



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/1316/>

Article:

Hansson, B., Åkesson, M., Slate, J. et al. (2005) Linkage mapping reveals sex-dimorphic map distances in a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, 272 (1578). pp. 2289-2298. ISSN: 1471-2954

<https://doi.org/10.1098/rspb.2005.3228>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

Bengt Hansson^{1,*}, Mikael Åkesson², Jon Slate³ and Josephine M. Pemberton¹

¹*Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK*

²*Department of Animal Ecology, Ecology Building, Lund University, 223 62 Lund, Sweden*

³*Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 3TN, UK*

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.* 2004a). 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.* 2000b, 2004a,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.* 2004c).

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

* Author for correspondence (bengt.hansson@zoekol.lu.se).

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 ($2n=80$; Derjushva *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.* 2000a). 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.* 2004a). 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.* 2000b, 2004b,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.* (2000b) and Richardson *et al.* (2000). The PCR-mix contained 4 pmol of each primer, 1×NH₄-buffer, 15 nmol MgCl₂, 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 <i>et al.</i> (2000 <i>b</i>)
Aar2	AF234986	796	109	60	TGTCTGCAGCAGCTCTGG	GACCTGAAGCGTTCCGTAA	—	Hansson <i>et al.</i> (2000 <i>b</i>)
Aar3	AF234987	772	868	54	GCATCTGGTCTCCGATTGTT	TTTGGGTTACATCTGAGTGTGC	7	Hansson <i>et al.</i> (2000 <i>b</i>)
Aar4 (Mcy μ 4)	AF234988 (U82388)	791	684	53	GATGACTAAGGTCTCTGGTGTG (ATAAGATGAC- TAAGGTCTCTGGTG)	GTTTGTGCATCAATTAGTCATG (TAGCAATTGTCTATCATGGTTTG)	6	Hansson <i>et al.</i> (2000 <i>b</i>) Double <i>et al.</i> (1997)
Aar5 (G7)	no sequence (DQ115906)	792	1126	56	GAGCTCTGTATGTGCGTG (GAGCTCTGTATGTGCGTGCG)	TCTGAGTGGACTCAGGAGT (CTGAGTGGACTCAGGAGTGC)	—	Hansson <i>et al.</i> (2000 <i>b</i>) I. Nishiumi (unpublished data)
Aar8 (Esc μ 6)	AF234991 (X77082)	758	700	56	TAGTGATGCCCTGCTAGGTA (CATAGTGATGCCCTGCTAGG)	AAGTGCTCCTTAATATTTGGCA (GCAAGTGCTCCTTAATATTTGG)	5	Hansson <i>et al.</i> (2000 <i>b</i>) Hanotte <i>et al.</i> (1994)
Ase7	AJ287390	769	732	60	AATCAACTTCAAATGCTCACAG	ACTACATGACTCCAGGCTCAG	1	Richardson <i>et al.</i> (2000)
Ase8rd (Ase8)	no sequence (AJ287391)	746	689	55	TACCTCTCCTTGGCTGAG (TACCTCTCCTTGGCTGAGCA)	CCAGCCCTAGCTGTTTCA (CCAGCCCTAGCTGTTTCAACC)	5	This study Richardson <i>et al.</i> (2000)
Ase9	AJ287392	779	924	60	GACTGAAGTCTTTCTGGCTTC	CACCAGGAATACAAGTCCATTG	1	Richardson <i>et al.</i> (2000)
Ase10	AJ287393	769	701	TD	CATTGGGGTACTATGGAAAGACC	TCCTGAGTGGAAAGGAACATAGG	3	Richardson <i>et al.</i> (2000)
Ase11	AJ287394	785	1196	60	TCCCCAATCTCTCAATTCC	AGTTCTAAGCCTGCCTGTGC	—	Richardson <i>et al.</i> (2000)
Ase12	AJ287395	755	366	60	TCAAGGAAACACAACACTACAGCC	TTTCCTCACAGCCTTGACTG	11	Richardson <i>et al.</i> (2000)
Ase15	AJ287398	796	218	55	CTGAACCCAAACATAAGCACAC	GCTCCAAACACGCCAGAG	7	D. S. Richardson <i>et al.</i> (unpublished data), see Hansson <i>et al.</i> (2004 <i>c</i>) and http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-birdmarkers.html
