Gene Order and Recombination Rate in Homologous Chromosome Regions of the Chicken and a Passerine Bird

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Genome structure has been found to be highly conserved between distantly related birds and recent data for a limited part of the genome suggest that this is true also for the gene order (synteny) within chromosomes. Here, we confirm that synteny is maintained for large chromosomal regions in chicken and a passerine bird, the great reed warbler *Acrocephalus arundinaceus*, with few rearrangements, but in contrast show that the recombination-based linkage map distances differ substantially between these species. We assigned a chromosomal location based on sequence similarity to the chicken genome sequence to a set of microsatellite loci mapped in a pedigree of great reed warblers. We detected homologous loci on 14 different chromosomes corresponding to chicken chromosomes Gga1–5, 7–9, 13, 19, 20, 24, 25, and Z. It is known that 2 passerine macrochromosomes correspond to the chicken chromosome Gga1. Homology of 2 different great reed warbler linkage groups (LG13 and LG5) to Gga1 allowed us to locate the split to a position between 20.8 and 84.8 Mb on Gga1. Data from the 5 chromosomal regions (on Gga1, 2, 3, 5, and Z) with 3 or more homologous loci showed that synteny was conserved with the exception of 2 large previously unreported inversions on Gga1/LG5 and Gga2/LG3, respectively. Recombination data from the 9 chromosomal regions in which we identified 2 or more homologous loci (accounting for the inversions) showed that the linkage map distances in great reed warblers were only 6.3% and 13.3% of those in chickens for males and females, respectively. This is likely to reflect the true interspecific difference in recombination rate because our markers were not located in potentially low-recombining regions: several linkage groups covered a substantial part of their corresponding chicken chromosomes and were not restricted to centromeres. We conclude that recombination rates may differ strongly between bird species with highly conserved genome structure and synteny and that the chicken linkage map may not be suitable, in terms of genetic distances, as a model for all bird species.

Introduction

The genome sequence of the red jungle fowl *Gallus gallus*, the ancestor of the domestic chicken *Gallus domesticus*, was recently released (International Chicken Genome Sequencing Consortium 2004). This facilitated whole-genomic comparisons with the previously sequenced genomes of human and mouse (International Chicken Genome Sequencing Consortium 2004). The release of the chicken sequence is also expected to have important consequences for future research on other bird species (Ellegren 2005). Comparative work on chicken and several passerines has shown that their genome size (Gregory 2007) and karyotype (Shields 1982; Derjusheva et al. 2004; Itoh and Arnold 2005) have been highly conserved despite 80–100 Myr of independent evolution (Shetty et al. 1999). The main cytogenetic alterations detected so far are that chicken chromosome 1 (Gga1) is homologous to 2 passerine macrochromosomes, whereas chicken chromosome 4 (Gga4) is homologous to 1 macrochromosome and 1 microchromosome in passerines (Derjusheva et al. 2004; Itoh and Arnold 2005). Recent comparative data in chicken and the great reed warbler *Acrocephalus arundinaceus* for parts of chicken chromosomes 2, 3, and 5 (Dawson et al. 2006) and for the Z chromosome in chicken, zebra finch *Taeniopygia guttata* (Itoh et al. 2006) and collared flycatcher *Ficedula albicollis* (Backström, Brandstrom, et al. 2006), respectively, also suggest that gene order (synteny) has been highly conserved, with few rearrangements, between these distantly related birds. Together, these results strongly suggest that the physical map of the chicken (International Chicken Genome Sequencing Consortium 2004) can be used as a framework to predict the genome organization in passerines (Ellegren 2005; Dawson et al. 2006). In the context of evolutionary biology, the chicken genome sequence may be of particular value in underpinning molecular-based approaches, such as linkage mapping and quantitative trait loci (QTL) analyses, in passerine birds. Many passerine study systems have already provided significant insights to evolutionary biology, especially in quantitative genetics (Merilä et al. 2001), natural (Richman and Price 1992) and sexual selection (Norris 1993), hybridization (Veen et al. 2001), speciation (Irwin et al. 2001), and development (Abzhanov et al. 2004).

It would be valuable to evaluate not only genome structure and synteny, but also whether recombination rates and linkage map distances have been conserved to the same extent as the genome structure in chicken and passerines, in order to aid the successful design of linkage mapping experiments and QTL analyses in passerines. The linkage map of the chicken (~4,000 cM; Groenen et al. 2000) is somewhat larger in size than in human (~3,700 cM; Dib et al. 1996), despite a much smaller genome size in chicken (~1.2×10^9 bp in chicken; ~3×10^9 bp in human; International Chicken Genome Sequencing Consortium 2004). This results partly from chicken having 16 more chromosomes (38 vs. 22 autosomes), including both macro- and microchromosomes, each with at least one obligate crossing-over during meiosis (i.e., all autosomes, including the microchromosomes, are expected to span at least 50 cM; Rodionov et al. 1992) and partly from a higher recombination rate per base in chicken for chromosomes of similar size (International Chicken Genome Sequencing Consortium

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2004; Schmid et al. 2005). At present, it is not known whether the comparatively high recombination rate and large linkage map in relation to genome size in chicken is a general phenomenon in birds. In fact, we may suspect the contrary because the recombination rate is not always strongly conserved between taxa; for example, it varies between human and chimpanzees (Winckler et al. 2005), between different *Drosophila* species (True et al. 1996), and even between subspecies and strains of *Arabidopsis thaliana* (Sanchez-Moran et al. 2002).

In the present study, we compare genome structure, synteny, and map distances between a recently derived partial linkage map of a passerine bird, the great reed warbler (Hansson et al. 2005; Åkesson et al. 2007) and homologous chromosomal regions of the chicken (International Chicken Genome Sequencing Consortium 2004; http://pre.ensembl.org/Gallus_gallus/index.html). We have previously compared synteny for a small region of the genome covered by a subset of the markers included in the present study (Dawson et al. 2006). The great reed warbler is a heterochiasmate species with an approximately 2-fold higher rate of recombination in females than in males (Hansson et al. 2005). Although this feature is not shared with chicken, where the sexes have similar recombination rates (Groenen et al. 2000), heterochiasmy is a well-known phenomenon in other species, including human and mouse (Dietrich et al. 1996; Kong et al. 2002; reviewed by Lenormand and Dutheil 2005). Our present comparative genomic analysis of chicken and great reed warblers was possible because several microsatellites that had been mapped in great reed warblers had homologous sequences in the chicken (see below). We assigned a chromosomal location in chicken to microsatellite loci mapped in great reed warblers based on their sequence similarity to the chicken genome sequence by Blast analyses. The rationale was similar to that of Dawson et al. (2006), but in the present study, we were able to use a much larger data set of homologous loci by using data from recently mapped great reed warbler loci and by using refined Blast methods. We first conducted a direct Blast search of the microsatellites to the chicken genome assembly and, second, loci that remained unmapped were matched against National Center for Biotechnology Information's (NCBI's) library of zebra finch *T. guttata* whole-genome shotgun (WGS) sequences, which in turn were compared against the chicken genome sequence and assigned a chromosomal location in chicken (see below). In the present paper, we were particularly interested in evaluating whether the linkage map of the chicken can serve as a predictor of recombination fractions and linkage map distances in other birds and whether genome structure and synteny have been conserved across a larger proportion of the genome and to identify any major chromosomal rearrangements.

Materials and Methods

Great Reed Warbler Pedigree, Genotyping and Linkage Map

The great reed warbler *A. arundinaceus* is a large-sized reed warbler of the family Sylviidae (Helbig and Seibold 1999). The species is a long-distance migrant that winters in Africa and breeds in reed lakes in Eurasia (Bensch 1996; Hansson et al. 2003). Its karyotype is not yet described, but other studied passerines (e.g., chaffinch *Fringilla coelebs*, blackbird *Turdus merula*, redwing *Turdus iliacus*, and zebra finch) have similar numbers of macro- and microchromosomes ($2n = 80$; Derjusheva et al. 2004; Itoh and Arnold 2005) to chicken ($2n = 78$; Masabanda et al. 2004). As in all birds, male great reed warblers are homogametic (ZZ), whereas females are heterogametic (ZW).

In the present study, we use linkage map distances from the mapping study of a great reed warbler pedigree population at Lake Kvismaren, southern central Sweden (Åkesson et al. 2007; cf., Hansson et al. 2005), where a detailed ecological study has been ongoing since 1983 (Bensch 1996; Hasselquist 1998; Hansson, Bensch, Hasselquist 2000). All details concerning the pedigree and genotyping can be found in Hansson et al. (2005) and in Åkesson et al. (2007), but for clarity some details are briefly summarized here. Genotypes came from an extended pedigree containing 812 individuals. Alleles of a large number of markers (microsatellites, amplified fragment length polymorphism (AFLPs), indels, and single nucleotide polymorphism (SNP) were polymerase chain reaction (PCR)–amplified and then separated and visualized using an ABI 3100 or an ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA). Genotypes were analyzed in GENEMAPPER 3.0 (Applied Biosystems). The linkage groups were constructed in CRIMAP 2.4 (Lander and Green 1987). This program calculates 2-point recombination fractions, provides logarithmic odds (LOD) scores for recombination estimates, and tests marker order. We assigned autosomal markers to linkage groups by calculating the recombination fractions between all pairs of markers with the TWOPOINT option in CRIMAP; markers were considered as significantly linked at a threshold of LOD > 3.0. The Z-linked loci were assigned to this linkage group on the basis of every female (being hemizygous, ZW) having only a single allele at codominant loci and on the basis of the segregation patterns in the pedigree for the dominant AFLP markers. We then determined the most parsimonious ordering of groups of linked loci with the options BUILD, FLIPSn, and FIXED. There was pronounced heterochiasmy in great reed warblers, and all map distances were derived from sex-specific analyses (Hansson et al. 2005). Map distances are given in Kosambi centimorgans (cM).

