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Linkage mapping reveals sex-dimorphic map distances in a passerine bird

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Linkage maps are lacking for many highly influential model organisms in evolutionary research, including all passerine birds. Consequently, their full potential as research models is severely hampered. Here, we provide a partial linkage map and give novel estimates of sex-specific recombination rates in a passerine bird, the great reed warbler (*Acrocephalus arundinaceus*). Linkage analysis of genotypic data at 51 autosomal microsatellites and seven markers on the Z-chromosome (one of the sex chromosomes) from an extended pedigree resulted in 12 linkage groups with 2–8 loci. A striking feature of the map was the pronounced sex-dimorphism: males had a substantially lower recombination rate than females, which resulted in a suppressed autosomal map in males (sum of linkage groups: 110.2 cM) compared to females (237.2 cM; female/male map ratio: 2.15). The sex-specific recombination rates will facilitate the building of a denser linkage map and cast light on hypotheses about sex-specific recombination rates.

Keywords: genetic map; linkage; recombination; heterochiasmy; bird; microsatellite

1. INTRODUCTION

Passerine birds are highly influential model organisms in evolutionary research. Work on this group has provided significant insights in, for example, the evolution of mating systems (Komdeur 1992), sex-ratios (Badyaev *et al.* 2002), inbreeding and inbreeding depression (Keller *et al.* 1994), life-history trade-offs (Gustafsson & Sutherland 1988), heritability and quantitative genetics (Merilä *et al.* 2001), natural (Richman & Price 1992) and sexual selection (Norris 1993), hybridization (Veen *et al.* 2001) and speciation (Irwin *et al.* 2001). However, the passerines' full potential as general research models is severely hampered by the fact that their genomes are not yet characterized. Recently, Derjusheva *et al.* (2004) used fluorescent *in situ* hybridization to reveal high chromosome conservation between chicken (*Gallus gallus*), pigeon (*Columba livia*) and two passerine birds, chaffinch (*Fringilla coelebs*) and redwing (*Turdus iliacus*). This suggests that the physical map of chicken (International Chicken Genome Sequencing Consortium 2004) can be used as a framework for genome organization in other bird species (Ellegren 2005). However, a physical map only reflects how genes are organized on chromosomes, whereas the actual association between genes is set by antagonistic counterplay involving selection, mutation and drift on the one hand, and recombination on the other. Recombination maps are highly desirable in evolutionary genetic research, but are not currently available for passerines. To date, in passerines, even first-generation recombination linkage maps based on moderate numbers of molecular markers, and basic evaluations of recombination rates and map sizes, are lacking.

In the present study, we provide a partial linkage map and give novel estimates of sex-dimorphic map distances in a passerine bird, the great reed warbler (*Acrocephalus arundinaceus*). We used this species because a two-decade long study of the breeding ecology of a Swedish great reed warbler population provided us with a unique extended pedigree in which true genetic mothers and fathers were fully resolved for all offspring and for which DNA samples were available (Hasselquist 1998; Arlt *et al.* 2004; Hansson *et al.* 2004*a*). Moreover, previous molecular work on the great reed warbler offered us a first set of microsatellite loci with which to study segregation (Hansson *et al.* 2000*b*, 2004*a*,*c*). In addition, we saw the potential to detect many more informative markers in the focal species by using already published microsatellite primers from the closely related Seychelles warbler (*Acrocephalus sechellensis*; Richardson *et al.* 2000) and from among the many microsatellites developed in many other passerines (e.g. Petren 1998; Saladin *et al.* 2003) and from primers derived from chicken sequence data (Sætre *et al.* 2003). Finally, we chose to study the great reed warbler because of its previous importance in various fields of molecular-based avian research, including extra-pair paternity and realized fitness, inbreeding and inbreeding depression, offspring sex-ratios and parasite–host interactions (Bensch *et al.* 1994; Hasselquist *et al.* 1996; Westerdahl *et al.* 2000, 2004; Hansson *et al.* 2004*c*).

In several previous mapping studies, it has been observed that the size of the linkage map differs when based on maternal and paternal meioses, respectively (e.g. Dietrich *et al.* 1996; Sakamoto *et al.* 2000; Kong *et al.* 2002). This is due to sex-specific recombination rates, a phenomenon termed 'heterochiasmy'. Despite the fact that heterochiasmy was documented early in the last century (Haldane 1922; Huxley 1928), there is no

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consensus as to which of the several proposed hypotheses may explain its occurrence (reviewed in Lenormand 2003). In the present study, we investigate heterochiasmy when building the map by using sex-specific linkage analyses, and then discuss our findings in relation to the proposed hypotheses.

2. MATERIAL AND METHODS

(a) *Study species and pedigree*

The great reed warbler (*A. arundinaceus*) is a large-sized reed-warbler of the family Sylviidae (Helbig & 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). Great reed warblers are facultatively socially polygynous and males may form pair bonds with up to five females in a season (Catchpole *et al.* 1985; Hasselquist 1998). The karyotype is not yet described, but two other passerines, chaffinch and redwing, have similar numbers of macro- and microchromosomes ($2n=80$; Derjusheva *et al.* 2004) as chicken ($2n=78$; Masabanda *et al.* 2004) and other Galliformes (Japanese quail, *Coturnix japonica*: $2n=78$, Kayang *et al.* 2004; turkey, *Meleagris gallopavo*: $2n=80$, Burt *et al.* 2003). In birds, males are the homogametic (ZZ) and females the heterogametic sex (ZW).

