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A Genetic and Cytogenetic Map for the Duck (*Anas platyrhynchos*)

Yinhua Huang,* Yonghui Zhao,* Chris S. Haley,† Shengqiang Hu,‡ Jinping Hao,‡ Changxin Wu§ and Ning Li*,[1]

**State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100094, People's Republic of China,* †*Roslin Institute, Roslin, Midlotthian EH25 9PS, United Kingdom,* ‡*Gold Star Duck Production, Beijing 100076, People's Republic of China and* §*College of Animal Science and Technology, China Agricultural University, Beijing 100094, People's Republic of China*

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ABSTRACT

A genetic linkage map for the duck (*Anas platyrhynchos*) was developed within a cross between two extreme Peking duck lines by linkage analysis of 155 polymorphic microsatellite markers, including 84 novel markers reported in this study. A total of 115 microsatellite markers were placed into 19 linkage groups. The sex-averaged map spans 1353.3 cM, with an average interval distance of 15.04 cM. The male map covers 1415 cM, whereas the female map covers only 1387.6 cM. All of the flanking sequences of the 155 polymorphic loci—44 monomorphic loci and a further 41 reported microsatellite loci for duck—were blasted against the chicken genomic sequence, and corresponding orthologs were found for 49. To integrate the genetic and cytogenetic map of the duck genome, 28 BAC clones were screened from a chicken BAC library using the specific PCR primers and localized to duck chromosomes by FISH, respectively. Of 28 BAC clones, 24 were detected definitely on duck chromosomes. Thus, 11 of 19 linkage groups were localized to 10 duck chromosomes. This genetic and cytogenetic map will be helpful for the mapping QTL in duck for breeding applications and for conducting genomic comparisons between chicken and duck.

MOLECULAR genetic maps will provide insight into the genome organization and chromosomal localization of cloned genes and also a framework for the identification and localization of major genes associated with economically important traits (Crittenden *et al.* 1993). The rapid progress being made in the development of genetic maps for humans and mice has led to a recent boom in the construction of genome maps for many farm animals. High-density linkage maps are now available for many species, such as pigs, cattle, sheep, and goats. In contrast, mapping studies in avian species are much less advanced except in the chicken. To construct saturated genetic maps for more bird species, the isolation of many polymorphic genetic markers, particularly microsatellite markers, is a prerequisite. Although many chicken markers were available, it was difficult to screen the microsatellite marker for the duck by cross-species amplification from this species because of the poor conservation of the microsatellite sequences between the species (Huang *et al.* 2005).

Microsatellites or simple sequence repeats are tandem repeated motifs of 1–6 bases found in all prokaryotic and eukaryotic genomes and present in both coding and noncoding regions. Despite the fact that the mechanism of evolution is still unclear, microsatellites are being widely employed in forensics, genetic mapping, population genetics, evolutionary studies, and investigation of social systems (Chakraborty *et al.* 1997; Buchholz *et al.* 1998; Chowdhury and Bansal 2001; Jernej and Branka 2001; Burt *et al.* 2003; Harry *et al.* 2003; Huang *et al.* 2004; Knutson *et al.* 2004).

The Peking duck, the most common type of duck breed for meat, was exported to the United States and Britain from China in the early part of the last century. Many ducks raised for their meat have originated from this breed. Ducks, belonging to the order Anseriformes, diverged from the chicken (Galliformes) ~110 million years ago (Tuinen and Hadly 2004). According to paleontological data, the main radiation of the modern duck took place during the Miocene, 5–23 million years ago (Olson 1985). Ducks, together with the ostrich, emu, peacock, turkey, quail, and other birds, play a major role in studies of bird evolution. Up to now, most available molecular data concerning ducks have come from evolutionary studies based on the analysis of mitochondrial DNA sequence (Cooper *et al.* 1996; Liu *et al.* 1996; Sraml *et al.* 1996; Johnson and Sorenson 1998, 1999; Donne-Gousse *et al.* 2002). However, information about genetic markers in the duck is limited (Buchholz *et al.* 1998; Maak *et al.* 2000, 2003; Paulus and Tiedemann 2003; Stai and Hughes 2003; Huang *et al.* 2005). In addition, comparative mapping between the duck and chicken genome gains importance as the