In total, we have genotype data for 62 microsatellite loci in great reed warblers (53 autosomal loci on LG1–LG15; 6 unlinked autosomal loci, and 3 Z-linked loci; see below). In the present study, we used map distances of the most parsimonious map in the most recent great reed warbler mapping analysis, which included data from both microsatellite and AFLP markers and a few indels and SNPs (Åkesson et al. 2007). The map consists of 21 autosomal LGs (LG1–LG21) each with 2–15 markers (in total 53 microsatellite loci and 50 AFLPs) and spans 552 cM in males and 858 cM in females (Åkesson et al. 2007; cf., Hansson et al. 2005). The LGZ consists of 12 markers (3 microsatellites, 3 indels, 1 SNP, and 5 AFLPs) and is 155 cM (Åkesson et al. 2007; cf. Hansson et al. 2005).

The updated linkage map (Åkesson et al. 2007) includes 10 new linkage groups (LG12–LG21) compared with the previous microsatellite-based map (Hansson et al. 2005) and includes 3 additional microsatellites (*Titgata02*, *VeCr08*, and *DkiD124*) on LG3, 2 (*Pocc8* and *Ase13*) on LG6, and a few microsatellites on some newly assigned linkage groups (*Ase11* on LG12, *Ase27* and *Ase43* on LG13, *Ase57* on LG14, and *Sjr4* on LG15). Six autosomal microsatellites [*Aar2*, *Ase16*, *G7* (=*Aar5*), *Gf08*, *Lswμ9*, and *ZL18*] are unlinked to all other markers and may be located on unique chromosomes or chromosome arms (Åkesson et al. 2007). The gene order and map distances are similar for most parts of the linkage groups that appear in both Hansson et al. (2005) and Åkesson et al. (2007), but for LG3 (the homologue of Gga2; from here on the chicken chromosomes are referred to by their Gga-number) the gene order of some loci changed when the order was reassessed after adding more markers (Hansson et al. 2005: *Gf15–PAT MP 2-43–Ase32–Ase44–PmaTGAn42–Ase10–Pdoμ4–LOX1*; Åkesson et al. 2007: *060F–PAT MP 2-43–Gf15–Pdoμ4–Ase10–Titgata02–VeCr08–PmaTGAn42–Ase32–Ase44–DkiD124–LOX1–136G–360A–216C*).

Detection of Homologous Single-Copy Chicken–Passerine Sequences

Dawson et al. (2006) conducted Blast analyses of 550 passerine microsatellite sequences, including most loci used in the great reed warbler mapping study of Hansson et al. (2005), on the 1st draft chicken genome sequence (International Chicken Genome Sequencing Consortium 2004; http://www.ncbi.nlm.nih.gov/genome/guide/chicken/). With the Blast settings in Dawson et al. (2006) (the "relaxed sequence similarity search" at an E value $<1 \times 10^{-05}$) it was possible to detect single homologous sequences and thus to predict a single chicken chromosomal location, for 23 great reed warbler microsatellite loci and 4 indels (supplementary appendix 1 in Dawson et al. 2006).

In the present study, we mapped 8 additional markers in the great reed warbler pedigree (*Ase13*, *Ase16*, *Ase43*, *DkiD124*, *Lswμ9*, *Pocc8*, *Titgata02*, and *VeCr08*; Åkesson et al. 2007). We also assigned more loci mapped in the great reed warbler pedigree to chicken chromosomes (see below) than did Dawson et al. (2006) for the following reasons. First, we used sequence data for 2 newly published loci (*Titgata02* and *VeCr08*). Second, we obtained sequence data for loci where such data were previously completely missing (*PAT MP 2-43*, *G7* [=*Aar5*], *G61*, and *Sjr4*) and additional sequence data for locus *Pdoμ4* where the original sequence data submitted to GenBank was limited. For the former loci (i.e., *PAT MP 2-43*, *G7* [=*Aar5*], *G61*, and *Sjr4*), we amplified the locus, ligated the PCR-amplified product into pUC-based plasmid, and sequenced it in the forward and reverse orientation using M13 primers to obtain a consensus sequence (accession numbers are provided in table 1). The original (shorter) sequence data for locus *Pdoμ4* was run through a Blast search but remained unmapped by Dawson et al. (2006). Only the region of sequence between the *Pdoμ4* forward and reverse primers had been submitted to European Molecular Biology Laboratory's sequence database (EMBL) (X93505—422 bp; Neumann and Wetton 1996), as opposed to the entire sequence of the *Mbo*I restriction fragment originally isolated (Neumann 1996). In an attempt to assign a location in the chicken genome for the locus *Pdoμ4*, the additional sequence data for the *Mbo*I restriction fragment was obtained and submitted to EMBL (AM287191—538 bp; extra sequence data kindly provided by Neumann K, personal communication; see Neumann 1996). Third, we performed a Blast search of the sequence data against the most recent chicken genome assembly ("WASHU 2.1" released in May 2006; http://pre.ensembl.org/Gallus_gallus/index.html), which has a higher proportion of sequence data assigned to named chromosomes than the previous draft (increase from ca. 85% to ca. 95%), and particularly so for the Z chromosome (increase from 33.6 to 74.6 Mb), so more hits were expected to be assigned to named chromosomes, though it should be noted that no extra sequence data were added in the new chicken genome assembly release. Fourth, for all loci we applied the most relaxed Blast method used in Dawson et al. (2006) previously used for mapping the sex-linked loci: the Ensembl WU-Blast software (Gish W. 1996–2004; http://blast.wustl.edu) using the Ensembl chicken genome browser and the "distant homologies" search setting, that is, the default setting that is optimized for detecting homology between divergent taxa (http://www.ensembl.org/Gallus_gallus/blastview; an important difference to the Blast method used for the autosomal loci in Dawson et al. (2006) is that the word size is lower, 9 instead of 11). We accepted matches that had an E value of $<1 \times 10^{-5}$ and when more than one significant match occurred the best hit had to be $<1 \times 10^{-10}$ and the next nearest hit had to be more than 1×10^{-10} weaker. When the length of a significant match was less than 70 bp, as was the case for locus *Pdoμ6* only, we confirmed the match with the method below utilizing homologous zebra finch sequences (table 1). All contaminating vector or linker sequences were identified where possible and removed before the sequences were run through a Blast search. Finally, all loci that could not be directly assigned a location in the chicken genome were mapped using the zebra finch orthologue of the locus. The orthologous zebra finch sequence was identified by performing a cross-species megaBLAST search in NCBI's zebra finch WGS database (http://www.ncbi.nlm.nih.gov/projects/genome/seq/BlastGen/BlastGen.cgi?pid=12898). Matches that had an E value of $<1 \times 10^{-5}$ were accepted. The homologous zebra finch sequences (including their flanks) were much longer (ca. 700–900 bp) than the original microsatellite sequences, and therefore, when matched against the chicken genome (using the Ensembl distant homologies search settings as above), many could be assigned a location in the chicken genome.

Linkage Map Distances in Chicken

The homologs of the passerine microsatellite loci are not included on the chicken linkage map, and therefore, their genetic map distances were estimated as follows. For each chicken chromosome, we used the publicly available genetic and physical map data of all sequence tagged site (STS) markers (which are mainly microsatellites; http://

Table 1
Location of the Microsatellite and Indel Loci Mapped in Great Reed Warblers on the Chicken Genome Assigned Using a Blast Analysis of the Source Passerine Species Sequence against the Chicken Genome (v2.1, Ensembl Release 42, December 2006)