In the present study, we use pedigree data and DNA from a great reed warbler population at Lake Kvismaren, southern central Sweden (59°10′N, 15°25′E), where a detailed ecological study of the whole population has been ongoing since 1983 (Bensch 1996; Hasselquist 1998; Hansson *et al.* 2000*a*). In this population, the territories of great reed warblers have been visited on a daily basis throughout the breeding season. Almost all breeding birds and unpaired territorial males have been captured in mist nets, marked with individual-specific combinations of aluminium and colour plastic rings, and blood-sampled (*ca* 25 µl blood were obtained from the tarsus vein). This ringing scheme allowed us to track the whole breeding population and collect data on the reproductive success of individual birds. We visited nests approximately every third day to measure clutch size, hatching success and number of fledglings. Nestlings were ringed, measured, weighed and blood-sampled nine days after hatching.

The pedigree consists of 151 broods from the years 1987 to 1998. In all of these broods, we have investigated the occurrence of extra-pair young and assigned true maternity and paternity to all extra-pair and legitimate young. This was done by using either minisatellite DNA-fingerprinting or microsatellite genotyping (Hasselquist *et al.* 1996; Arlt *et al.* 2004; Hansson *et al.* 2004*a*). In the present study, only five offspring from two broods were extra-pair young and in these cases the true genetic father was included in the pedigree. Great reed warblers are highly philopatric (Hansson *et al.* 2002) and several offspring entered the pedigree as parents in subsequent years. Moreover, several adults bred in more than one year and/or with more than one partner per year. In this study, we genotyped a subset of individuals for a large number of loci (table 1). In total, 812 unique individuals were included in the mapping pedigree. Eighty-three different males and 96 different females were included as parents ($\chi^2_1=1.24$, $p>0.1$). There was an even sex-ratio among the 693 nestlings (359 males and 334 females, i.e. 51.8% males; $\chi^2_1=0.9$, $p>0.1$).

(b) *Molecular markers and genotyping*

We isolated DNA from blood samples with phenol/chloroform-isoamylalcohol extraction (Bensch *et al.* 1994).

We have previously screened 3–8 unrelated great reed warblers for polymorphism at 130 passerine microsatellite markers, including five which were specifically developed from great reed warbler microsatellite clones (Hansson *et al.* 2000*b*, 2004*b*,*c*; Richardson *et al.* 2000), and specifically for this study we screened an additional 88 microsatellites. This effort resulted in 51 polymorphic autosomal microsatellites and three polymorphic microsatellites on the Z-chromosome (table 1). Some of these markers had segregating non-amplifying alleles ('null alleles'); we tried to eliminate these problematic alleles by re-designing the primers (see table 1). This was done by excluding two bases in the 3′-end of the original primers because we suspected that the null alleles resulted from mismatches between template and primers at these sites. To increase the number of markers on the Z-chromosome, we tested 20 primer pairs derived from sequence data of Z-linked chicken genes (Sætre *et al.* 2003). We sequenced the PCR-products of four unrelated great reed warblers at loci that yielded a single product of expected length (DYEnamics sequence kit, QIAGEN). This survey yielded three loci with length polymorphism (BRM12, BRM15, CHDZ20; table 1) and one locus with single-nucleotide polymorphism ('SNP'; locus VLDLR9; table 1). In total, we ended up with 51 autosomal and seven Z-linked markers (table 1).

Alleles were amplified in standard PCRs following Hansson *et al.* (2000*b*) and Richardson *et al.* (2000). The PCR-mix contained 4 pmol of each primer, 1×NH_4-buffer, 15 nmol $MgCl_2$, 2 nmol dNTP, 0.5 U BIOTAQ DNA-polymerase (Bioline) and 1–5 ng template in 10 µl reaction volume. PCR-condition was as follows: 94 °C for 2 min, then 35 cycles at 94 °C for 30 s/T_A for 30 s/72 °C for 30 s, followed by 72 °C for 10 min; where T_A is the locus-specific annealing temperature (table 1). The SNP (locus VLDLR9) was scored by using two allele-specific forward primers (2 pmol of each primer in PCR) and one reverse primer (4 pmol in PCR), where the 3′-ends of the forward primers were made to match each of the SNP-alleles and the 5′-ends differed in length by two bases (table 1), which resulted in a designed length polymorphism (Hansson & Kawabe 2005). One primer in each primer combination was labelled with fluorescent dye and the PCR-products were separated and the alleles detected in an ABI 3730 capillary sequencer (Applied Biosystems). When possible, several loci per individual were run simultaneously in the sequencer, and each run included a size ladder (ABI GeneScan LIZ500; Applied Biosystems). Genotypes were read in GENEMAPPER v. 3.0 (Applied Biosystems).