Ase18	AJ276375	793	359	60	ATCCAGTCTTCGCAAAGGCC	TGCCCCAGAGGGAAGAAG	1	Richardson <i>et al.</i> (2000)
Ase21	AJ276378	745	759	58	TTAGAACCATTGATAGTTGCCAC	ATGGGTTTCTTGGGGAAGAG	6	Richardson <i>et al.</i> (2000)
Ase27	AJ276384	419	165	60	TTAACATGTCATGCTCCTGC	AGTCAAGGTACAGGCTAGATAGCC	—	Richardson <i>et al.</i> (2000)
Ase32	AJ276635	661	747	58	AATGAGCAATACCATGACAGC	GATCTTTCAGTCAGGAACAAGC	3	Richardson <i>et al.</i> (2000)
Ase34	AJ276636	774	649	60	GTTATTCTTTTGGCCCTCAGC	GGAGACCCACACCAATGC	8	Richardson <i>et al.</i> (2000)
Ase38rd (Ase38)	no sequence (AJ276640)	750	682	55	ATCCGAGAACCCAATCAC (ATCCGAGAACCCAATCACTT)	GCAGCATTACAGTCTCAAAGA (GCAGCATTACAGTCTCAAAGAAC)	5	This study Richardson <i>et al.</i> (2000)
Ase42	AJ276644	777	1180	60	CATGGGTAGGTTGGGATGTC	AGGTGAGGGTATGCAAACATG	9	Richardson <i>et al.</i> (2000)
Ase44	AJ276646	772	762	60	TTCCCGTAATTATGACCTCTCTTG	ACCAGAACTTGTTGTCTGGGAG	3	Richardson <i>et al.</i> (2000)
Ase48	AJ276777	482	241	58	TTTATTTCTCTGGACTGGAACAATC	GAACATTGGGCTACTGGGC	6	Richardson <i>et al.</i> (2000)
Ase50	AJ276779	803	780	60	CTGTGGAATGCTGTCTGGC	ATGGACTCCCCTAACTTGC	Z	Richardson <i>et al.</i> (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	AATTCCTAGACAGGCAGC	TCACTGGAGAGCCAAATTCC	1	Richardson <i>et al.</i> (2000)
Ase53rd (Ase53)	no sequence (AJ276782)	481	186	53	ATGGAGAATTCTGGGTGC (ATGGAGAATTCTGGGTGCTG)	CCCAATAATGAGGTAACACC (CCCAATAATGAGGTAACACCAA)	4	This study Richardson <i>et al.</i> (2000)
Ase55	AJ276784	729	292	60	GTGTGGACTCTGGTGGCTC	TCCCAAAGCACTCAAACCTAGG	1	Richardson <i>et al.</i> (2000)
Ase56	AJ276785	746	343	60	TTCCTGAGAAGTGAGAATGTG	GTCCTTGATTGATTACAGGCT	5	Richardson <i>et al.</i> (2000)
Ase57rd (Ase57)	no sequence (AJ276786)	776	1090	50	GCAAGTGCAGATGTTTCC (GCAAGTGCAGATGTTTCCCT)	CCAAAGCAGGACAATGC (CCAAAGCAGGACAATGCTG)	—	This study Richardson <i>et al.</i> (2000)
Ase58	AJ276787	772	1046	60	ATCCAGGGATTGGGCAG	CTCAAAGCGAAATTGAGCAGT	8	Richardson <i>et al.</i> (2000)
Ase60	AJ276789	775	1180	55	CATGAAAAGGAACTCTCCAGC	TTCCATCTCTGTTCTACTGCG	1	Richardson <i>et al.</i> (2000)
Ase61	AJ276790	459	191	54	AGGATTTTTAATGGGATATACA- CATCTG	AGCCACATTTTAGCCACAG	7	Richardson <i>et al.</i> (2000)
Ase63	AJ276792	717	513	60	TTTGGGGTTTAGGAATAGCAGA	GGCTTCAGCCTGAGAAAGTC	5	Richardson <i>et al.</i> (2000)
Ase64	AJ276793	766	1152	TD	CCACCTTTTCACTGTTGGGAG	TTCAGCCAGTCAGTGTAGCC	10	Richardson <i>et al.</i> (2000)
BRM12rd- A ^b (BRM12)	DQ073912 (X91638)	784 ^b	1019 ^b	55	CAACACACTGGCAAACCAG (CCCTATCTCATCATTGTTCC)	TGCACACCTGACAAAAGAGTG (CACAGAAGGAGCCCATTGT)	Z	This study Sætre <i>et al.</i> (2003) and G.-P. Sætre <i>et al.</i> (unpublished data)
BRM12rd- B ^b (BRM12)	DQ073912 (X91638)	783 ^b	722 ^b	52	ATGCGTTTTCTTTCTCAA (CCCTATCTCATCATTGTTCC)	AAAAATAACAACACAACCTCCTCAA (CACAGAAGGAGCCCATTGT)	Z	This study Sætre <i>et al.</i> (2003) and G.-P. Sætre <i>et al.</i> (unpublished data)
BRM15rd (BRM15)	DQ073913 (X91638)	788	1143	55	AGCACCTTTGAACAGTGGTT (AGCACCTTTGAACAGTGGTT)	AAATCCCTAAGTACCAGTGCAAA (TACTTTATGGAGACGACGGA)	Z	This study Sætre <i>et al.</i> (2003) and G.-P. Sætre <i>et al.</i> (unpublished data)
Cdi38	AB089175	765	895	54	ACATCTTCGGCACGGCT	GAGCTGGAAGTGGTGGGA	2	Otsuka <i>et al.</i> (2003)