Locus	EMBL Accession Number	Locus Reference	Chromosome (Gga)[a]	Chromosome Location (bp)	*E* Value[b]	Unique Significant Location
Microsatellites						
Aar1	AF234985	Hansson, Bensch, Hasselquist, Lillandt, et al. 2000	—	—	NS	No
Aar2	AF234986	Hansson, Bensch, Hasselquist, Lillandt, et al. 2000	5	60, 308, 028	9×10^{-32}	Yes
Aar3	AF234987	Hansson, Bensch, Hasselquist, Lillandt, et al. 2000	4	75, 159, 351	3×10^{-16}	Yes
Ase7	AJ287390	Richardson et al. 2000	3	98, 595, 550	5×10^{-16}	Yes
Ase8	AJ287391	Richardson et al. 2000	—	—	Multiple hits	No
Ase9	AJ287392	Richardson et al. 2000	3	75, 597, 289	1×10^{-28}	Yes
Ase10	AJ287393	Richardson et al. 2000	2	105, 261, 296	3×10^{-32}	Yes
Ase11	AJ287394	Richardson et al. 2000	21	4, 833, 043	4×10^{-12}	Yes
Ase12	AJ287395	Richardson et al. 2000	7	30, 980, 828	1×10^{-16}	Yes
Ase13	AJ287396	Richardson et al. 2000	10	18, 918, 032	7×10^{-07}	No
			5	27, 206, 279	5×10^{-06}	Multiple
Ase15	AJ287398	Richardson et al. 2000	—	—	NS	No
Ase16	AJ276374	Richardson et al. 2000	—	—	NS	No
Ase18	AJ276375	Richardson et al. 2000	3	23, 864, 832	2×10^{-7}	Yes
Ase21	AJ276378	Richardson et al. 2000	5	12,293,574	3×10^{-116}	Yes
Ase27	AJ276384	Richardson et al. 2000	1	20,835,257	5×10^{-09}	No
			Un	39,731,226	1×10^{-08}	Multiple
Ase32	AJ276635	Richardson et al. 2000	2	58, 577, 008	5×10^{-06}	Yes
Ase34	AJ276636	Richardson et al. 2000	8	17, 468, 168	5×10^{-21}	Yes
Ase38	AJ276640	Richardson et al. 2000	—	—	NS	No
Ase42	AJ276644	Richardson et al. 2000	—	—	NS	No
Ase43	AJ276645	Richardson et al. 2000	—	—	NS	No
Ase44	AJ276646	Richardson et al. 2000	2	124, 996, 129	3×10^{-07}	Yes
Ase48[c]	AJ276777	Richardson et al. 2000	5	35, 872, 300	7×10^{-07}	Yes
Ase50	AJ276779	Richardson et al. 2000	Z	54,088,008	2×10^{-60}	Yes
Ase51	AJ276780	Richardson et al. 2000	—	—	NS	No
Ase53	AJ276782	Richardson et al. 2000	—	—	NS	No
Ase55	AJ276784	Richardson et al. 2000	3	63, 955, 332	2×10^{-41}	Yes
Ase56	AJ276785	Richardson et al. 2000	—	—	NS	No
Ase57	AJ276786	Richardson et al. 2000	—	—	NS	No
Ase58	AJ276787	Richardson et al. 2000	—	—	Multiple hits	No
Ase60	AJ276789	Richardson et al. 2000	3	53, 187, 565	3×10^{-51}	Yes
Ase61	AJ276790	Richardson et al. 2000	—	—	NS	No
Ase63	AJ276792	Richardson et al. 2000	—	—	NS	No
Ase64	AJ276793	Richardson et al. 2000	9	6, 167, 593	2×10^{-06}	Yes
Cdi38	AB089175	Otsuka et al. 2003	19	8, 142, 086	2×10^{-20}	Yes
Cuμ02	AF122890	Gibbs et al. 1999	24	5, 384, 752	9×10^{-23}	Yes
Cuμ04	AF122891	Gibbs et al. 1999	5	33, 265, 716	7×10^{-39}	Yes
Cuμ28	AF122894	Gibbs et al. 1999	—	—	NS	No
DkiD124	AY769691	King et al. 2005	—	—	NS	No
Escμ6 (=Aar8)	X77082	Hanotte et al. 1994	1	143, 949, 849	5×10^{-8}	Yes
G61[d]	DQ179379	Nishiumi et al. 1996	—	—	NS	No
G7 (=Aar5)[d]	DQ115906	(Nishiumi I, unpublished data)	—	—	Multiple hits	No
Gf08	AF081932	Petren 1998	—	—	NS	No
Gf15	AF081939	Petren 1998	—	—	NS	No
HrU5	X84090	Primmer et al. 1995	—	—	NS	No
LOX1[e]	Y16820	Piertney et al. 1998	2	130, 451, 211	3×10^{-45}	Yes
Lswμ9	AF129092	Winker et al. 1999	5	61, 177, 123	4×10^{-12}	Yes
Mcyμ4 (=Aar4)	U82388	Double et al. 1997	5	34, 635, 468	3×10^{-10}	Yes
MSLP2	AB031374	Ishibashi et al. 2000	5	49,013,616	8×10^{-07}	Yes
PAT MP 2-43[d]	AM056063	Otter et al. 1998	2	27, 855, 891	4×10^{-15}	Yes
Pdoμ4[d]	AM287191	Neumann and Wetton 1996	2	107, 385, 063	1×10^{-30}	Yes
Pdoμ6[f]	Y15125	Neumann and Wetton 1996	1	84, 764, 542	5×10^{-06}	Yes
PmaTGAn42	AY260540	Saladin et al. 2003	2	62, 574, 195	5×10^{-28}	Yes
Pocc8	U59119	Bensch et al. 1997	—	—	NS	No
Ppi2	AJ272375	Martinez et al. 1999	9	14, 348, 449	1×10^{-31}	Yes
Sjr4[d]	DQ179381	(McDonald DB, Potts WK, unpublished data)	—	—	NS	No
Titgata02	AY792958	Wang et al. 2005	2	105, 859, 093	2×10^{-09}	Yes
VeCr08	AY542881	Stenzler et al. 2004	2	77, 036, 019	1×10^{-16}	Yes
WBSW7	AF130434	McRae and Amos 1999	—	—	NS	No

Table 1
Continued

Locus	EMBL Accession Number	Locus Reference	Chromosome (Gga)[a]	Chromosome Location (bp)	*E* Value[b]	Unique Significant Location
ZL18	AF076668	Degnan et al. 1999	—	—	NS	No
ZL44	AJ517996	Frentiu et al. 2003	13	13, 202, 211	2×10^{-08}	Yes
ZL45	AJ517997	Frentiu et al. 2003	—	—	NS	No
ZL54	AJ518005	Frentiu et al. 2003	—	—	NS	No
Indels						
BRM12	DQ073912	Hansson et al. 2005	Z	27, 168, 555	9×10^{-32}	Yes
BRM15	DQ073913	Hansson et al. 2005	Z	27, 172, 824	3×10^{-09}	Yes
CHD1Z-20	DQ073914	Hansson et al. 2005	W_random	437, 456	9×10^{-13}	No
Chicken CHD	AF004397	Griffiths and Korn 1997	Z	50, 202, 981	$10^{-\infty}$	
			W_random	453, 095	8×10^{-269}	
VLDLR9	DQ073915	Hansson et al. 2005	Z	27, 362, 359	5×10^{-14}	Yes

NOTE.—Primer sequences for the unpublished loci are *G7* (*Aar5*): F = GAGCTCTGTATGTGCGTG; R = TCTGAGTGGACTCAGGAGT (see Hansson, Bensch, Hasselquist, Lillandt et al. 2000) *Sjr4*: F = TCCAGGCTGTGCTTGCACTTG; R = TGCCAGACCACCAACTAAATC (see Hansson et al. 2000).

[a] Gga: Chicken chromosome number. Z = Z chromosome. Un = "GgaUnknown," that is, actual chromosome number not yet assigned.

[b] Blast method (see text for details): Ensembl's distant homologies search settings (W = 9); NS (nonsignificant): *E* value $\geq 1 \times 10^{-05}$.

[c] *Ase48*, sequence originally submitted contained a double insert, but hit attributable to microsatellite-containing fragment (Dawson et al. 2006).

[d] New sequence data was obtained for loci *G61*, *G7* (=*Aar5*), *PAT MP 2-43*, and *Sjr4*. Additional sequence data was obtained for *Pdoμ4* (see text for details).

[e] *LOX1*, sequence contaminated with vector; removed before the Blast search (Dawson et al. 2006).

[f] *Pdoμ6*, the match to the chicken genome was significant, but the length of the match to the chicken genome was only 49 bp. The location in chicken on Gga1 was confirmed by a Blast analysis of the homologous zebra finch WGS sequence [homologous zebra finch sequence gnl|ti|1231564242 produced a 328 bp hit by the forward flank to Gga1, location 84,764,109 (1.5×10^{-39}) and a 339-bp hit by the reverse flank to Gga1, location 84,763,843 (1.4×10^{-56})].

www.ncbi.nlm.nih.gov/genome/guide/chicken/). In chicken, the recombination rate varies only slightly over the chromosomes (Schmid et al. 2005). Therefore, we generated a linear regression equation for each chromosome (using linkage map distance as dependent variable and physical map distance as independent variable) and used this to interpolate the genetic map positions for the homologous sequences in chicken. The approximate location of centromeres was based on Schmid et al. (2005).

Results

Homologous Chicken–Great Reed Warbler Sequences

The direct Blast search of the 62 microsatellites and 4 indels mapped in great reed warbler using the Ensembl chicken genome browser (distant homologies search settings; *E* value of $<1 \times 10^{-05}$) resulted in 37 loci with a single homologous sequence in the chicken genome (table 1). However, the great reed warbler sequence (EMBL accession number: DQ073914) of the indel locus *CHD1Z-20*, which is Z-linked in the great reed warbler, matched a sequence on chicken chromosome "W_random." Because the homologous CHD genes are known to be located on both the W and the Z chromosome in chicken and other birds (e.g., Griffiths and Korn 1997), we used the location at GgaZ achieved by a Blast of the chicken CHD sequence (EMBL accession number: AF004397) and not the match on W_random obtained for the great reed warbler sequence. Two loci, *Ase13* and *Ase27*, had 2 matching chicken sequences (table 1). *Ase13* produced significant matches by 2 different parts of the sequences to 2 different named chicken chromosomes. Part of the forward flank matched a Gga5 location and part of the reverse flank matched a location on Gga10 (table 1). A possible explanation for this is that during the cloning and isolation of this locus, one of the flanks was contaminated with an extra Seychelles warbler (*Acrocephalus sechellensis*) DNA insert at an unidentifiable (blunt-ended) ligation point, and this extra insert maps to a different part of the genome compared with the rest of the sequence. Extra Seychelles warbler (*Mbo*I) DNA inserts had been previously recognized and removed in other clones in this genomic library (*Ase12* and *Ase48*; Dawson DA, unpublished data). Locus *Ase27* had one matching sequence on Gga1 and the exact same 118 bp sequence region matched chromosome "Unknown" (i.e., an assembly of sequences not yet assigned to chromosome; http://pre.ensembl.org/Gallus_gallus/index.html). Because, the matching sequence on Gga1 and on the Unknown chromosome were 100% identical in terms of base pair composition and many large 100–1000 bp sections of Gga1 are duplicated on the Unknown chromosome, the most probable reason for a duplicate copy on the Unknown chromosome is that the sequence assigned to Gga1 was not removed from the Unknown chromosome when the genome sequence was compiled. Alternatively *Ase13* and *Ase27* may have been duplicated in the genome, in which case it is difficult to know which loci represent the orthologous ones.