We genotyped 50.4±8.7 s.d. loci per individual, and 706.1±136.1 s.d. individuals per locus (table 1). The number of informative meioses (which increases with number of genotyped individuals and with increasing genetic variability) ranged from 75 at locus ZL45 to 1196 at locus Ase11 (table 1).

(c) *Linkage analyses*

The segregation of alleles in the pedigree was evaluated (detection of genotype and scoring errors, and null alleles) by eye and by running the data in PEDCHECK v. 1.1 (http://hpcio.cit.nih.gov/lserver/PEDCHECK.html; O'Connell & Weeks 1998). All scoring errors were corrected and new

Table 1. Information on markers.
(All loci are microsatellites with the exceptions of three indels (BRM12, BRM15 and CHDZ20) and one SNP (VLDLR9; see §2*b*). Number of genotyped individuals (N_{GI}), number of informative meioses (N_{IM}), annealing temperature used in PCR (T_A), primer sequences, linkage group (LG) and reference are given for each locus. In cases where re-designed primers were used, the original locus name and primer sequences are given in parenthesis.)

locus	EMBL accession number	N_{GI}	N_{IM}	T_A[a]	F-primer (5′–3′)	R-primer (5′–3′)	LG	reference
Aar1	AF234985	764	953	62	TGAGGAGCAGCTGGGAAG	GCAGCAGGAGCTCAGGAG	Z	Hansson *et al.* (2000*b*)
Aar2	AF234986	796	109	60	TGTCTGCAGCAGCTCTGG	GACCTGAAGCGTTTCCGTAA	—	Hansson *et al.* (2000*b*)
Aar3	AF234987	772	868	54	GCATCTGGTCTCCGATTGTT	TTTGGGTTACATCTGAGTGTGC	7	Hansson *et al.* (2000*b*)
Aar4	AF234988	791	684	53	GATGACTAAGGTCTCTGGTGTG	GTTTGTGCATCAATTAGTCATG	6	Hansson *et al.* (2000*b*)
(Mcyμ4)	(U82388)				(ATAAGATGAC-TAAGGTCTCTGGTG)	(TAGCAATTGTCTATCATGGTTTG)		Double *et al.* (1997)
Aar5	no sequence	792	1126	56	GAGCTCTGTATGTGCGTG	TCTGAGTGGACTCAGGAGT	—	Hansson *et al.* (2000*b*)
(G7)	(DQ115906)				(GAGCTCTGTATGTGCGTGCG)	(CTGAGTGGACTCAGGAGTGC)		I. Nishiumi (unpublished data)
Aar8	AF234991	758	700	56	TAGTGATGCCCTGCTAGGTA	AAGTGCTCCTTAATATTTGGCA	5	Hansson *et al.* (2000*b*)
(Escμ6)	(X77082)				(CATAGTGATGCCCTGCTAGG)	(GCAAGTGCTCCTTAATATTTGG)		Hanotte *et al.* (1994)
Ase7	AJ287390	769	732	60	AATCAACTTCAAATGCTCACAG	ACTACATGACTCCAGGCTCAG	1	Richardson *et al.* (2000)
Ase8rd	no sequence	746	689	55	TACCTCTCCTTGGCTGAG	CCAGCCCTAGCTGTTTCA	5	This study
(Ase8)	(AJ287391)				(TACCTCTCCTTGGCTGAGCA)	(CCAGCCCTAGCTGTTTCACC)		Richardson *et al.* (2000)
Ase9	AJ287392	779	924	60	GACTGAAGTCCTTTCTGGCTTC	CACCAGGAATACAAGTCCATTG	1	Richardson *et al.* (2000)
Ase10	AJ287393	769	701	TD	CATTGGGGTACTATGGAAAGACC	TCCTGAGTGGAAGGAACATAGG	3	Richardson *et al.* (2000)
Ase11	AJ287394	785	1196	60	TCCCCAAATCTCTCAATTCC	AGTTCTAAGCCTGCCTGTGC	—	Richardson *et al.* (2000)
Ase12	AJ287395	755	366	60	TCAAGGAAACACAACTACAGCC	TTTCCTCACAGCCTTGACTG	11	Richardson *et al.* (2000)
Ase15	AJ287398	796	218	55	CTGAACCCAAACATAAGCACAC	GCTCCAAACACGCCAGAG	7	D. S. Richardson *et al.* (unpublished data), see Hansson *et al.* (2004*c*) and http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-birdmarkers.html
Ase18	AJ276375	793	359	60	ATCCAGTCTTCGCAAAAGCC	TGCCCCAGAGGGAAGAAG	1	Richardson *et al.* (2000)
Ase21	AJ276378	745	759	58	TTAGAACCATTTGATAGTTGCCAC	ATGGGTTTCTTGGGGAAGAG	6	Richardson *et al.* (2000)
Ase27	AJ276384	419	165	60	TTAACATTGCATGCTCCTGC	AGTCAAGGTACAGGCTAGATAGCC	—	Richardson *et al.* (2000)
Ase32	AJ276635	661	747	58	AATGAGCAATACCATGACAGC	GATCTTTCAGTCAGGAACAAGC	3	Richardson *et al.* (2000)
Ase34	AJ276636	774	649	60	GTTATTCTTTTGGCCCTCAGC	GGAGACACCACACCAATGC	8	Richardson *et al.* (2000)
Ase38rd	no sequence	750	682	55	ATCCGAGAACCCAATCAC	GCAGCATTACAGTCTCAAAGA	5	This study
(Ase38)	(AJ276640)				(ATCCGAGAACCCAATCACTT)	(GCAGCATTACAGTCTCAAAGAAC)		Richardson *et al.* (2000)
Ase42	AJ276644	777	1180	60	CATGGGTAGGTTGGGATGTC	AGGTGAGGGTATGCAAACATG	9	Richardson *et al.* (2000)
Ase44	AJ276646	772	762	60	TTCCCGTAATTATGACCTCTCTTG	ACCAGAACTTGTTGTCTGGGAG	3	Richardson *et al.* (2000)
Ase48	AJ276777	482	241	58	TTTATTTCCTGGACTGGAACAATC	GAACATTGGGCTACTGGGC	6	Richardson *et al.* (2000)
Ase50	AJ276779	803	780	60	CTGTGGAATGCTGTCTGGC	ATGGACTCCCGTCTAACTTGC	Z	Richardson *et al.* (2000)