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[1]*Corresponding author:* State Key Laboratory for Agrobiotechnology, China Agricultural University, Yuanmingyuan, West Rd. 2, Haidian District, Beijing 100094, People's Republic of China.
E-mail: ninglbau@public3.bta.net.cn

Chicken Genome Project moves toward functional genomics, *i.e.*, the functional characterization of large regions of sequenced DNA. A good comparative genetic map based on the orthologous microsatellite markers will provide the substrates for major gene identification (Reed *et al.* 2005a,b). Up to now, most available comparative data between ducks and chickens have come from the analysis of chromosome painting (Guttenbach *et al.* 2003). Herein, the preliminary genetic map was developed in an inbred Peking duck resource population (line VI cross with line V) on the basis of linkage analysis of 103 novel genetic markers from the microsatellite-enriched library (Huang *et al.* 2005), together with 137 reported microsatellite loci (Cathey *et al.* 1996; Fields and Scribner 1997; Buchholz *et al.* 1998; Maak *et al.* 2000; Genet *et al.* 2003; Stai and Hughes 2003; Huang *et al.* 2005). In addition, the preliminary genetic map was integrated with the cytogenetic map based on fluorescence *in situ* hybridization (FISH) using 28 chicken heterologous BAC clones.

MATERIALS AND METHODS

Resource population: In collaboration with Gold Star Duck Production (Beijing), an experimental population with a total of 224 G_2 individuals was created containing 12 full-sib families of a cross between two extreme Peking duck lines (line LVI and LV). Four male individuals of generation 1 were the offspring of LVI, and 12 female individuals of generation 1 were the offspring of LV. A routine phenol/chloroform extraction method was used to extract and purify the duck genomic DNA. The DNA was quantified using agarose gel electrophoresis. The DNA concentration was estimated by comparison with molecular standard markers.

Sequencing of clones and designing of primers: A library enriched for $(CA)_n$, $(CAG)_n$, $(GCC)_n$, and $(TTTC)_n$ was constructed from a female Peking duck (Huang *et al.* 2005). Plasmids, extracted from clones of the microsatellite-enriched library, were sequenced with the BigDye kit on PRISM 377 DNA sequencers (ABI, Columbia, MD). Oligo6.0 was used to design PCR products ranging from 100 to 400 bp. One primer in each pair was labeled with either 6-FAM or HEX fluorescent dye (AuGCT Biotechnology, Beijing, People's Republic of China).

Optimization of mutiplex PCR and muti-run: The annealing temperature of the microsatellite primers was determined using an authorized Thermal Cycler (Eppendorf, Hamburg, Germany). DNA amplification was performed in a total volume of 10 μl, with 40 ng duck DNA, 50 mm KCl, 1.5 mm MgCl2, 10 mm Tris, HCl (pH 8.3), 1 mm tetramethylammonium chloride, 0.1% Triton X-100, 0.01% gelatin, 200 mm dNTP, 0.2–2 pmol of each primer, and 2.5 units Taq polymerase. The PCR reaction conditions were denaturing for 5 min at 94°, followed by 94° for 40 sec, 58° ± 10° for 30 sec or 1 min, and 72° for 30 sec or 1 min, with a final 30-min elongation step at 72°. PCR primer pairs with similar annealing temperatures and different amplification product sizes were combined in mutiplex PCR reactions. Primer pairs unsuitable for mutiplex PCR were used in independent reactions; however, the products could be run in the same lane (muti-run) of the gel if their sizes were sufficiently different (>60 bp).