For 19 of the 29 loci that remained unmapped after the direct Blast search, we found homologous zebra finch sequences (table 2) using a cross-species megaBLAST search in NCBI's zebra finch WGS database (http://www.ncbi.nlm.nih.gov/projects/genome/seq/BlastGen/BlastGen.cgi?pid=12898). Of these 19 loci, 9 had a single homologous sequence in the chicken genome (table 2). *G61* is Z-linked in great reed warblers but the homologous zebra finch sequence mapped to Gga4 (table 2; discussed below). Utilizing the zebra finch sequences did not change the results for *Ase13* and *Ase27*: the flanking regions of *Ase13* matched different zebra finch sequences, which in turn mapped to Gga5 and Gga10, respectively, whereas the zebra finch sequence equivalent of *Ase27* matched both Gga1 and GgaUnknown (table 2).

Table 2
Location of the Microsatellite Loci on the Chicken Genome (v2.1, Ensembl Release 42, December 2006) Assigned via Blast Analyses of the Homologous Zebra Finch WGS Sequences

Locus	EMBL	Homologous Zebra Finch Sequence	Length of Zebra Finch Sequence (bp)	Zebra Finch *E* Value[a]	Chicken Chromosome Number (Gga)[b]	Chicken Chromosome Location (bp)	Chicken *E* Value[c]	Unique Location in Chicken
Aar1	AF234985	gnl|ti|1424280761	898	8×10^{-84}	—	—	Multiple hits	No
Ase8	AJ287391	gnl|ti|1241398207	846	2×10^{-93}	—	—	NS	No
Ase13	AJ287396	gnl|ti|1290408300	878	6×10^{-28}	10	18, 917, 269	6×10^{-92}	No
		gnl|ti|1258878665	886	6×10^{-17}	5	27, 206, 074	2×10^{-26}	Multiple
Ase15	AJ287398	gnl|ti|1397722329	714	1×10^{-10}	—	—	Multiple hits	No
Ase16	AJ276374	—		NS	—	—	—	No
Ase27	AJ276384	gnl|ti|1230196198	933	5×10^{-92}	1	20, 835, 246	4×10^{-34}	No
					Un	39, 731, 040	3×10^{-35}	Multiple
Ase38	AJ276640	gnl|ti|1424471476	853	2×10^{-43}	1	195, 613, 090	5×10^{-17}	Yes
Ase42	AJ276644	gnl|ti|1398948451	941	3×10^{-76}	13	7, 168, 832	6×10^{-37}	Yes
Ase43	AJ276645	gnl|ti|1231562782	852	3×10^{-94}	—	—	NS	No
Ase51	AJ276780	gnl|ti|1255142960	835	8×10^{-117}	—	—	NS	No
Ase53	AJ276782	—		NS	—	—	—	No
Ase56	AJ276785	gnl|ti|1424474211	807	1×10^{-29}	—	—	Multiple hits	No
Ase57	AJ276786	—		NS	—	—	—	No
Ase58	AJ276787	gnl|ti|1395987884	860	2×10^{-24}	8	9, 475, 564	1×10^{-22}	Yes
Ase61	AJ276790	gnl|ti|1238868702	862	7×10^{-38}	—	—	NS	No
Ase63	AJ276792	gnl|ti|1422995470	976	8×10^{-85}	1	165, 186, 140	1×10^{-28}	Yes
Cuμ28	AF122894	gnl|ti|1385164192	928	5×10^{-110}	7	28, 992, 667	8×10^{-36}	Yes
DkiD124	AY769691	gnl|ti|1374758564	762	2×10^{-81}	—	—	Multiple hits	No
G61[c]	DQ179379	gnl|ti|1423061228	823	7×10^{-22}	4	5, 311, 183	3×10^{-49}	Yes
G7 (=Aar5)[c]	DQ115906	gnl|ti|1224444506	797	3×10^{-34}	25	930, 526	6×10^{-27}	Yes
Gf08	AF081932	—		NS	—	—	—	No
Gf15	AF081939	gnl|ti|1285549343	873	1×10^{-36}	2	43, 482, 483	9×10^{-30}	Yes
HrU5	X84090	—		NS	—	—	—	No
Pocc8	U59119	gnl|ti|1423178969	828	2×10^{-61}	5	16, 222, 460	7×10^{-18}	Yes
Sjr4[c]	DQ179381	—		NS	—	—	—	No
WBSW7	AF130434	gnl|ti|1405401644	646	1×10^{-47}	—	—	Multiple hits	No
ZL18	AF076668	—		NS	—	—	—	No
ZL45	AJ517997	gnl|ti|1253384330	722	4×10^{-123}	—	—	NS	No
ZL54	AJ518005	gnl|ti|1287053601	945	1×10^{-08}	—	—	Multiple hits	No

[a] Blast method (see text for details): NCBI's cross-species megaBLAST search of the zebra finch WGS database; NS: *E* value $\geq 1 \times 10^{-05}$.
[b] Blast method (see text for details): Ensembl's distant homologies search settings (W = 9); NS: *E* value $\geq 1 \times 10^{-05}$.
Z = Z chromosome. Un = "GgaUnknown," that is, chromosomes not yet assigned a number.
[c] New sequence data was obtained for loci *G61*, *G7* (=*Aar5*), and *Sjr4*.

This means that we could use comparative data for 46 of the 66 loci (70%; including *CHD1Z-20* and *G61*, but excluding the potentially duplicated loci *Ase13* and *Ase27*). Note that we do, however, present data for the homologous sequence on Gga1 for *Ase27* (table 1; fig. 1). This is because, using the previous chicken genome assembly (draft 1.0), 4 loci each displayed homology to both a named chromosome and the unknown chromosome (*Ase11*, *Ase21*, *Ase27*; supplementary appendix 1 in Dawson et al. [2006]; and *VeCr08*), but when blasted against the present chicken genome assembly (draft 2.1), 3 of these (*Ase11*, *Ase21*, and *VeCr08*) produced only a single unique hit to the named chromosome (table 1), suggesting that when sequences are reassigned to a named chromosome during genome assembly they are not always removed from the Unknown chromosome. This suggests that *Ase27* may be located at a unique location on chromosome 1; the next release of the chicken genome sequence may resolve this.

Synteny in Homologous Chicken–Great Reed Warbler Regions

Loci from the same great reed warbler linkage group matched sequences on a single chicken chromosome in the Blast analyses (fig. 1 and table 3), with the exception of locus G61 on LGZ that mapped to Gga4, whereas the other loci on LGZ mapped to GgaZ. Likewise, most loci on the same chicken chromosome matched sequences on a single great reed warbler linkage group (fig. 1 and table 3), with the exceptions of 1) Gga1, represented by 2 great reed warbler linkage groups (LG5 and LG13), 2) Gga4, represented by markers from LG7 and LGZ, and 3) Gga5, represented by markers on LG6 and 2 unlinked markers (fig. 1 and table 3). This result strongly suggests that the chromosome structure has been highly conserved between chicken and passerines.

Locus *Ase27* mapped to one end of Gga1 (and as mentioned above also had a 2nd hit on the GgaUnknown chromosome), whereas the markers forming the linkage group LG5 mapped onto the opposite end of Gga1 (fig. 1 and table 3). This suggests that *Ase27* (and locus *Ase43* and 4 AFLP markers that together with *Ase27* constitute LG13; Åkesson et al. 2007) and LG5 are located on the 2 different passerine macrochromosomes, which, as mentioned above, are both homologous to Gga1 (Derjusheva et al. 2004; Itoh and Arnold 2005). Thus, the separation of the reported split of Gga1 to form 2 macrochromosomes should be located on Gga1 at a position between 20.8 and 84.8 Mb (between *Ase27* and *Pdoμ6*; fig. 1 and table 3;).