CHDZ20rd (CHDZ20)	DQ073914 (AF004397)	782	803	52	GCCCCAAAGTAAACTTGGAA (GAAGAGAGCTGAAACTCGG)	TGATTAGCAGGAGAGCCATTT (TCATCTTCATCCATATTGG)	Z	This study Sætre <i>et al.</i> (2003) and G.-P. Sætre <i>et al.</i> (unpublished data)
Cu μ 02	AF122890	262	211	60	CCTTGGATTGCTTCCAAATG	CCAATTTCTGCAGACTCTTTC	4	Gibbs <i>et al.</i> (1999)
Cu μ 04	AF122891	744	216	55	AATTGCATAAATGTGATCCAC	AAATGAAATGTGGTAGAATTCC	6	Gibbs <i>et al.</i> (1999)
Cu μ 28	AF122894	755	329	60	GAGGCACAGAAATGTGAATT	TAAGTAGAAGGACTTGATGGCT	12	Gibbs <i>et al.</i> (1999)
G61	no sequence	797	1172	56	GAGCAGAAGCTACAGAAATC	GCAAAGTCTGATTGTAAGCAG	Z	Nishiumi <i>et al.</i> (1996)
Gf08	AF081932	744	417	62	TGGGAGAGCAAGGTGGGAACAG	TGGAGTGGTGAATAACCAGCAGG	—	Petren (1998)
Gf15	AF081939	702	460	62	CTCCACCTCCCCTAAGTCTGCTACC	CAACACCTGGAGTGGAAAGTGCC	3	Petren (1998)
HrU5	X84090	641	116	45	TCAACAAGTGTCAATTAGGTTT	AACTTAGATAAGGAAGGTATAT	5	Primmer <i>et al.</i> (1995)
LOX1	Y16820	719	639	54	ATGATGGTAAGTCTAATGAAAGC	CCACACACATTCATCTATTG	3	Piertney <i>et al.</i> (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 <i>et al.</i> (2000)
PAT MP	no sequence	749	321	60	ACAGGTAGTCAGAAATGGAAAG	GTATCCAGAGTCTTTGCTGATG	3	Otter <i>et al.</i> (1998)
2-43								
Pdo μ 4	X93505	332	297	54	CGATAAGCTTGGATCAGGACTAC	CTTGGGAAGAGAATGAGTCAGGA	3	Neumann & Wetton (1996)
Pdo μ 6	Y15125	220	99	59	CTGATCATGTGTAGATGTAA-GACTGC	CAGATCCTTAAGCAGGAAGTTAGG	5	Griffith <i>et al.</i> (1999)
PmaT-GAn42	AY260540	747	1127	57	ACTTCCACATGCCAGTTTCC	TGTTAAGGCAGAGAGGTGGG	3	Saladin <i>et al.</i> (2003)
Ppi2	AJ272375	789	1122	53	CACAGACCATTGGAAGCAGA	GCTCCGATGGTGAATGAAGT	10	Martinez <i>et al.</i> (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 <i>et al.</i> (2003) and G.-P. Sætre <i>et al.</i> (unpublished data)
WBSW7	AF130434	751	266	54	TCTGGAGTTCTGGGACCTGT	CTCACTCAACAGCAGGACCA	6	McRae & Amos (1999)
ZL18	AF076668	721	810	58	CTGCAGAGGAGCTCAGGTAAC	CTCGGTGCTGCCAGAACTCAG	—	Degnan <i>et al.</i> (1999)
ZL44	AJ517996	698	460	53	CTGTCCCTGCCCTCTCATC	ACCATGGCAGAGGCACCAA	9	Frentiu <i>et al.</i> (2003)
ZL45	AJ517997	756	74	58	CCGGAGCACCACGCACAGC	GTTTGGGTCCAAGCGCCTCGAG	9	Frentiu <i>et al.</i> (2003)
ZL54	AJ518005	681	486	58	CACGACTTCTCAAGCAGAC	GAGCCTTGACAAAACGGAC	2	Frentiu <i>et al.</i> (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 $\text{LOD} > 3.0$. 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 $\text{LOD} > 3.0$) 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 $\text{LOD} > 3.0$ 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 $\text{LOD} > 3.0$ to at least one other locus. There were 86 pairs with $\text{LOD} > 3.0$ 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 1a). The number of informative meioses was not associated with probability that a marker either scored $\text{LOD} > 3.0$ or $\text{LOD} \leq 3.0$ 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 $\text{LOD} > 3.0$, 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 1a), 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 $\text{LOD} > 3.0$ to the other loci (ranging between $\text{LOD} 3.6$ for Aar1 versus BRM15 and 67.1 for BRM12 versus BRM15). This linkage group spanned 45.3 cM (figure 1b). 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 ($\Delta\text{LOD} = 3.57$; figure 1b).