Linkage analysis: Mutiplex PCR products or independent PCR products were diluted by 10–70 times. A mixture of 1 μl diluted PCR product, 12 μl deoinized formadide (Amresco), and 0.2 μl Genescan-350 ROX or Genescan-500 ROX (ABI) internal standard was run on a 3100 pop-4 (ABI) using a 3100 genetic analyzer (ABI). The fragment sizes of PCR products were analyzed using the Genescan 3.7 and Genemapper 1.1 software (ABI). Although the genotypes were performed automatically by Genemapper 1.1 software after the panel and bin were defined, all individual genotypes were checked manually twice (each time before and after export to Excel). In addition, typing errors detected with the CRIMAP program (using the PREPARE option) were rechecked within Genescan 3.7 and corrected where necessary.

The genotyping data were extracted from Excel worksheets and reformatted into the Gen file for the CRIMAP Version 2.4 linkage analysis program (Green *et al.* 1990). Initially, a two-point linkage analysis in which polymorphic markers were analyzed against each other (LOD score ≥3.0) was performed. Then markers that distributed into the same linkage group were used to build the map using the CRIMAP-ALL option. Next the order of the framework loci was checked using the CRIMAP–FLIPS function. Finally, the marker distance on a linear map was determined by the CRIMAP–BUILD option.

The linkage maps were drawn using MapChart 2.1 software (Voorrips 2002).

Identifying the orthologous microsatellite DNA of duck in the chicken genome: The flanking sequences of duck microsatellite DNA were analyzed in a megaBLAST search against the National Center for Biotechnology Information with the default parameters on http://www.ncbi.nlm.nih.gov/genome/seq/ggablast.html. The unique match sequences with an *E*-value smaller than e-25 from chicken were used as orthologs to the duck microsatellite DNA.

Screening and preparing probes: The BAC clones used for FISH mapping were screened by 4D, two-step PCR from a *Hin*dIII, female white-silk chicken BAC library with inserts from vector pBeloBAC11 using specific PCR primers (http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixII) which were designed from the orthologous sequences of the duck microsatellite in chicken genome (Liu *et al.* 2003). Screened positive clones were confirmed further by sequencing analysis of PCR products. A routine phenol/chloroform extraction method was used to extract and purify the chicken BAC genomic DNA. The BIONICK labeling system was used to prepare DNA probes combined with biotin-14-dATP (Invitrogen, San Diego).

FISH: Metaphase chromosomes were prepared from fetal fibroblast cultures of Peking duck by standard cytogenetic techniques. The method of FISH was modified from Coppieters *et al.* (1994). Briefly, chromosome slides were hardened at 65° for 2 hr, denatured at 70° for 2.5 min in 70% formamide, 2× SSC, and dehydrated in an ethanol series at −20°. Probe, which was labeled with biotin-14-dATP, was coprecipitated with a 50-fold excess of salmon sperm DNA and a 50-fold excess of sonicated chicken genome DNA. The denatured precipitation was dissolved in hybridization solution to a final concentration of 50 ng/μl and prehybridized for 40 min at 37°. Hybridization was performed for 48 hr at 37° in a humid chamber. Probes were detected with FITC-conjugated avidin (Vector, Burlingame, CA) and signals were amplified by biotinlated antiavidin (Vector). Chromosomes were counterstained with 0.5 μg/ml propidium iodide. Images were taken with an epifluorescence microscope equipped with a DP70 CCD camera (Olympus, Tokyo) and dealt with software Video TesT-FISH (Video TesT, St. Petersburg, Russia).

RESULTS

Isolation and characterization of microsatellite loci: To isolate the specific microsatellite markers for duck, a

total of 1025 plasmids, extracted from the microsatellite-enriched library (Huang *et al.* 2005), were sequenced according to the procedures described in materials and methods; 753 (73.46%) were found to contain $(CA)_n$, $(CAG)_n$, $(GCC)_n$, $(TTTC)_n$, $(TC)_n$, $(TCC)_n$, $(TTCC)_n$, $(TTTTC)_n$, $(TTGCC)_n$, $(TTCCTC)_n$, $(AAC)_n$, $(AAAC)_n$, $(TA)_n$, or $(TTTA)_n$ microsatellites with five or more repeats, and 5 contained $(TCTCTTTC)_n$, $(TTTCCCTCTTTC)_n$, or $(CTTCTTTC)_n$ with six or more repeats. Of the 758 sequences, 539 (71.11%) contained >12 bp of uninterrupted core repeats. Of the 539 clones, 93 were found to contain two microsatellites and 92 were duplicates; 223 (41.37%) did not provide sufficient sequence information in the region flanking the repeat for development of PCR primers, and 224 were used to design for primers. Of the 224 pairs of primers, 103 were suitable for genotyping with the expected size fragments after the modification of annealing temperature or extension time (see http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixI). Polymorphisms of the markers were detected in 31 unrelated G_0 individuals according to the optimized mutiplex PCR. Of the 103 primer pairs, 84 were polymorphic, and for the remaining 19 loci, only one allele was found in the population.