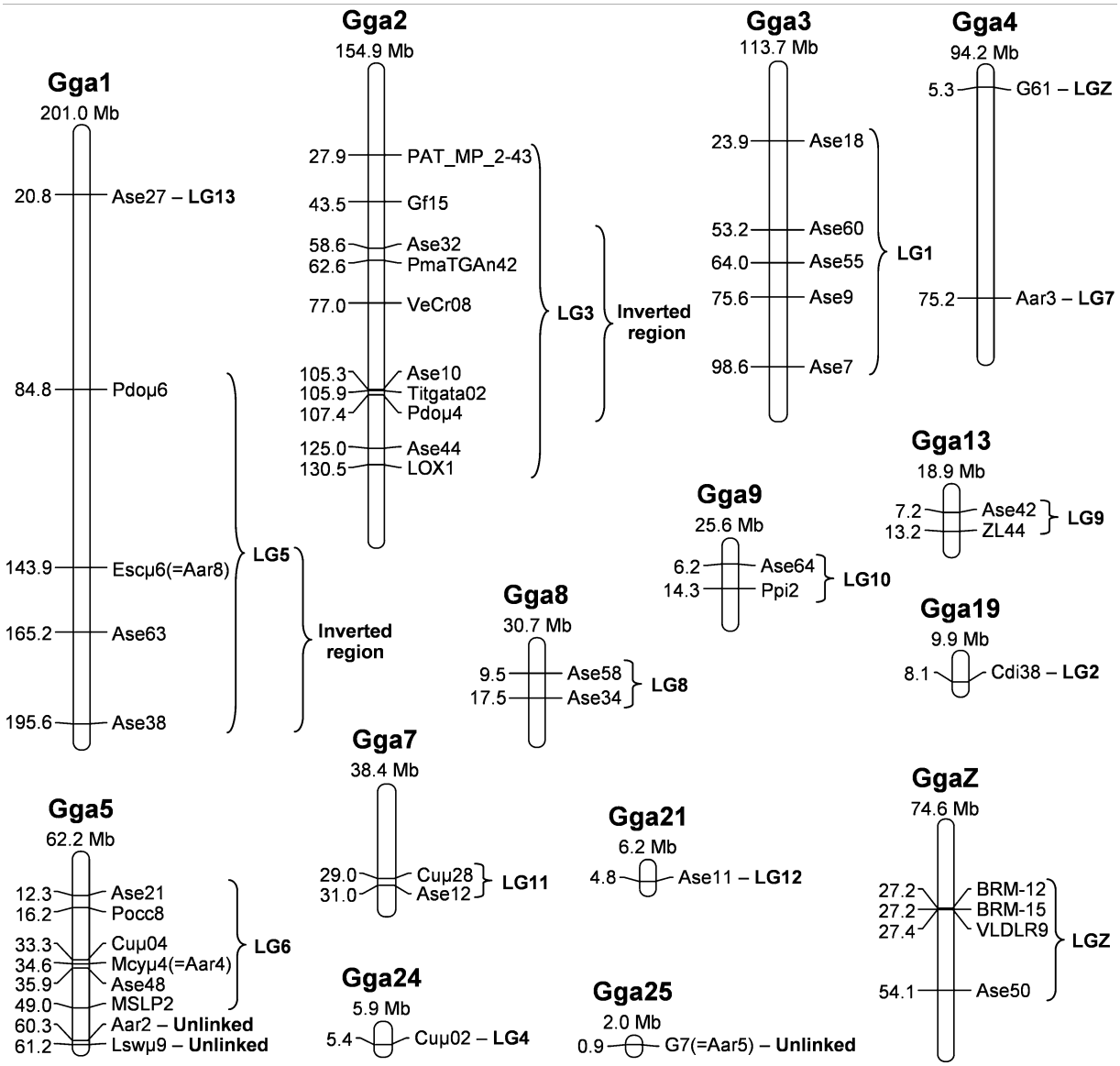

FIG. 1.—Location of the homologous loci of microsatellites mapped in great reed warblers on the chicken chromosomes (Gga). Each chicken chromosome corresponded to a unique great reed warbler linkage group with 3 exceptions: Gga1 was represented by LG5 and LG13, Gga4 by LG7 and LGZ, and Gga5 by LG6 and 2 loci unlinked in great reed warblers. The inverted regions of Gga1–LG5 and Gga2–LG3 are indicated (see text and fig. 2 for details).

Surprisingly, locus *G61*, which is Z-linked in great reed warbler, mapped to Gga4. Gga4 is known to be homologous to 1 macrochromosome and 1 microchromosome in passerines (Derjusheva et al. 2004; Itoh and Arnold 2005), but there are no data that suggest that Gga4 has homologous regions in the passerine Z chromosome, which may suggest that this match is spurious. Alternatively, the match is correct and may instead mirror a more complex history of locus *G61* (e.g., a duplication, followed by a translocation and a deletion in chicken).

The Blast analysis located *Aar2* and *Lswμ9* on Gga5 (tables 1 and 3) but these markers were not significantly linked to the markers on LG6 (match Gga5) or with each other in the 2-point analysis (Åkesson et al. 2007). This may be explained by the fact that these markers (*Aar2* and *Lswμ9*) and also other markers on LG6 had few informative meioses and therefore low power in the linkage analyses (Hansson et al. 2005; Åkesson et al. 2007) or that these loci are located in the telomeric region, which may have a higher recombination rate, as found in other species (Nachman and Churchill 1996). Indeed, these 2 loci are located close to the end of Gga5 in chicken (positions 60.3 and 61.2 Mb, respectively; Gga5 has a total size 62.2 Mb; fig. 1). Alternatively, this may indicate that homology has been less conserved for this region of Gga5. In contrast, the chromosomal region of Gga5 from 12.3–49.0 Mb appears to be remarkably well conserved, as indicated by the *E* values and the conserved synteny for the loci mapped to this region (table 3).

Nine different great reed warbler linkage groups and chicken chromosomes shared at least 2 homologous loci

Table 3
Microsatellite Linkage Map Distances in Great Reed Warbler, the Assigned Physical Position of the Homologous Sequences in Chicken, and the Estimated Map Distances in Chicken

	Great Reed Warbler Linkage Location			Homologous Chicken Chromosome Sequence Location		Homologous Chicken Linkage Location		Chromosome Coverage for Homologous Region Coverage (%); Total Size of Gga (Mb)[d]
Locus	LG[a]	Map Location in ♂/♀ (cM)[a]	Cumulative Distance in ♂/♀ (cM)	Gga[b]	Start Position(bp)	Estimated Position (cM)[c]	Cumulative Distance (cM)	
Ase18	1	27/0	0/0	3	23, 864, 832	81	0	
Ase60	1	35/21	8/21	3	53, 187, 565	162	81	
Ase55	1	36/24	9/24	3	63, 955, 332	191	110	
Ase9	1	37/27	10/27	3	75, 597, 289	223	142	
Ase7	1	41/36	14/36	3	98, 595, 550	287	206	66%; 114 Mb
Ase51	1	69/89		—	Not mapped			
ZL54	2	0/0		—	Not mapped			
Cdi38	2	26/39		19	8, 142, 086			
PAT MP 2-43	3	8/5	0/0	2	27, 855, 891	96	0	
Gf15	3	9/5	1/0	2	43, 482, 483	137	41	
Pdoμ4	3	11/12	—[e]	2	107, 385, 063	304	—[e]	
Ase10	3	12/14	—[e]	2	105, 261, 296	299	—[e]	
Titgata02	3	12/16	—[e]	2	105, 859, 093	300	—[e]	
VeCr08	3	14/17	—[e]	2	77, 036, 019	225	—[e]	
PmaTGAn42	3	14/18	—[e]	2	62, 574, 195	187	—[e]	
Ase32	3	15/18	—[e]	2	58, 577, 008	176	—[e]	
Ase44	3	15/18	7/13	2	124, 996, 129	350	254	
DkiD124	3	17/18		—	Not mapped			
LOX1	3	27/29	19/24	2	130, 451, 211	365	269	66%; 155 Mb
Ase53	4	0/0		—	Not mapped			
Cuμ02	4	23/54		24	5, 384, 752			
Pdoμ6	5	11/15	—[f]	1	84, 764, 542	251	—[f]	
Ase38	5	17/15	0/0	1	195, 613, 090	555	142	
Ase56	5	19/15		—	Not mapped			
Ase63	5	21/23	4/8	1	165, 186, 140	471	58	
HrU5	5	22/26		—	Not mapped			
Escμ6 (=Aar8)	5	22/31	5/16	1	143, 949, 849	413	0	26%; 201 Mb
Ase8	5	25/37		—	Not mapped			
Ase13	6	32/132		(10) (5)	(18, 918, 032) (27, 206, 279)			
Ase21	6	37/142	0/0	5	12, 293, 574	35	0	
Pocc8	6	37/142	0/0	5	16, 222, 460	47	12	
Cuμ04	6	39/151	2/9	5	33, 265, 716	98	63	
Mcyμ4 (=Aar4)	6	39/154	2/12	5	34, 635, 468	102	67	
Ase48	6	39/154	2/12	5	35, 872, 300	106	71	
WBSW7	6	39/159	2/17	—	Not mapped			
MSLP2	6	40/162	3/20	5	49, 013, 616	146	111	59%; 62 Mb
Ase61	7	0/0		—	Not mapped			
Ase15	7	5/0		—	Not mapped			
Aar3	7	8/11		4	75, 159, 351			
Ase58	8	4/37	0/0	8	9, 475, 564	19	0	
Ase34	8	6/43	2/6	8	17, 468, 168	48	29	26%; 31 Mb
Ase42	9	0/0	0/0	13	7, 168, 832	26	0	
ZL44	9	4/5	4/5	13	13, 202, 211	52	26	32%; 19 Mb
ZL45	9	16/5		—	Not mapped			
Ase64	10	23/14	0/0	9	6, 167, 593	39	0	
Ppi2	10	24/18	1/4	9	14,3 48, 449	78	39	30%; 26 Mb
Cuμ28	11	12/0	0/0	7	28,992,667	110	0	
Ase12	11	12/0	0/0	7	30, 980, 828	118	8	5%; 38 Mb
Ase11	12	0/0		21	4, 833, 043			
Ase27	13	14/47		(1) (Un)	(20, 835, 257) (39, 731, 226)	(73)		
Ase43	13	27/66		—	Not mapped			
Ase57	14	4/34		—	Not mapped			
Sjr4	15	0/0		—	Not mapped			
Aar1	Z	0/–		—	Not mapped			
BRM-12	Z	34/–	0/–	Z	27, 168, 555	64	0	
VLDLR-9	Z	34/–	0/–	Z	27, 362, 359	65	1	
BRM-15	Z	35/–	1/–	Z	27, 172, 824	(64)		
CHD1Z-20	Z	44/–		Z	50, 202, 981	137		