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

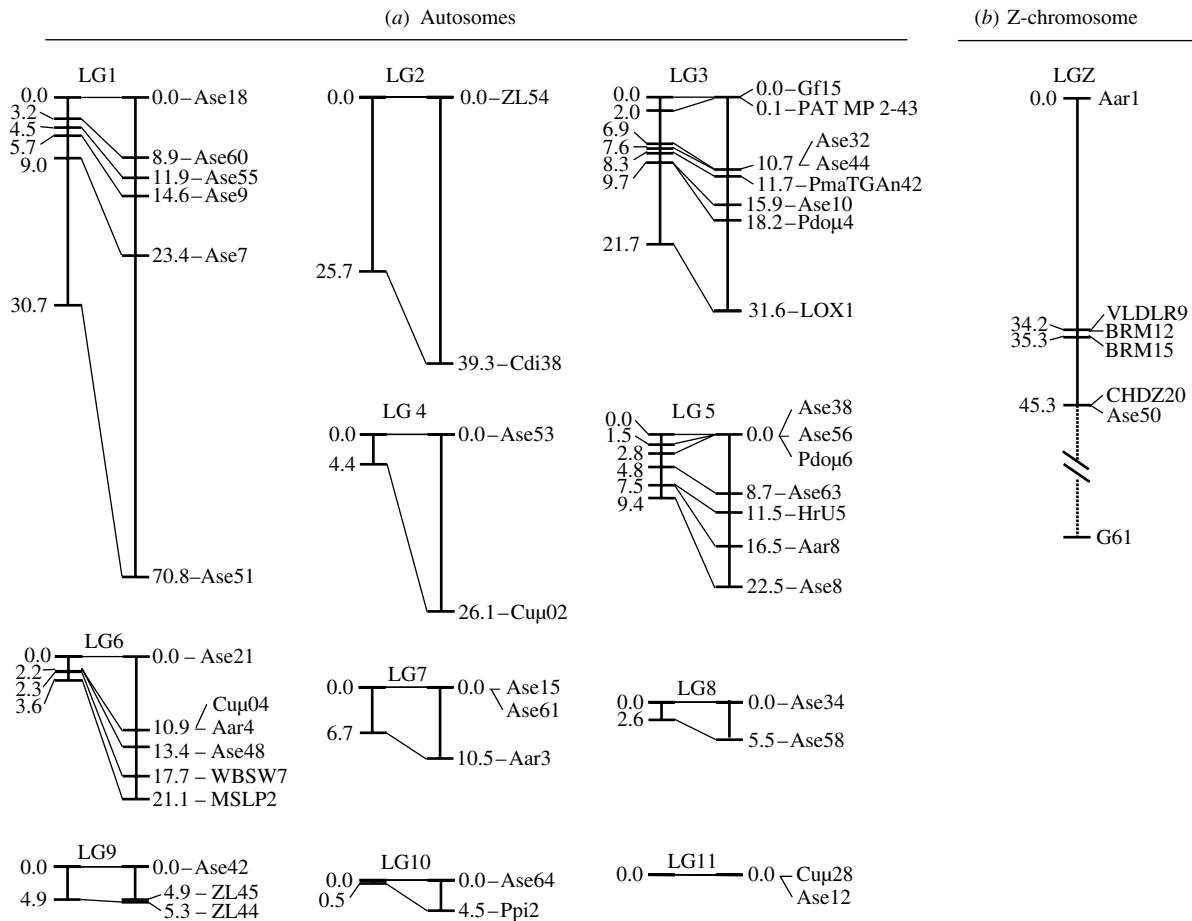


Figure 1. (a) Male- and female-specific autosomal recombination linkage map of great reed warbler (based on 43 microsatellite markers). Male maps are to the left in each linkage group. (b) Male-specific Z-chromosome linkage group (based on three microsatellite and four chicken-derived markers; see table 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 (Hultén 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 wide variety of passerines (e.g. Ppi2, Aar4/Mcyu4, Aar8/Escu6; 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.* 2004c), 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).