(*Continued.*)

Table 1 (*Continued*.)

locus	EMBL accession number	N_{GI}	N_{IM}	T_A[a]	F-primer (5′–3′)	R-primer (5′–3′)	LG	reference
Ase51	AJ276780	747	925	60	AATTCCCCTAGACAGGCAGC	TCACTGGAGAGCCAAATTCC	1	Richardson *et al.* (2000)
Ase53rd	no sequence	481	186	53	ATGGAGAATTCTGGGTGC	CCCAATAATGAGGTAACACC	4	This study
(Ase53)	(AJ276782)				(ATGGAGAATTCTGGGTGCTG)	(CCCAATAATGAGGTAACACCAA)		Richardson *et al.* (2000)
Ase55	AJ276784	729	292	60	GTGTGGACTCTGGTGGCTC	TCCCAAAGCACTCAAACTAGG	1	Richardson *et al.* (2000)
Ase56	AJ276785	746	343	60	TTCACTGAGAAGTGAGAATGTG	GTCCTTGATTGATTACAGGCT	5	Richardson *et al.* (2000)
Ase57rd	no sequence	776	1090	50	GCAAGTGCAGATGTTTCC	CCAAAGCAGGACAATGC	—	This study
(Ase57)	(AJ276786)				(GCAAGTGCAGATGTTTCCCT)	(CCAAAGCAGGACAATGCTG)		Richardson *et al.* (2000)
Ase58	AJ276787	772	1046	60	ATTCCAGGGATTGGGCAG	CTCAAAGCGAAATTGAGCAGT	8	Richardson *et al.* (2000)
Ase60	AJ276789	775	1180	55	CATGAAAAGGAACTCTCCAGC	TTCCATCTCTGTTCTACTGCG	1	Richardson *et al.* (2000)
Ase61	AJ276790	459	191	54	AGGATTTTTAATGGGATATACA-CATCTG	AGCCACATTTTAGCCCACAG	7	Richardson *et al.* (2000)
Ase63	AJ276792	717	513	60	TTTGGGGTTTAGGAATAGCAGA	GGCTTCAGCCTGAGAAAGTC	5	Richardson *et al.* (2000)
Ase64	AJ276793	766	1152	TD	CCACCTTTCATACTGGGGAG	TTCAGCCAGTCAGTGTAGCC	10	Richardson *et al.* (2000)
BRM12rd-A[b]	DQ073912	784[b]	1019[b]	55	CAACACACTGGCAAAACCAG	TGCACACCTGACAAAAGAGTG	Z	This study
(BRM12)	(X91638)				(CCCTATCTCATCATTGTTCC)	(CACAGAAGGAGCCCATTTGT)		Sætre *et al.* (2003) and G.-P. Sætre *et al.* (unpublished data)
BRM12rd-B[b]	DQ073912	783[b]	722[b]	52	ATGCGTTTTCCTTTCTCCAA	AAAAATAACAACACAACTCCTCAA	Z	This study
(BRM12)	(X91638)				(CCCTATCTCATCATTGTTCC)	(CACAGAAGGAGCCCATTTGT)		Sætre *et al.* (2003) and G.-P. Sætre *et al.* (unpublished data)
BRM15rd	DQ073913	788	1143	55	AGCACCTTTGAACAGTGGTT	AAATCCCTAAGTACCAGTGCAAA	Z	This study
(BRM15)	(X91638)				(AGCACCTTTGAACAGTGGTT)	(TACTTTATGGAGACGACGGA)		Sætre *et al.* (2003) and G.-P. Sætre *et al.* (unpublished data)
Cdi38	AB089175	765	895	54	ACATCTTCGGCACGGCT	GAGCTGGAAGTGGTGGA	2	Otsuka *et al.* (2003)
CHDZ20rd	DQ073914	782	803	52	GCCCCAAAGTAAACTTGGAA	TGATTAGCAGGAGAGCCATTT	Z	This study
(CHDZ20)	(AF004397)				(GAAGAGAGCTGAAACTCGG)	(TCATCTTCATCCATATTGG)		Sætre *et al.* (2003) and G.-P. Sætre *et al.* (unpublished data)
Cuμ02	AF122890	262	211	60	CCTTGGATTGCTTCCAAATG	CCAATTTCCTGCAGACTCTTTC	4	Gibbs *et al.* (1999)
Cuμ04	AF122891	744	216	55	AATTGCATAAATGTGATCCAC	AAATGAAATGTGGTAGAATTCC	6	Gibbs *et al.* (1999)
Cuμ28	AF122894	755	329	60	GAGGCACAGAAATGTGAATT	TAAGTAGAAGGACTTGATGGCT	12	Gibbs *et al.* (1999)
G61	no sequence	797	1172	56	GAGCAGAAGCTACAGAAATC	GCAAAGTCTGATTGTAAGCAG	Z	Nishiumi *et al.* (1996)
Gf08	AF081932	744	417	62	TGGGAGAGCAAGGTGGGAACAG	TGGAGTGGTGATTAAACCAGCAGG	—	Petren (1998)
Gf15	AF081939	702	460	62	CTCCACCTCCCACTAACTGCTACC	CAACACCTGGAGTGGAAGTGCC	3	Petren (1998)
HrU5	X84090	641	116	45	TCAACAAGTGTCATTAGGTTC	AACTTAGATAAGGAAGGTATAT	5	Primmer *et al.* (1995)
LOX1	Y16820	719	639	54	ATGATGGTAAGTCTAATGAAAGC	CCACACACATTCACTCTATTG	3	Piertney *et al.* (1998)