Table 3
Continued

Locus	Great Reed Warbler Linkage Location			Homologous Chicken Chromosome Sequence Location		Homologous Chicken Linkage Location		Chromosome Coverage for Homologous Region Coverage (%); Total Size of Gga (Mb)[d]
	LG[a]	Map Location in ♂/♀ (cM)[a]	Cumulative Distance in ♂/♀ (cM)	Gga[b]	Start Position(bp)	Estimated Position (cM)[c]	Cumulative Distance (cM)	
				(W)	(437,456)			
Ase50	Z	44/–	10/–	Z	54,088,008	149	85	36%; 75 Mb
G61	Z	155/–		(4)	(5,311,183)			
Aar2	—			5	60,308,028			
Lswμ9	—			5	61,177,123			
ZL18	—			—	Not mapped			
G7 (=Aar5)	—			25	930,526			
Ase16	—			—	Not mapped			
Gf08	—			—	Not mapped			

NOTE.—Also shown is chromosome coverage in chicken for each homologous region. Nine great reed warbler linkage group and chicken chromosomes (Gga) shared 2 or more loci.

[a] linkage group and map position are from a linkage map consisting of microsatellites and AFLP markers (Åkesson et al. 2007).

[b] Gga: Chicken chromosome number. Un = "GgaUnknown."

[c] Estimated from linear regression equations based on linkage and sequence positions of chicken STSs (Estimated position in cM = $a + b \times 10^{-6} \times$ start position in base pairs; GgaX/a/b: Gga1/15.61/2.75; Gga2/22.79/2.62; Gga3/18.24/2.53; Gga5/−2.23/3.02; Gga7/−0.43/3.82; Gga8/−16.20/3.69; Gga9/9.11/4.82; Gga13/−5.56/4.37; GgaZ/−21.44/3.16).

[d] Data from: http://www.ncbi.nlm.nih.gov/genome/guide/chicken/.

[e] Data not given because these markers are located in the inverted region.

[f] Data not given because this marker is in the rearranged region.

(LG–Gga: 1–3, 3–2, 5–1, 6–5, 8–8, 9–13, 10–9, 11–7, and Z-Z) and, of these, 5 regions shared at least 3 homologous loci (LG–Gga: 1–3, 3–2, 5–1, 6–5, and Z-Z; fig. 1 and table 3). Synteny was fully conserved for both LG1–Gga3 and LG6–Gga5 (fig. 1 and table 3;). LG3–Gga2 showed a large inversion involving at least 48.8 Mb of Gga2; (markers *Pdoμ4*, *Ase10*, *Titgata02*, *VeCr08*, *PmaTGAn42*, and *Ase32*; fig. 2). Within the inversion, synteny was also fully conserved with the exception of locus *Titgata02*, which was located between *Ase10* and *VeCr08* in the great reed warbler and between *Ase10* and *Pdoμ4* in chicken. Note, however, that for *Titgata02*, the map location assigned based on the mapping of the great reed warbler pedigree is not a single unambiguous location: a 2nd possible position, between *Pdoμ4* and *Ase10*, does not differ significantly from the most parsimonious position between *Ase10* and *VeCr08* indicated in figure 2 (Åkesson et al. 2007). Thus, we can conclude that synteny was conserved within the inversion for all so-called framework loci with a robust and unambiguous position on the great reed warbler map (i.e., *Ase10*, *VeCr08*, *PmaTGAn42*, and *Ase32*) and that if *Titgata02* was inverted within the inversion then this 2nd inversion was small. A 2nd inversion was detected for LG5–Gga1. Here, a 51.7-Mb region toward the end of Gga1 has been inverted in great reed warblers. There is a striking difference in linkage for markers *Pdoμ6* and *Ase38* in the 2 species, with very tight linkage in great reed warblers (6.4 cM in males and 0.0 cM in females) and wide separation in chicken (110.8 Mb; fig. 2). This may suggest that the split of Gga1 to form 2 macrochromosomes was followed by additional chromosomal rearrangements. If we ignore locus *G61* (and its match to Gga4), there was only slight deviation from synteny for LGZ–GgaZ involving the joint location of the loci *BRM-12*, *BRM-15*, and *VLDLR9*. However, these 3 loci are extremely tightly linked in great reed warblers (within 1 cM; table 3), and their relative ordering is not fully resolved (Åkesson et al. 2007). The most parsimonious ordering provided in table 3 does not differ from the order suggested by the chicken data (Åkesson et al. 2007).

Linkage Map Distances in Chicken and Great Reed Warblers

Available linkage map and sequence position data for 479 chicken STS markers were analyzed with linear regression to interpolate the linkage map positions for the homologous chicken sequences (table 3). Three of the 479 STSs were obvious outliers (detected by studentized residual test and by eye) in the linear regression analyses (locus–chromosome: *LEI0138*–Gga1, *ADL0314*–Gga1, and *MCW0324*–Gga2) and were therefore excluded from the data set before calculating regression lines. In total, we used data from 476 STSs on the 9 chromosomes (ranging from 19 STSs on Gga9 to 141 STSs on Gga1) to calculate regression lines and for estimating map positions of the homologous loci (table 3). The homologous regions covered between 5% (LG11–Gga7) and 66% (LG1–Gga3 and LG3–Gga2) of their respective chicken chromosome lengths (table 3, fig. 3).

Our result shows that the recombination-based linkage map distances were substantially smaller in great reed warblers than in chicken (table 3 and fig. 3). The summed map distance of male (58 cM) and female (111 cM) great reed warblers was only 6.3% and 13.3% of that of the chicken in

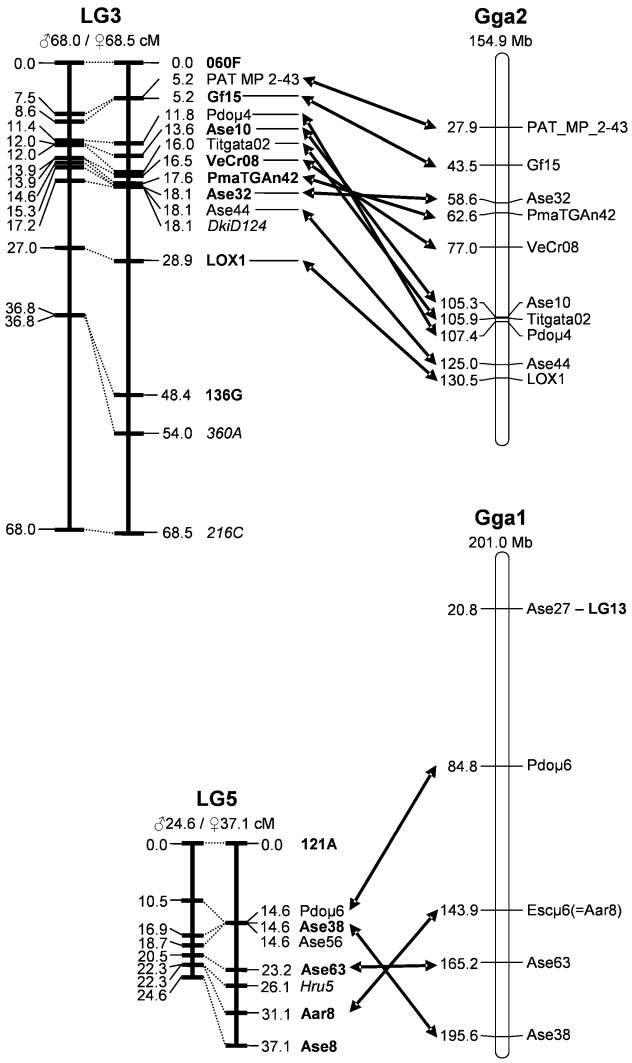

FIG. 2.—The inversions on LG3–Gga2 and LG5–Gga1. The great reed warbler linkage groups (LG3 and LG5) are shown with the male map to the left and the female map to the right (data from Åkesson et al. 2007). LG3 consists of 11 microsatellites and 4 AFLPs (e.g., *060F*), and LG5 consists of 7 microsatellites and 1 AFLP. The inverted regions span at least 48.8 Mb for Gga2 (between *Ase32* and *Pdoμ4*) and 51.7 Mb for Gga1 (between *Escμ6* and *Ase38*) in chicken. Great reed warbler marker names in bold font indicate framework loci, that is, loci with an unambiguous position; normal font indicates loci with 2 possible positions; and italics font indicates loci with more than 2 possible positions.

the homologous chromosomal regions (915 cM in males and 830 cM in females; table 2 and fig. 3). The great reed warbler had smaller map distance than chicken in all 9 chromosome comparisons (table 3), and the pattern was significant for both male and female great reed warbler map distance data (paired *t*-test: males, $t = 3.40$, degree of freedom [df] = 8, $P = 0.009$; females, $t = 2.99$, df = 7, $P = 0.020$). The inversion on LG3–Gga2 did not affect the recombination rate analysis because there were informative noninverted markers flanking both sides of it at either end of the chromosome (*PAT MP 2-43* and *Gf15*, and *Ase44* and *LOX1*, respectively; table 3); and for LG5–Gga1, we use data for the inverted region (table 3 and fig. 3).