All the 103 novel duck microsatellite sequences were submitted to GenBank (AY493281–AY493347 and AY587022–AY587057). Information for 103 novel microsatellite markers, including GenBank accession number, microsatellite repeat sequence, sequence of the PCR primers, optimal annealing temperature for PCR, length of PCR products, number of alleles, observed heterozygosity, as well as the polymorphism information content, are shown in Appendix I (http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixI).

In addition, 137 duck-specific microsatellite markers, which were reported by Fields and Scribner (1997), Buchholz *et al.* (1998), Maak *et al.* (2000, 2003), Genet *et al.* (2003), Stai and Hughes (2003), Paulus and Tiedemann (2003), and Huang *et al.* (2005), were amplified from Peking duck genomic DNA and 96 of them yielded specific amplified products after the modification of annealing temperature or extension time (see http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixI). Of these 96 loci, 71 exhibited sequence length polymorphisms, while for the remainder only one allele was observed in the 31 unrelated G_0 individuals.

Thus, a total of 155 polymorphic loci were genotyped in the resource population with 12 full-sib families. A total of 650 alleles were observed for these polymorphic loci, and the number of alleles per locus ranged from 2 to 18 with an average of 4.19. Among the polymorphic markers, the highest heterozygosity (0.97) was observed at CAUD019 and the lowest herterozygosity (0.03) at CAUD064 (http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixI). A total of 63 (40.65%) loci had heterozygosities >0.50. The polymorphism information content (PIC) of 155 loci ranged from 0.03 to 0.90. The percentage of the loci with a PIC >0.50 was 35.48% (55), with a PIC between 0.25 and 0.5, 49.03% (76); and with a PIC <0.25, 15.48% (24). In addition, 149 of 155 polymorphic loci for which the number of informative meioses varied from 9 to 427, the average being 196.33, were useful to linkage analysis. The remaining 6 loci were polymorphic but had no informative meioses in our pedigree and so were not useful for linkage analysis.

Genetic linkage map: A total of 115 markers were placed into 19 genetic groups, and 34 polymorphic markers were unlinked with any markers by two-point analysis with LOD scores >3.0 using CRIMAP Version 2.4 (Green *et al.* 1990) (see Figure 1 and Table 1). Thirteen linkage groups composed three or more loci, whereas the remaining 6 groups each contained 2 markers. The sex-averaged map spanned 1353.3 cM. The marker interval of each linkage group ranged from 0 to 62.2 cM. The markers were distributed among 90 distinct positions; thus the average marker interval was 15.04 cM. On this sex-averaged map, 17.78% (16) of the intervals between markers varied from 0 to 5 cM, 25.56% (23) ranged from 5 to 10 cM, 30% (27) varied from 10 to 20 cM, and 26.67% (24) were >20 cM.

Sex-specific maps have also been constructed. The length of the male map was 1415 cM, with an average intermarker distance of 16.26 cM, whereas the female map was 1387.6 cM, with the average intermarker spacing of 17.13 cM. The male map comprised linkage groups ranging in length from 0 to 347.2 cM, while the female map contained linkage groups with a length ranging 0 to 398.8 cM (see Table 1). The length of the male map is 1.02-fold that of the female map, and that of the sex-averaged map was shorter than both of the sex-specific maps and 0.98 times as long as the female map.