(*Continued*.)

Table 1 (*Continued*.)

locus	EMBL accession number	N_{GI}	N_{IM}	T_A[a]	F-primer (5′–3′)	R-primer (5′–3′)	LG	reference
MSLP2	AB031374	747	297	50	TAACTACAGCCAGTTAGAAG	TGAAGTTACTGGTAGCCTTTG	6	Ishibashi *et al.* (2000)
PAT MP 2–43	no sequence	749	321	60	ACAGGTAGTCAGAAATGGAAAG	GTATCCAGAGTCTTTGCTGATG	3	Otter *et al.* (1998)
Pdoμ4	X93505	332	297	54	CGATAAGCTTGGATCAGGACTAC	CTTGGGAAGAGAATGAGTCAGGA	3	Neumann & Wetton (1996)
Pdoμ6	Y15125	220	99	59	CTGATCATGTGTAGATGTAA-GACTGC	CAGATCCTTAAGCAGGAAGTTAGG	5	Griffith *et al.* (1999)
PmaT-GAn42	AY260540	747	1127	57	ACTTCCACATGCCAGTTTCC	TGTTAAGGCAGAGAGGTGGG	3	Saladin *et al.* (2003)
Ppi2	AJ272375	789	1122	53	CACAGACCATTCGAAGCAGA	GCTCCGATGGTGAATGAAGT	10	Martinez *et al.* (1999)
Sjr4	no sequence	479	642	54	TCCAGGCTGTGCTTGCACTTG	TGCCAGACCACCAACTAAATC	—	D. B. McDonald and W. K. Potts (unpublished data)
VLDLR9rd	DQ073915	783	1015	52	TCTGTTACCAAGTGTGGAATGG	TCGGTTGGTGAAAATCAGAC	Z	This study
(VLDLR9)	(X80207)				TGTTACCAAGTGTGGAATGC (AAGTGTGAATGTAGCCGTGG)	(TCGGTTGGTGAAAATCAGAC)		Sætre *et al.* (2003) and G.-P. Sætre *et al.* (unpublished data)
WBSW7	AF130434	751	266	54	TCTGGAGTTCTGGGACCTGT	CTCACTCAACAGCAGGACCA	6	McRae & Amos (1999)
ZL18	AF076668	721	810	58	CTGCAGAGGAGCTCAGGTAAC	CTCGGTGCTGCCAGAACTCAG	—	Degnan *et al.* (1999)
ZL44	AJ517996	698	460	53	CTGTCCCTGCCCTCTCATC	ACCATGGCAGAGGCACCAA	9	Frentiu *et al.* (2003)
ZL45	AJ517997	756	74	58	CCGGAGCACCACGCACAGC	GTTTGGGTCCAAGCGCCTCGAG	9	Frentiu *et al.* (2003)
ZL54	AJ518005	681	486	58	CACGACTTCTCAAGCAGAC	GAGCCTTGCACAAACGGAC	2	Frentiu *et al.* (2003)

[a] TD, Touch down PCR with T_A continuously falling from 60° to 50°.
[b] BRM12rdA and BRM12rdB score two indels at the same locus and were evaluated simultaneously; $N_{GI}=786$, $N_{IM}=1053$.

PCRs were run in some cases. As stated above, we re-designed some primers to avoid null alleles. At some loci, null alleles could not be completely eliminated, and we only included segregation that could be easily interpreted in these cases.

