and this applies in particular to less well-characterized nonmodel organisms. However, while the polymorphism content of microsatellites is clearly higher than that of SNPs, it might be anticipated that SNP-based maps will come to find an increased application in the future. First, the increasing number of completely sequenced genomes will facilitate the development of PCR-based markers in nonmodel organisms, as demonstrated in this study. Second, SNPs in genes have a clear advantage in comparative mapping, in that they make identification of synteny and conserved chromosomal segments straightforward, as also demonstrated here. Third, there is rapid progress in technology for large-scale SNP genotyping with increased throughput at decreased costs.

Conclusions: Obtaining the genome sequence of the chicken was an important step toward increased understanding of avian biology and this study has clearly revealed the usefulness of chicken as a model organism for genetic studies of other birds. Specifically, we have demonstrated an approach by which polymorphic markers in a focal species can be developed on the basis of sequence information from chicken and how such markers subsequently can be used for genetic mapping in a nonmodel organism. Moreover, our study reveals highly conserved synteny between the sex chromosomes of chicken and a passerine bird. Importantly, it should be possible to extend this approach to whole-genome coverage, selecting markers evenly distributed along all chicken chromosomes. We anticipate that this will pave the way for studies of other bird species, with the ultimate goal of using genetic maps for the identification of genes underlying phenotypic traits and the subsequent study of selection directly on genotypes instead of phenotypes. Such work is now in progress in collared flycatchers.

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