Orthologs of duck microsatellite sequence in the chicken genome: To search for orthologs, 240 flanking sequences of duck microsatellite DNA were blasted against chicken genomic sequence as described in materials and methods. The corresponding orthologous sequences were found for 49 of the 240 examined (see Table 2). The position in the duck genetic map, the physical position in the chicken genome, the physical position in the duck genome, primer sequences for the screening of chicken BAC clones, as well as repeats of the orthologous sequences are listed in Table 2.

Of the 49 duck microsatellite loci with orthologs in the chicken genome, 27 were placed into 15 genetic groups, 18 were unlinked with any markers, and the rest were not genotyped in the above resource population. Sequencing comparisons showed that the core repeats in the chicken for 25 of the orthologous loci were the same or similar to the corresponding one of the duck, but were different for 7 orthologous loci. Moreover, for the remaining 17 of these duck microsatellite loci the

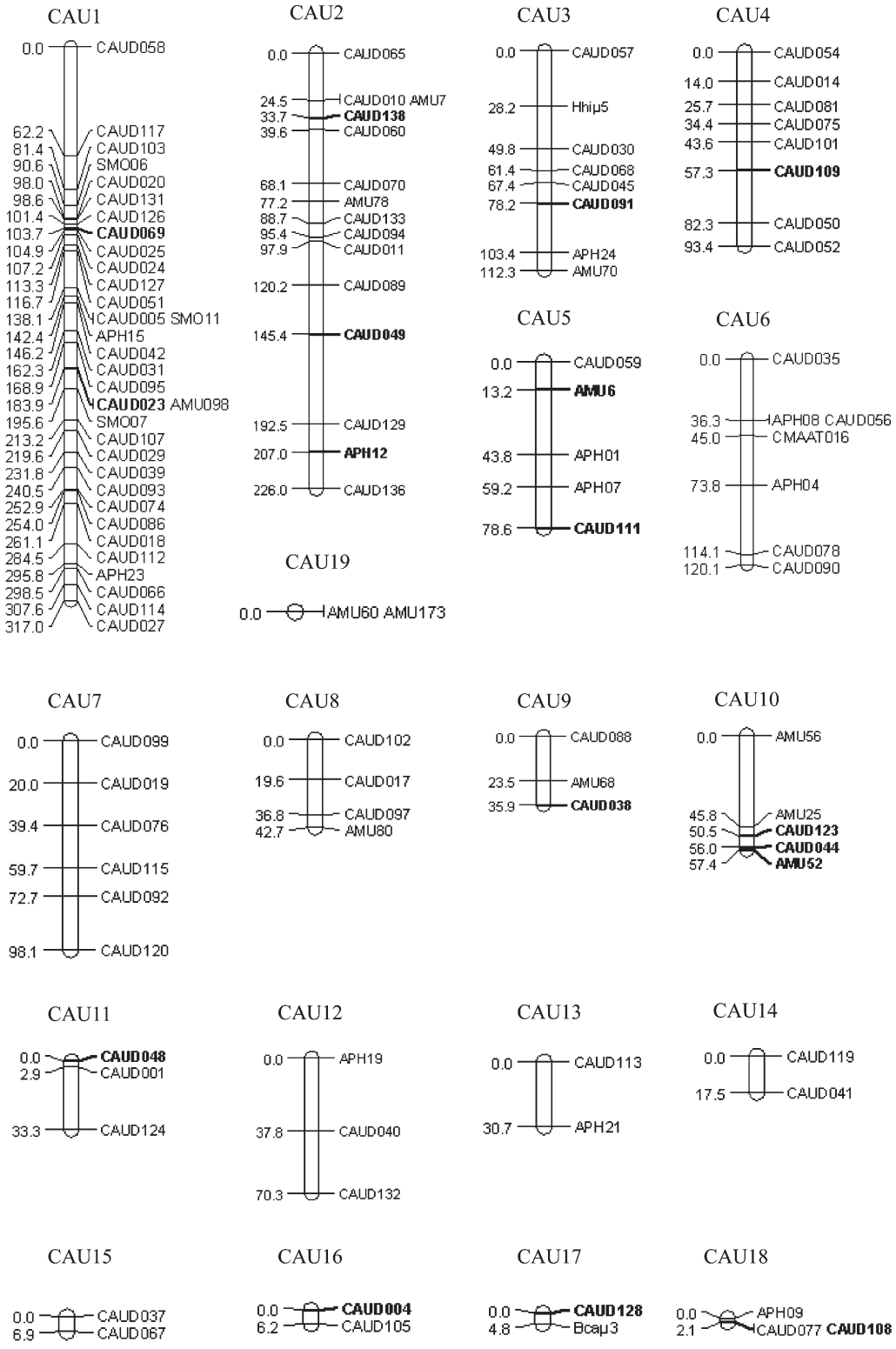

FIGURE 1.—Sex-average map in Kosambi centimorgans for the duck consisting of 19 linkage groups. The markers in boldface type were physically assigned to duck chromosomes by FISH with chicken BAC probe. Two principles were used in naming the linkage groups of duck: (1) physical positions on the duck and chicken chromosomes of the orthologous loci from the linkage groups and (2) coverage of the linkage groups.

core repeats were absent from the orthologous loci in the chicken.