The linkage map was constructed in CRIMAP v. 2.4 (http://biobase.dk/Embnetut/Crimap/analyse1.html; Lander & Green 1987). This program calculates two-point recombination fractions, provides logarithmic odds (LOD) scores for recombination estimates, and tests marker order. In line with previous mapping studies (e.g. Kayang *et al.* 2004; Samollow *et al.* 2004), we assigned markers to linkage groups at a threshold of LOD > 3.0. Autosomal and Z-linked loci were evaluated separately. For the autosomal loci, we first calculated the recombination fractions between all pairs of markers with the TWOPOINT option in CRIMAP, and then determined the most parsimonious ordering of significantly linked loci (i.e. groups of pair-wise markers with LOD > 3.0) with the options FLIPSN and FIXED. The analyses revealed pronounced heterochiasmy, and all our results are from sex-specific analyses. One of the loci on the Z-chromosome (G61) was not linked at LOD > 3.0 to any other locus, but when we had determined the most parsimonious ordering of the other six Z-linked loci, we could use the combined dataset and the FIXED option in CRIMAP to determine the relative position of this locus. All map distances are given in Kosambi centiMorgans (cM).

3. RESULTS

Forty-three of the 51 autosomal loci (84.3%) scored LOD > 3.0 to at least one other locus. There were 86 pairs with LOD > 3.0 and among these pairs LOD scores ranged from 4.3 (Ase18 versus Ase55) to 201.6 (Ase64 versus Ppi2). The 43 linked autosomal loci were placed on the map (figure 1*a*). The number of informative meioses was not associated with probability that a marker either scored LOD > 3.0 or LOD ≤ 3.0 with another marker (logistic regression: $\chi^2_1 = 0.02$, $p = 0.89$), which suggests that most apparently unlinked markers were indeed located on unique chromosomes or chromosome arms.

There was pronounced heterochiasmy. Between pairs of loci with LOD > 3.0, the recombination rate in females (0.101 ± 0.099 s.d.) was almost twice that of males (0.052 ± 0.066 s.d.). These loci constituted 11 autosomal linkage groups with 2–8 loci, with an average size of 10.0 (± 10.8 s.d.) cM in males and 21.6 (± 20.6 s.d.) cM in females (paired *t*-test: $t = 3.2$, d.f. = 10, $p = 0.009$). In total, the autosomal linkage map was much smaller in males (110.2 cM) compared to females (237.2 cM; figure 1*a*), with a female-to-male map ratio of 2.15.

Among the Z-linked markers, six out of seven (i.e. all loci excluding G61) scored LOD > 3.0 to the other loci (ranging between LOD 3.6 for Aar1 versus BRM15 and 67.1 for BRM12 versus BRM15). This linkage group spanned 45.3 cM (figure 1*b*). When the combined information from the other six loci was used, there was significant support for locating G61 together with Ase50 and CHDZ20 rather than together with Aar1 at the other end of the chromosome (ΔLOD = 3.57; figure 1*b*).

4. DISCUSSION

In the present study, we have constructed a partial linkage map in a passerine bird, the great reed warbler. Our analyses revealed a pronounced heterochiasmy in the species: males had substantially lower meiotic recombination rate than females. This resulted in a reduced male autosomal linkage map compared to that of the females, and a female-to-male map length ratio of 2.15.

Heterochiasmy has previously been documented in several animals and plants, and the phenomenon was first described by Haldane (1922) and Huxley (1928). They hypothesized that in species where the sexes differed much in recombination rate, it is usually the heterogametic sex that has suppressed recombination (the 'Haldane–Huxley rule'). In support of this rule, reduced recombination rate in the heterogametic sex has been found in several mammals, with a female-to-male map ratio of 1.56–1.65 in human (Broman *et al.* 1998; Kong *et al.* 2002), 1.36–1.41 in dog (*Canis familiaris*; Mellersh *et al.* 1997; Neff *et al.* 1999), 1.30–1.55 in pig (*Sus scrofa*; Archibald *et al.* 1995) and 1.25 in mouse (*Mus musculus*; Dietrich *et al.* 1996). In fish, heterochiasmy seems to be particularly strong, where, for example, rainbow trout (*Oncorhynchus mykiss*) has a female-to-male map ratio of 3.25 (Sakamoto *et al.* 2000).

In chicken, Japanese quail and turkey, which to our knowledge are the only other bird species with available maps, the sexes have almost identical map distances (Groenen *et al.* 2000; Burt *et al.* 2003; Kayang *et al.* 2004). Thus, the highly sex-dimorphic linkage map in great reed warblers contrasts strongly with that found in chicken and other Galliformes. Moreover, in birds females are the heterogametic sex and therefore the sex-bias observed in great reed warblers—that females have higher recombination rate than males—opposes the Haldane–Huxley rule. Stauss *et al.* (2003) studied recombination between three allozyme loci in a single linkage group in two other passerines, great tit (*Parus major*) and blue tit (*P. caeruleus*). Although, genome-wide recombination rates should not be inferred from a single linkage group (Lynn *et al.* 2000; Sakamoto *et al.* 2000), their data indicate that heterochiasmy occurs in other passerines: the female-to-male recombination ratio was 1.91 in great tits and 0.44–0.56 in blue tits (Stauss *et al.* 2003). Thus, the great tit may be another exception to the Haldane–Huxley rule among birds. Other firmly established exceptions are found in marsupials, insects and plants (Lenormand 2003; Samollow *et al.* 2004). In the short-tailed opossum (*Monodelphis domestica*), for instance, males are the heterogametic sex but females have suppressed recombination (female/male map ratio: 0.50; Samollow *et al.* 2004).