We thank all field workers at Lake Kvismaren, especially S. Bensch, D. Hasselquist, and B. Nielsen for their long-term efforts. Also, we are grateful to T. Burke, D. Richardson, G.-P. Sætre and T. Borge for sharing previously unpublished primers; to D. A. Dawson for primer and microsatellite sequence information, data for orthologous loci in great reed warblers and chicken and the bird marker database at <http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-bird-markers.html>; to T. Thomasson for efficient genotyping; to D. Beraldi for support with PEDCHECK and CRIMAP runs; and to S. Bensch and D. Hasselquist for initiating the great reed warbler project and supporting all steps of this work. The manuscript was greatly improved by the comments of two anonymous reviewers. The work was supported by a post-doctoral grant from the Swedish Research Council and STINT (to BH), by a Marie-Curie post-doctoral fellowship (to BH/JMP), the National Environmental Research Council (to L. E. B. Kruuk), Lunds Djurskyddsfond (to BH), Berggrens Stiftelse (to BH), Schwartz Stiftelse (to BH), and Kvismare Bird Observatory (report no. 138).

REFERENCES

- Archibald, A. L. *et al.* 1995 The pigmap consortium linkage map of the pig (*Sus scrofa*). *Mamm. Genome* **6**, 157–175. (doi:10.1007/BF00293008.)
- Arlt, D., Hansson, B., Bensch, S., von Schantz, T. & Hasselquist, D. 2004 Breeding synchrony does not affect extra-pair paternity in great reed warblers. *Behaviour* **141**, 863–880. (doi:10.1163/1568539042265699.)
- Badyaev, A. V., Hill, G. E., Beck, M. L., Dervan, A. A., Duckworth, R. A., McGraw, K. J., Nolan, P. M. & Whittingham, L. A. 2002 Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* **295**, 316–318. (doi:10.1126/science.1066651.)
- Baverstock, P. R., Adams, M., Polkinghorne, R. W. & Gelder, M. 1982 A sex-linked enzyme in birds—Z-chromosome conservation but no dosage compensation. *Nature* **296**, 763–766. (doi:10.1038/296763a0.)
- Bensch, S. 1996 Female mating status and reproductive success in the great reed warbler: is there a potential cost of polygyny that requires compensation? *J. Anim. Ecol.* **65**, 283–296.
- Bensch, S., Hasselquist, D. & von Schantz, T. 1994 Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler. *Evolution* **48**, 317–326.
- Bernstein, H., Hopf, F. & Michod, R. 1988 Is meiotic recombination and adaptation for repairing DNA, producing genetic variability, or both? In *The evolution of sex* (ed. R. Michod & B. Levin), pp. 139–160. Sunderland, MA: Sinauer.
- Broman, K. W., Murray, J. C., Sheffield, V. C., White, R. L. & Weber, J. L. 1998 Comprehensive human genetic maps: Individual and sex-specific variation in recombination. *Am. J. Hum. Genet.* **63**, 861–869. (doi:10.1086/302011.)
- Burns, J. L., Jackson, D. A. & Hassan, A. B. 2001 A view through the clouds of imprinting. *FASEB J.* **15**, 1694–1703. (doi:10.1096/fj.010085rev.)
- Burt, D. W., Morrice, D. R., Sewalem, A., Smith, J., Paton, I. R., Smith, E. J., Bentley, J. & Hocking, P. M. 2003 Preliminary linkage map of the Turkey (*Meleagris gallopavo*) based on microsatellite markers. *Anim. Genet.* **34**, 399–409. (doi:10.1046/j.1365-2052.2003.01033.x.)
- Catchpole, C. K., Leisler, B. & Winkler, H. 1985 Polygyny in the great reed warbler, *Acrocephalus arundinaceus*: a possible case of deception. *Behav. Ecol. Sociobiol.* **16**, 285–291. (doi:10.1007/BF00310992.)