Integration of genetic and cytogenetic map: To integrate the genetic and cytogenetic map in the duck genome, 28 BAC clones were screened by 4D, two-step PCR from a chicken BAC library using the specific PCR primers and were localized to duck chromosomes by FISH, respectively. Fluorescent signals were detected on duck chromosomes in 24 clones (see Figure 2; http://www.genetics.org/cgi/content/full/genetics.105.053256/AppendixII). Among them, 3 clones (CB0509A6, CB0041G5, and CB0234B11) were situated on duck chromosome 1, 3 clones (CB0742G10, CB0729A5, and CB0828G1) on chromosome 2, 2 clones (CB0593C11

TABLE 1

Parameters of the duck genetic and cytogenetic maps

Linkage group	Locus no.	Size average	Size		Physically localized locus no.[a]
			Male	Female	
1	33	317.0	347.2	398.8	2
2	15	226	188.7	257.6	3
3	8	112.3	126.8	102.9	1
4	8	93.4	122.7	78.1	1
5	5	78.6	74.9	98.2	2
6	7	120.1	109.8	136.9	0
7	6	98.1	113.8	81	0
8	4	42.7	46.2	9	0
9	3	35.9	42.5	31.4	1
10	5	57.4	47.6	69.7	3
11	3	33.3	50.2	16.8	1
12	3	70.3	37.2	70.8	0
13	2	30.7	41.3	19.7	0
14	2	17.5	30.2	5.7	0
15	2	6.9	22.4	1.9	0
16	2	6.2	7.8	6	1
17	2	4.8	4.8	0	1
18	3	2.1	1	3.1	1
19	2	0	0	0	0
Total	115	1353.3	1415	1387.6	17

[a] The number of orthologous loci assigned to the duck chromosome by FISH using chicken BAC probes.

and CB0385F11) on chromosome 3, 2 clones (CB1342G10 and CB0432E11) on chromosome 4, 2 clones (CB0839F6 and CB0480D5) on chromosome 5, 1 clone (CB0375F10) on chromosome 7, 1 clone (CB0857E5) on chromosome 8, 1 clone (CB0096E9) on chromosome 9, 5 clones (CB0320C6, CB0478A3, CB0664E4, CB0098H2, and CB0228F1) on chromosome 10, and 4 clones (CB0222B3, CB0082E7, CB0247E2, and CB0168F8) on the other small chromosomes. Thus, 9 of 19 linkage groups (CAU1, CAU2, CAU4, CAU5, CAU9, CAU10, CAU11, CAU16, and CAU18) were assigned to 9 pairs of duck chromosomes, respectively. Two unlinked groups (CAU3 and CAU17) were placed on the same duck chromosome 3. Moreover, 1 unlinked marker was mapped on chromosome 1, 1 on chromosome 4, 1 on chromosome 7, 1 on chromosome 8, 2 on chromosome 10, and 1 on one pair of the microchromosome.