Discussion

We have conducted comparative gene mapping in great reed warblers and chicken by using data from 46 of the 66 loci (70%) mapped in great reed warblers. As previously suggested from a smaller data set of homologous chicken–great reed warbler loci (Dawson et al. 2006), our results confirm that genome structure and synteny have been remarkably conserved between chicken and great reed warblers, with the exception of the newly identified rather large inversions on LG3–Gga2 and LG5–Gga1. However, there are as yet large regions of the genome to be comparatively analyzed including the entirety of Gga6 and the regions of poor coverage across the chromosomes 1, 4, 7, and 8. Moreover, we are with the present low-density map unlikely to detect small-scale inversions. It therefore seems likely that future studies will identify other large- and small-scale inversions in passerines and chicken.

Loci from the same great reed warbler linkage groups matched sequences on a single chicken chromosome in the Blast analyses (with the exception of *Ase13* on LG6 that had a match to both Gga5 and Gga10 and *Ase27* on LG13 that mapped to both Gga1 and GgaUnknown). Likewise, most loci on the same chicken chromosome matched sequences on a single great reed warbler linkage group, with the exception of the following 1) chromosome Gga1, which was, if we consider the homologous sequence on Gga1 for *Ase27*, represented by 2 great reed warbler linkage groups (LG5 and LG13), 2) Gga4, represented by markers from LG7 and LGZ, and 3) Gga5, represented by markers on LG6 and 2 unlinked markers. This result strongly suggests that the chromosome structure has been highly conserved between chicken and passerines. Synteny was fully conserved for both LG1–Gga3 and LG6–Gga5 and possibly for LGZ–GgaZ. For LG3–Gga2, we detected a previously unknown large inversion, involving 6 loci and at least 48.8 Mb up to 81.5 Mb (32–53%) (fig. 2). Four of the 6 inverted great reed warbler loci (*Ase10*, *VeCr08*, *PmaTGAn42*, and *Ase32*) and 2 flanking loci on each side of the inversion (*060F* and *Gf15*, and *LOX1* and *136G*, respectively) have unambiguous map locations and are so-called framework loci (Åkesson et al. 2007). A 2nd inversion was detected for LG5–Gga1. Here a 51.7-Mb region near the end of Gga1 has been inverted in great reed warblers. We can rule out the possibility that these inversions were due to errors in the assembly of the chicken genome sequence because the sequence orientation was supported by the order of the STSs on the chicken linkage map. The detected inversions suggest that one may expect occasional intrachromosomal rearrangements between chicken and passerines, in addition to the already-described main cytogenetic alterations (i.e., that Gga1 is homologous to 2 passerine macrochromosomes and that Gga4 is homologous to 1 macrochromosome and 1 microchromosome in passerines; Derjusheva et al. 2004; Itoh and Arnold 2005).

Because only one marker on LG7 mapped to Gga4, we were unable to identify the location of the split of this chromosome into 2 passerine chromosomes. If we accept the match of Ase27 to Gga1, our result identifies the region where Gga1 splits into 2 macrochromosomes to be between 20.8 and 84.8 Mb, and adding new markers will provide the

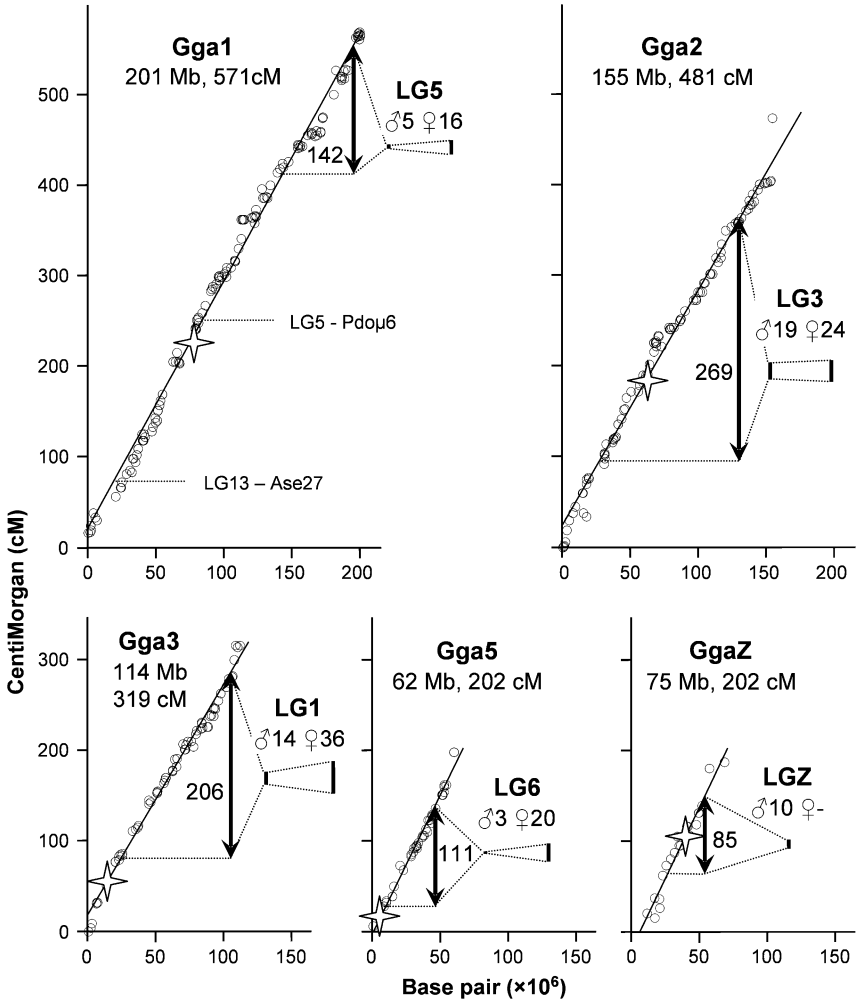

FIG. 3.—Linkage map distance (cM) in relation to physical distance (bp) for STS markers on 5 chicken chromosomes (Gga1, 2, 3, 5, and Z). The linear regression equations were used to estimate linkage map positions of homologous loci in chicken. Indicated are: linkage map distances of the homologous regions in chicken (arrows) and great reed warblers (vertical short lines; LG1, 3, 5, 6, and Z) and centromere locations (stars).

potential to pinpoint more precisely where this split has occurred. In addition, the detected inversion on LG5–Gga1 may suggest that the split of Gga1 was followed by additional chromosome rearrangements. Two markers at the end of Gga5 in chicken are unlinked to the other markers on this chromosome (LG6) in great reed warblers, which may suggest that the end of Gga5 (60.3–61.2 Mb region) comprises a separate passerine microchromosome. Alternatively, linkage between these markers and markers on LG6 could not be verified because these loci had few informative meioses and consequently low statistical power (Hansson et al. 2005; Åkesson et al. 2007) or because they were located very close to the telomere (which is the case in chicken; see fig. 1), which may have a higher recombination rate, as found for telomeric markers in other species, for example, *Drosophila* (Nachman and Churchill 1996).

There are so far no comparable data available for the autosomes of other passerine species, but 2 independent studies have recently conducted comparative analyses of the Z chromosome in chicken and zebra finch *T. guttata* (Itoh et al. 2006) and collared flycatcher *F. albicollis* (Backström, Brandstrom, et al. 2006), respectively. In accordance with our present findings, there was a high degree of synteny between chicken and each of these passerines, with few rearrangements (one in each comparison). As in our study, Backström, Brandstrom, et al. (2006) compare passerine linkage map data to chicken sequence data. However, they used data from a previous chicken genome assembly (draft 1.1), which differed substantially, particularly so for the Z chromosome, from the recently released assembly (draft 2.1) used in the present study. This complicates a direct comparison between their and our results for this chromosome, although the high degree of synteny in both studies supports our conclusions of conserved synteny.

We observed that there is variation between chromosomes in the number of loci being mapped and, for instance, not a single locus was mapped on Gga6 and relatively few loci on Gga1, 4, 7, and 8 (see fig. 1). In contrast, both Gga2 and Gga5 have a high number of mapped markers per base pair of sequence. It is possible that the number of loci mapped per base pair of sequence indicates which regions of the

genome are more conserved and that the strength (*E* value) of the Blast hits may show how strongly each region is conserved between chicken and passerine. In regions where more sequence variation between passerine and chicken has been observed, it may be acceptable to reduce the word size (W) in future Blast analyses to enable more markers to be mapped. For example, an Ensembl Blast search for the Darwin's finch (*Geospiza fortis*) microsatellite sequence for locus *Gf15* in the chicken genome that used the distant homologies settings and a word size of 6 instead of 9 enabled the chromosomal location for this locus to be identified on Gga2 at 43.5 Mb with a confident *E* value of 4×10^{-10}. This location is in agreement with the assigned location that we obtained by identifying a longer homologous zebra finch genome sequence and comparing this with chicken (table 2). Because it is not always possible to identify an homologous zebra finch sequence, reducing the word length to assign a chromosomal location locus is a useful approach. However, changing the Blast settings to lower the threshold should be done with caution because this increases the risk of including non-homologous loci in the analyses.