Consequently, there is no clear-cut association between heterogamety and heterochiasmy, and other (not necessarily exclusive) hypotheses have to be explored to explain the phenomenon of sex-dimorphic map distances. Several other hypotheses exist (reviewed in Lenormand 2003; Samollow *et al.* 2004). For example, Samollow *et al.* (2004) suggested that systematic differences between placental mammals and marsupials in the X-chromosome inactivation process may explain the opposing female-to-male map ratios of these groups of animals. In marsupials (females have suppressed recombination), it is always the paternally inherited X-chromosome that is inactivated in females, whereas in placentals some genes on the paternal and some genes on the maternal X-chromosome are inactivated. If this scenario were to explain the pattern

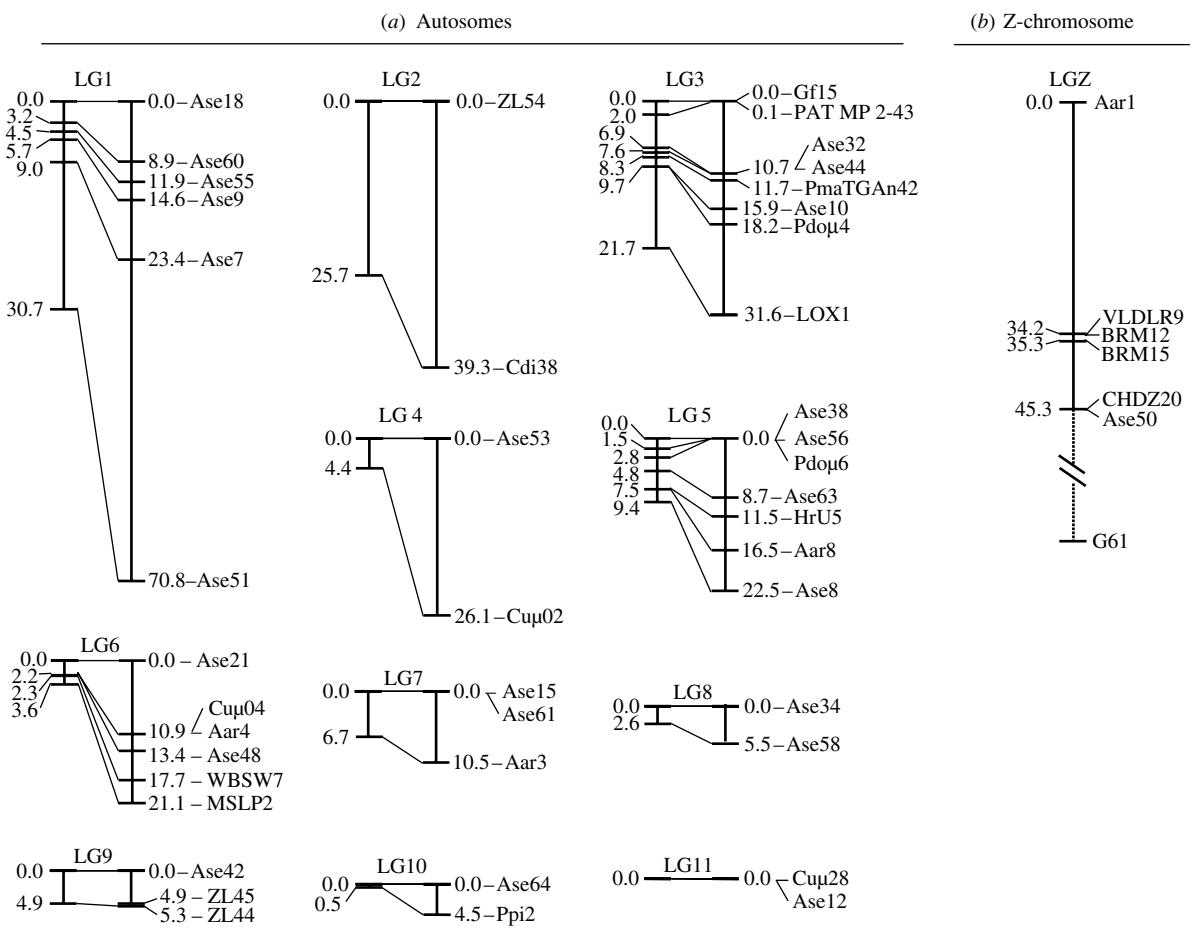

Figure 1. (*a*) Male- and female-specific autosomal recombination linkage map of great reed warbler (based on 43 microsatellite markers). Male maps are to the left in each linkage group. (*b*) Male-specific Z-chromosome linkage group (based on three microsatellite and four chicken-derived markers; see table 1). Note that the location of G61 is preliminary (see §3). Locus positions are given by their distance in cM.

found in the present study (suppressed recombination in males), the maternally inherited Z-chromosome should be inactivated in male offspring. However, work on chicken, spotted turtle-dove (*Streptopelia chinensis*) and house sparrow (*Passer domesticus*) suggests that neither of the Z-chromosomes are inactivated (Baverstock *et al.* 1982; Kuroda *et al.* 2001; but see McQueen *et al.* 2001), and although further research on other species is necessary to confirm that this is a general pattern in birds, the current data in birds fail to support this hypothesis for heterochiasmy.