- Christians, E., Boiani, M., Garagna, S., Dessy, C., Redi, C. A., Renard, J. P. & Zuccotti, M. 1999 Gene expression and chromatin organization during mouse oocyte growth. *Dev. Biol.* **207**, 76–85. (doi:10.1006/dbio.1998.9157.)
- Dawson, D. A., Burke, T., Hansson, B., Pandhal, J., Hale, M. C., Hinten, G. H. & Slate, J. 2006 A predicted microsatellite map of the passerine genome based on chicken–passerine sequence similarity. *Mol. Ecol.* (In press.)
- Degnan, S. M., Robertson, B. C., Clegg, S. B. & Moritz, C. C. 1999 Microsatellite primers for studies of gene flow and mating systems in white-eyes (*Zosterops*). *Mol. Ecol.* **8**, 159–160. (doi:10.1046/j.1365-294X.1999.00799.x.)
- Derjushva, S., Kurganova, A., Habermann, F. & Gaginskaya, E. 2004 High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds. *Chromosome Res.* **12**, 715–723. (doi:10.1023/B:CHRO.0000045779.50641.00.)
- Dietrich, W. F. *et al.* 1996 A comprehensive genetic map of the mouse genome. *Nature* **380**, 149–152. (doi:10.1038/380149a0.)
- Double, M. C., Dawson, D., Burke, T. & Cockburn, A. 1997 Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairywren *Malurus cyaneus*. *Mol. Ecol.* **6**, 691–693. (doi:10.1046/j.1365-294X.1997.00228.x.)
- Ellegren, H. 2005 The avian genome uncovered. *Trends Ecol. Evol.* **20**, 180–186. (doi:10.1016/j.tree.2005.01.015.)
- Flint, J. & Mott, R. 2001 Finding the molecular basis of quantitative traits: successes and pitfalls. *Nat. Rev. Genet.* **2**, 437–445. (doi:10.1038/35076585.)
- Frentiu, F. D., Lange, C. L., Burke, T. & Owens, I. P. F. 2003 Isolation of microsatellite loci in the Capricorn silvereye, *Zosterops lateralis chlorocephalus* (Aves: Zosteropidae). *Mol. Ecol. Notes* **3**, 462–464. (doi:10.1046/j.1471-8286.2003.00484.x.)
- Gibbs, H. L., Tabak, L. M. & Hobson, K. 1999 Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Mol. Ecol.* **8**, 1551–1552. (doi:10.1046/j.1365-294x.1999.00673.x.)
- Griffith, S. C., Stewart, I. R. K., Dawson, D. A., Owens, I. P. F. & Burke, T. 1999 Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? *Biol. J. Linn. Soc.* **68**, 303–316. (doi:10.1006/bjil.1999.0343.)
- Groenen, M. A. M. *et al.* 2000 A consensus linkage map of the chicken genome. *Genome Res.* **10**, 137–147.
- Gustafsson, L. & Sutherland, W. J. 1988 The cost of reproduction in the collared flycatcher *Ficedula albicollis*. *Nature* **335**, 813–815. (doi:10.1038/335813a0.)
- Haldane, J. B. S. 1922 Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* **12**, 101–109.
- Hanotte, O., Zanon, C., Greig, C., Dixon, A. & Burke, T. 1994 Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Mol. Ecol.* **3**, 529–530.
- Hansson, B. & Kawabe, A. 2005 A simple method to score single nucleotide polymorphisms based on allele-specific PCR and primer-induced fragment-length variation. *Mol. Ecol. Notes* **5**, 692–696. (doi:10.1111/j.1471-8286.2005.01033.x.)
- Hansson, B., Bensch, S. & Hasselquist, D. 2000a Patterns of nest predation contribute to polygyny in the great reed warbler. *Ecology* **81**, 319–328.
- Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B.-G., Wennerberg, L. & von Schantz, T. 2000b Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Mol. Ecol.* **9**, 1529–1538. (doi:10.1046/j.1365-294x.2000.01028.x.)
- Hansson, B., Bensch, S., Hasselquist, D. & Nielsen, B. 2002 Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler. *Oecologia* **130**, 536–542. (doi:10.1007/s00442-001-0831-2.)