Our results clearly show that the great reed warbler has substantially shorter linkage map distances than chicken at the compared homologous chromosomal regions. This contrast is probably a result of a true interspecific difference in recombination rate, but first we have to rule out some obvious alternative explanations. It may be hypothesized that the detected difference was caused simply by the linkage groups being located in low-recombining parts of the great reed warbler genome, such as the centromeres (Hulten 1974; Lynn et al. 2000). We may expect such a bias in this type of analysis because linkage between typed markers is most readily detected when the recombination rate is low. However, against this hypothesis, several of the linkage groups covered a substantial part of their corresponding chicken chromosomes (up to 66%; table 3) and were not restricted to centromeres (figs. 1 and 3). Another possible explanation for the observed differences in recombination rate is that the great reed warbler has a substantially smaller genome than chicken. However, although we cannot rule out this possibility completely, it is difficult to imagine a 5 to 10-fold genome reduction in the great reed warbler without the loss of too many functionally important genes, especially considering that chicken already has a comparatively compact genome (1.25 pg; Gregory 2007). Moreover, the genome sizes of 62 other passerine species studied so far (mean 1.37 pg; range 1.04–1.93 pg; the 4 species of Sylviidae range between 1.09–1.47 pg) are comparable to or at least not much smaller than that of chicken (Gregory 2007).

Cytological work in many species suggests that each chromosome will have at least one chiasma for efficient chromosome pairing and segregation (Rodionov et al. 1992). Although there are exceptions to this general picture, most notably the lack of recombination in *Drosophila* males, species with at least some recombination seem to have at least one chiasma per chromosome. This is the case in chicken, which means that all chromosomes (except the W chromosome) are expected to span approximately 50 cM (Rodionov et al. 1992; Ellegren 2005; Schmid et al. 2005; Groenen MAM, personal communication), and explains why microchromosomes have a much higher recombination rate per base pair than the macrochromosomes (International Chicken Genome Sequencing Consortium 2004). If this applies also to great reed warblers, then each linkage group should be 50 cM or larger. This would imply that the microchromosomes may be of similar length and have similar high rate of recombination in the 2 species. Nevertheless, it appears safe to conclude that the contrasting genetic map distances for the macrochromosomes (table 3 and fig. 3) result from true interspecific differences in recombination rate. This is not a controversial result because research on other taxa has shown that the recombination rates sometimes differ substantially, even between closely related species and subspecies (True et al. 1996; Sanchez-Moran et al. 2002; Winckler et al. 2005).

One may ask why these 2 bird species with strongly conserved genome structure and highly maintained synteny have such a pronounced difference in recombination rate for the macrochromosomes. The chicken mapping families almost all originate from domesticated lines that have undergone several generations of strong directional selection; one mapping family was derived from a backcross between a partially inbred jungle fowl line and a highly inbred white leghorn line, whereas the other 2 families were derived from highly inbred lines (Groenen et al. 2000). The domestication process and inbreeding may select for high recombination. Some recombination rate models predict a higher recombination rate in inbred populations, and it has been observed cytologically that inbreeding plant species can have greater chiasma frequency compared with outcrossing relatives (reviewed in Charlesworth et al. 1977, 1979). However, if inbreeding selected for a rapid increase in the recombination rate in chicken, one might expect the different families used in the mapping analyses to differ more in map size than they actually did (Groenen et al. 2000). Moreover, a previous study comparing inbreeding and outcrossing species (*A. thaliana* and *A. lyrata*) detected only slightly higher rates of recombination in the inbreeder (Hansson et al. 2006). Thus, although a hypothesis based on selection for increased rate of recombination during domestication in chicken may explain our observation, alternative hypotheses cannot be excluded. For instance, high diversity (i.e., greater sequence differences between alleles) in outbred populations has been suggested to inhibit recombination (Borts and Haber 1987). However, even though one may expect low sequence diversity in chicken due to directional selection during domestication, this hypothesis cannot be tested until comparable sequence data have been gathered in both species. Also, even though the great reed warbler currently has a very large population size, suggesting it could harbor extensive sequence diversity, data from both mitochondrial and nuclear genes suggest that its long-term population size is much smaller, and its genetic diversity reduced because of population contractions during the last, and probably previous, glaciations (Bensch and Hasselquist 1999; Hansson and Richardson 2005). Another scenario is that the processes causing the pronounced heterochiasmy in great reed warblers (Hansson et al. 2005) have simultaneously caused a general decline in recombination rate. Unfortunately, this hypothesis is also difficult to test because it is based on, and requires, estimates of

haploid selection pressures and selection on imprinted genes (Lenormand and Dutheil 2005).

As mentioned above, the linkage maps of chicken and human are of similar size (~4000 cM in chicken, Groenen et al. [2000]; ~3700 cM in Human, Dib et al. [1996]), despite a much smaller genome size in chicken (~1.2×10^9 bp in chicken and ~3×10^9 bp in human; International Chicken Genome Sequencing Consortium 2004). This results partly from chickens having more chromosomes, each with at least one obligate crossing-over during meiosis (Rodionov et al. 1992), and partly from a higher recombination rate per base in chicken (International Chicken Genome Sequencing Consortium 2004; Schmid et al. 2005). Edwards and Dillon (2004) studied recombination in the red-winged blackbird *Agelaius phoeniceus* within a restricted region of the chromosome corresponding to Gga16 (a microchromosome). The evaluation of the per-site recombination rate suggested that recombination in blackbirds is up to 2 orders of magnitude higher than in humans, resembling those in *Drosophila* (and then also chicken) more than those in humans (Edwards and Dillon 2004). However, studies of fine-scale recombination rate in human, for instance, have shown that the presence of recombination hotspots makes it difficult to predict the large-scale pattern of recombination from that deduced at the smaller scale (Nachman 2002; Ptak et al. 2004; Winckler et al. 2005). Furthermore, the recombination rate may differ substantially between the macro- and microchromosomes, so it remains to be evaluated whether the result applies also to the macrochromosomes. Moreover, the blackbird study explored sequence and haplotype based data with a coalescent approach and the resulting estimates of recombination rate depend on assumptions about parameters that are difficult to estimate, such as coalescence time and effective population size (Edwards and Dillon 2004). It is therefore too early to conclude that the overall recombination rate in red-winged blackbirds is comparable to the high levels found in chicken.

Instead, our data suggest that recombination rates differ between some bird species and that the chicken linkage map may not be suitable, in terms of genetic distances, as a model for all bird species. If other passerines also have as low a recombination rate (this study) and as pronounced heterochiasmy (Hansson et al. 2005) as the great reed warbler, it will be easier to build basic linkage maps in this group than if recombination were as frequent as in chicken. Relatively few markers will be needed to cover the total genome sufficiently. For instance, the 9 homologous regions, which can be mapped by less than 41 microsatellites in great reed warblers, correspond to a total of 317 Mb in chicken (table 3). This implies a coverage of 44% for these 7 chromosomes (317 Mb of a total of 721 Mb) and of 26% for the whole genome in chicken (317 Mb of a total of ~1,200 Mb). However, a low recombination rate also implies some restrictions. For instance, in the context of parentage analyses and marker-based inbreeding analyses it is crucial to use independent loci. If markers are picked randomly, a low recombination rate will increase the risk of cosegregation. This problem may be circumvented by using chicken sequence data to design primers to amplify polymorphisms at predicted locations of the genome, for example, on different chromosomes or chromosome arms (Ellegren 2005; Dawson et al. 2006). In QTL analyses, a low recombination rate would facilitate the work initially by increasing the amount of information per marker and thereby increase the probability to detect QTLs (Lynch and Walsh 1998). However, once a QTL has been detected, little can be concluded about the number and position of the underlying functional genes because their true locations may be dispersed over a large part of the chromosome due to the imprecision of genetic mapping.

The low recombination rate in great reed warblers is interesting in light of the previously detected association between marker-based heterozygosity and variation in fitness-associated traits in the species (Hansson et al. 2001, 2004). This heterozygosity–fitness correlation was hypothesized to be caused by relatively high levels of linkage disequilibrium in the study population (Hansson et al. 2004) and not by partial inbreeding (cf. Hansson and Westerberg 2002; Slate et al. 2004). Preliminary analyses (Hansson B, Csilléry K, unpublished data) suggest that the level of linkage disequilibrium in the great reed warbler is comparable to the high levels detected in some extremely bottlenecked populations, such as the Scandinavian wolf population (Bensch et al. 2006) and in some domesticated species (McRae et al. 2002; Sutter et al. 2004), rather than those in human and other wild bird and wild mammal populations (Dunning et al. 2000; Winckler et al. 2005; Backström, Qvarnstrom, et al. 2006). One possibility is that the strong linkage disequilibrium observed in the studied great reed warbler population was caused by the species' recent bottleneck-and-expansion in the region (Hansson et al. 2004). A low recombination rate will maintain linkage disequilibrium also between loci separated by relatively large physical distances, thus increasing the possibility that a locus will be a marker for linked fitness loci. Low recombination rate could also lead to a general depletion of the nucleotide diversity, for example, due to background selection, hitchhiking, or a combination of these processes. A correlation between recombination rate and nucleotide diversity has been demonstrated for a variety of organisms, from plants to humans (e.g., Begun and Aquadro 1992; Kraft et al. 1998; Nachman 2001), which points to the joint effects of linkage and selection in shaping patterns of genetic variation (Nachman 2002).

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