Another group of hypotheses for heterochiasmy is based on selection. It is well known that recombination may evolve to optimize the allelic associations between loci as an epistatic response to natural selection—a process that will shape the sex-average rate of recombination (reviewed in Otto & Lenormand 2002; Rice 2002). In line with this theory, selection may also cause the sexes to diverge in recombination rate. Trivers (1988) proposed that sexual selection may cause heterochiasmy. He suggested that genes and combinations of genes that pass through the sex with highest variance in reproductive success would be superior on average and thus a reduced rate of recombination will be selected for in this sex. Lenormand (2003) suggested that heterochiasmy can result when epistasis varies with sex during the haploid phase or between chromosomes inherited from the father and the mother during the diploid phase ('sex-of-origin effect'). This scenario requires that genes are expressed during the haploid phase, which is common in plants but uncommon in animals (Christians *et al.* 1999; Xu *et al.* 1999), or a mechanism such as imprinting that produces the sex-of-origin effect in diploids, but imprinting probably only affects a very small proportion of the genome (*ca* 0.1% of the genes are imprinted in mammals; Burns *et al.* 2001). In polygynous species such as the great reed warbler, males are likely to have higher variance in reproduction success than females. Thus, Trivers' (1988) ideas about heterochiasmy and sexual selection may fit our observation. However, Lenormand's (2003) suggestion may also be correct; more recombination in females could result from the fact that the haploid phase is shorter in females than in males and that there is little scope for selection to reduce recombination in females.

It has also been suggested that heterochiasmy may occur for mechanistic reasons due to sex-differences in the internal or external environment. For instance, recombination rates may be affected by the metabolic rate or ambient temperature (Bernstein *et al.* 1988; Plomion & O'Malley 1996). However, it is difficult with this hypothesis to explain heterochiasmy in hermaphrodites where both male and female meiosis may occur simultaneously, and in plants where timing of meiosis is similar in the two sexes. Still, the hypothesis may apply in species with such sex-differences, but relevant data and predictions are lacking for passerines. Clearly, more data on, for

example, timing of meiosis in males and females, haploid gene-expression and imprinting are needed to understand the occurrence of heterochiasmy in great reed warblers and other organisms.

To our surprise a very high proportion of the autosomal markers tested (84.3%) were significantly linked to at least one other marker. This could partly stem from the fact that we genotyped many individuals and therefore achieved high statistical power. Our result could also be explained in terms of non-random distribution of microsatellites in the genome. If our markers were predominantly located in areas with low recombination rate, they would be more probably associated with other markers in this type of linkage analysis. In chicken, there is an even distribution of microsatellites over macrochromosomes and intermediate-sized chromosomes, whereas the microchromosomes, large parts of the sex chromosomes, and most centromeres and telomeres have very low densities of microsatellites (Primmer *et al.* 1997). Microchromosomes constitute only a moderate part of the genome and the general scarcity of microsatellites on these chromosomes is not sufficient to explain why we ended up with a very high proportion of linked markers. Neither can very low densities of microsatellites near centromeres explain our finding. The recombination rate is reduced around centromeres (Hulten 1974; Lynn *et al.* 2000), so a low density of markers in this region would have reduced the chances of finding linked markers. Instead, comparative data in great reed warblers and chicken suggest another explanation as to why many of our markers were significantly linked, namely that the recombination rate is relatively low in great reed warblers. Based on data for orthologous loci on seven chromosomes (Dawson *et al.* 2006), we have provisionally estimated that the recombination rates in male and female great reed warblers were only *ca* 17% and 32% that of the chicken, respectively (B. Hansson, D. A. Dawson, M. Åkesson, T. Burke, J. M. Pemberton and J. Slate, unpublished data).

We have used markers that we know are polymorphic in many other passerines. Several of the microsatellites developed in the Seychelles warbler are polymorphic in other *Acrocephalus* spp. (Richardson *et al.* 2000), and many other markers are frequently used in parental analyses of a vide variety of passerines (e.g. Ppi2, Aar4/Mcyμ4, Aar8/Escμ6; Hanotte *et al.* 1994; Double *et al.* 1997; Martinez *et al.* 1999; see also http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-birdmarkers.html). These markers can be used as framework loci to link future passerine maps and enable comparative work on passerine genomes. The pronounced heterochiasmy has practical implications for experimental design: it will facilitate the building of a high-resolution map in great reed warblers—work that we have already initiated by genotyping individuals in the pedigree with the AFLP-technique (M. Åkesson, unpublished data). Potentially, our and other future passerine linkage maps will cast light on important issues in evolutionary research (Ellegren 2005), including hypotheses of heterozygosity-fitness correlations (Hansson *et al.* 2004*c*), causes of heterochiasmy (Lenormand 2003), distribution of quantitative trait loci (Flint & Mott 2001; Slate *et al.* 2002) and avian genome organization (Hurst *et al.* 2004).

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