- Hansson, B., Gavrillov, E. & Gavrillov, A. 2003 Hybridisation between great reed warblers *Acrocephalus arundinaceus* and clamorous reed warblers *A. stentoreus*: morphological and molecular evidence. *Avian Sci.* **3**, 145–151.
- Hansson, B., Bensch, S. & Hasselquist, D. 2004a Do female great reed warblers seek extra-pair fertilizations to avoid inbreeding? *Proc. R. Soc. B* **271**(Suppl. 5), S290–S292. (doi:10.1098/rsbl.2004.0164.)
- Hansson, B., Bensch, S. & Hasselquist, D. 2004b Lifetime fitness of short- and long-distance dispersing great reed warblers. *Evolution* **58**, 2546–2557.
- Hansson, B., Westerdahl, H., Bensch, S., Hasselquist, D. & Åkesson, M. 2004c Does linkage disequilibrium generate heterozygosity–fitness correlations in great reed warblers? *Evolution* **58**, 870–879.
- Hasselquist, D. 1998 Polygyny in the great reed warbler: a long-term study of factors contributing to male fitness. *Ecology* **79**, 2376–2390.
- Hasselquist, D., Bensch, S. & von Schantz, T. 1996 Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* **381**, 229–232. (doi:10.1038/381229a0.)
- Helbig, A. J. & Seibold, I. 1999 Molecular phylogeny of the Palearctic-African *Acrocephalus* and *Hippolais* warblers (Aves: Sylviidae). *Mol. Phylogenet. Evol.* **11**, 246–260. (doi:10.1006/mpev.1998.0571.)
- Hulten, M. 1974 Chiasma distribution at diakinesis in normal human male. *Hereditas* **76**, 55–78.
- Hurst, L. D., Pal, C. & Lercher, M. J. 2004 The evolutionary dynamics of eukaryotic gene order. *Nat. Rev. Genet.* **5**, 299–310. (doi:10.1038/nrg1319.)
- Huxley, J. S. 1928 Sexual differences of linkage in *Gammarus chevreuxi*. *J. Genet.* **20**, 145–156.
- International Chicken Genome Sequencing Consortium 2004 Sequencing and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716. (doi:10.1038/nature03154.)
- Irwin, D. E., Bensch, S. & Price, T. D. 2001 Speciation in a ring. *Nature* **409**, 333–337. (doi:10.1038/35053059.)
- Ishibashi, Y., Mikami, O. & Abe, S. 2000 Isolation and characterization of microsatellite loci in the Japanese marsh warbler *Locustella pryeri*. *Mol. Ecol.* **9**, 373–375. (doi:10.1046/j.1365-294x.2000.00874-5.x.)
- Kayang, B. B., Vignal, A., Inoue-Murayama, M., Miwa, M., Monvoisin, J. L., Ito, S. & Minvielle, F. 2004 A first-generation microsatellite linkage map of the Japanese quail. *Anim. Genet.* **35**, 195–200. (doi:10.1111/j.1365-2052.2004.01135.x.)
- Keller, L. F., Arcese, P., Smith, J. N. M., Hochachka, W. M. & Stearns, S. C. 1994 Selection against inbred song sparrows during a natural population bottleneck. *Nature* **372**, 356–357. (doi:10.1038/372356a0.)
- Komdeur, J. 1992 Importance of habitat saturation and territory quality for the evolution of cooperative breeding in the Seychelles warbler. *Nature* **358**, 493–495. (doi:10.1038/358493a0.)
- Kong, A. *et al.* 2002 A high-resolution recombination map of the human genome. *Nat. Genet.* **31**, 241–247.
- Kuroda, Y., Arai, N., Arita, M., Teranishi, M., Hori, T., Harata, M. & Mizuno, S. 2001 Absence of Z-chromosome inactivation for five genes in male chickens. *Chromosome Res.* **9**, 457–468. (doi:10.1023/A:1011672227256.)
- Lander, E. S. & Green, P. 1987 Construction of multilocus genetic-linkage maps in humans. *Proc. Natl Acad. Sci. USA* **84**, 2363–2367.

- Lenormand, T. 2003 The evolution of sex dimorphism in recombination. *Genetics* **163**, 811–822.
- Lynn, A., Kashuk, C., Petersen, M. B., Bailey, J. A., Cox, D. R., Antonarakis, S. E. & Chakravarti, A. 2000 Patterns of meiotic recombination on the long arm of human chromosome 21. *Genome Res.* **10**, 1319–1332. (doi:10.1101/gr.138100.)
- McQueen, H. A., McBride, D., Miele, G., Bird, A. P. & Clinton, M. 2001 Dosage compensation in birds. *Curr. Biol.* **11**, 253–257. (doi:10.1016/S0960-9822(01)00070-7.)
- McRae, S. B. & Amos, W. 1999 Characterization of hypervariable microsatellites in the cooperatively breeding white-browed sparrow weaver *Plocepasser mahali*. *Mol. Ecol.* **8**, 903–904.
- Martinez, J. G., Soler, J. J., Soler, M., Møller, A. P. & Burke, T. 1999 Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution* **53**, 269–278.
- Masabanda, J. S. *et al.* 2004 Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. *Genetics* **166**, 1367–1373. (doi:10.1534/genetics.166.3.1367.)
- Mellersh, C. S. *et al.* 1997 A linkage map of the canine genome. *Genomics* **46**, 326–336. (doi:10.1006/geno.1997.5098.)
- Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. 2001 Cryptic evolution in a wild bird population. *Nature* **412**, 76–79.
- Neff, M. W., Broman, K. W., Mellersh, C. S., Ray, K., Acland, G. M., Aguirre, G. D., Ziegler, J. S., Ostrander, E. A. & Rine, J. 1999 A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics* **151**, 803–820.
- Neumann, K. & Wetton, J. H. 1996 Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. *Mol. Ecol.* **5**, 307–309. (doi:10.1046/j.1365-294X.1996.00095.x.)
- Nishiumi, I., Yamagishi, S., Maekawa, H. & Shimoda, C. 1996 Paternal expenditure is related to brood sex ratio in polygynous great reed warblers. *Behav. Ecol. Sociobiol.* **39**, 211–217. (doi:10.1007/s002650050283.)
- Norris, K. 1993 Heritable variation in a plumage indicator of viability in male great tits *Parus major*. *Nature* **362**, 537–539. (doi:10.1038/362537a0.)
- O'Connell, J. R. & Weeks, D. E. 1998 PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am. J. Hum. Genet.* **63**, 259–266. (doi:10.1086/301904.)
- Otsuka, R., Nishiumi, I. & Wada, M. 2003 Characterisation of 12 polymorphic microsatellite loci in the Japanese bush warbler *Cettia diphone*. *Mol. Ecol. Notes* **3**, 44–46. (doi:10.1046/j.1471-8286.2003.00345.x.)
- Otter, K., Ratcliffe, L., Michaud, D. & Boag, P. T. 1998 Do female black-capped chickadees prefer high-ranking males as extra-pair partners? *Behav. Ecol. Sociobiol.* **43**, 25–36. (doi:10.1007/s002650050463.)
- Otto, S. P. & Lenormand, T. 2002 Resolving the paradox of sex and recombination. *Nat. Rev. Genet.* **3**, 252–261. (doi:10.1038/nrg761.)
- Petren, K. 1998 Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. *Mol. Ecol.* **7**, 1782–1784.
- Piertney, S. B., Marquiss, M. & Summers, R. 1998 Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Mol. Ecol.* **7**, 1261–1263. (doi:10.1046/j.1365-294x.1998.00493.x.)
- Plomion, C. & O'Malley, D. M. 1996 Recombination rate differences for pollen parents and seed parents in *Pinus pinaster*. *Heredity* **77**, 341–350.
- Primmer, C. R., Møller, A. P. & Ellegren, H. 1995 Resolving genetic relationship with microsatellite markers: a parent-age testing system for the swallow *Hirundo rustica*. *Mol. Ecol.* **4**, 493–498.
- Primmer, C. R., Raudsepp, T., Chowdhary, B. P., Møller, A. P. & Ellegren, H. 1997 Low frequency of microsatellites in the avian genome. *Genome Res.* **7**, 471–482.
- Rice, W. R. 2002 Experimental tests of the adaptive significance of sexual recombination. *Nat. Rev. Genet.* **3**, 241–251. (doi:10.1038/nrg760.)
- Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2226–2231. (doi:10.1046/j.1365-294X.2000.105338.x.)
- Richman, A. D. & Price, T. 1992 Evolution of ecological differences in the Old World leaf Warblers. *Nature* **355**, 817–821. (doi:10.1038/355817a0.)
- Sætre, G. P., Borge, T., Lindroos, K., Haavie, J., Sheldon, B. C., Primmer, C. & Syvanen, A. C. 2003 Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proc. R. Soc. B* **270**, 53–59. (doi:10.1098/rspb.2002.2204.)
- Sakamoto, T. *et al.* 2000 A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. *Genetics* **155**, 1331–1345.
- Saladin, V., Bonfils, D., Binz, T. & Richner, H. 2003 Isolation and characterization of 16 microsatellite loci in the Great Tit *Parus major*. *Mol. Ecol. Notes* **3**, 520–522. (doi:10.1046/j.1471-8286.2003.00498.x.)
- Samolow, P. B., Kammerer, C. M., Mahaney, S. M., Schneider, J. L., Westenberger, S. J., VandeBerg, J. L. & Robinson, E. S. 2004 First-generation linkage map of the gray, short-tailed opossum, *Monodelphis domestica*, reveals genome-wide reduction in female recombination rates. *Genetics* **166**, 307–329. (doi:10.1534/genetics.166.1.307.)
- Slate, J., Visscher, P. M., MacGregor, S., Stevens, D., Tate, M. L. & Pemberton, J. M. 2002 A genome scan for quantitative trait loci in a wild population of red deer (*Cervus elaphus*). *Genetics* **162**, 1863–1873.
- Stauss, M., Tomiuk, J., Segelbacher, G., Driesel, S., Fietz, J., Bachmann, L. & Kompf, J. 2003 Sex-specific recombination rates in *Parus major* and *P. caeruleus*, an exception to Huxley's rule. *Heredity* **139**, 199–205. (doi:10.1111/j.1601-5223.2003.01698.x.)
- Trivers, R. 1988 Sex differences in rates of recombination and sexual selection. In *The evolution of sex* (ed. R. Michod & B. Levin), pp. 270–286. Sunderland, MA: Sinauer.
- Veen, T., Borge, T., Griffith, S. C., Sætre, G. P., Bures, S., Gustafsson, L. & Sheldon, B. C. 2001 Hybridization and adaptive mate choice in flycatchers. *Nature* **411**, 45–50. (doi:10.1038/35075000.)
- Westerdahl, H., Bensch, S., Hansson, B., Hasselquist, D. & von Schantz, T. 2000 Brood sex ratios, female harem status and resources for nestling provisioning in the great reed warbler. *Behav. Ecol. Sociobiol.* **47**, 312–318. (doi:10.1007/s002650050671.)
- Westerdahl, H., Hansson, B., Bensch, S. & Hasselquist, D. 2004 Between-year variation of MHC allele frequencies in great reed warblers: selection or drift? *J. Evol. Biol.* **17**, 485–492. (doi:10.1111/j.1420-9101.2004.00711.x.)
- Xu, H. L., Swoboda, I., Bhalla, P. L. & Singh, M. B. 1999 Male gametic cell-specific gene expression in flowering plants. *Proc. Natl Acad. Sci. USA* **96**, 2554–2558. (doi:10.1073/pnas.96.5.2554.)

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.