Among the 24 orthologous loci, which were located on duck chromosomes using chicken BAC clones, 3 (2 from CAU1 and 1 unlinked marker) were located on chicken chromosome 1, 3 (all from CAU2) on chicken chromosome 2, 2 (1 from CAU3 and 1 from CAU17) on chicken 3, 2 (1 from CAU4 and 1 from CAU18) on chicken chromosome 4, 2 (all from CAU5) on chicken chromosome 5, 1 (1 unlinked marker) on chicken chromosome 7, 1 (1 unlinked marker) on chicken chromosome 8, 1 (1 from CAU9) on chicken chromosome 9, 5 (3 from CAU10 and 2 unlinked markers) on chicken chromosome 10, 1 (1 from CAU11) on chicken chromosome 11, 1 (1 unlinked marker) on chicken chromosome 12, 1 (1 from CAU16) on chicken chromosome 26, and the other (1 unlinked marker) was not assigned to a chicken chromosome.

DISCUSSION

Evaluation of the mix-enriched library: The percentage of clones that contained microsatellite sequence (73.46%) in this enriched microsatellite library is similar to that in the ostrich-enriched microsatellite library (Tang *et al.* 2003) and higher than the percentage observed in the chicken-enriched microsatellite library (Cheng *et al.* 1995). Some highly enriched libraries (>90%) have been constructed in cichlid fish and plant (Jernej and Branka 2001; Carleton *et al.* 2002). Two reasons may account for the difference in enrichment level. One is that the procedure used to construct the library is different. The second is that the frequency of microsatellite DNA regions generally increases with increasing genome sizes (Gibbs *et al.* 1997; Garner 2002). In our enriched library, 41.37% of the clones, which contain microsatellite repeats, are not suitable to develop PCR primers because of the short flanking sequence. This percentage is higher than that observed in chicken and ostrich (Cheng *et al.* 1995; Tang *et al.* 2003). As in chicken and ostrich, small imperfect $(TG)_n$ microsatellites scattered in a 100- to 200-bp region and duplicated sequences are also found (Cheng *et al.* 1995; Tang *et al.* 2003).

In our modified enrichment procedure, four different probes of the microsatellite motifs were employed

TABLE 2

The position in duck genetic map, physical position in chicken, and core repeat of the orthologous loci

Locus	Linkage group	Score (bits)	*E*-value	Chromosome	Physical position in chicken genome	Repeat 1 in the chicken	Repeat 2 in the duck
AMU3	—	227	1.00e-57	Un	3919–4004	(CA)5CG(CA)9	(GC)3(AC)7
AMU6	5	292	4.00e-77	5	28655188–28655469	(AC)3AAG	(CA)11
AMU26	—	221	1.00e-55	21	3248787–3249060	A5C5	(AC)8
AMU52	10	227	5.22e-58	10	909588–909714	A16C(CA)6	(AC)9
AMU59	×	139	8.00e-31	6	964938–965018	(CA)3(CG)6	(CA)8
AMU60	19	356	3.00e-96	15	2366911–2367200	No	(CA)16
AMU63	×	191	9.00e-47	19	1794827–1794959	C4TC5	(AC)18
AMU87	—	314	1.00e-83	6	18939428–18939653	(AC)9	(GC)3(AC)5ATGC(AC)4
AMU144	×	125	3.00e-27	Un	02272–102379	A7	(AAAC)4(AAAA)2(AC)4AAAAC(AAAC)4
AMU171	×	396	1.00e-108	2	918238–6918696	T4C5	(AC)7
AMU174	—	675	0.00e+00	10	173670–174145	(AC)3AT(AC)5	(AC)6
AMU182	—	244	1.00e-62	1	2742097–2742628	No	(CA)8
APH12	2	377	1.00e-103	2	8156706–8156913	A23T2A8T5A5(GA)3A4GA3GA4	A13(AAAG)4
APH20	—	142	4.00e-32	8	2472612–2472916	No	(AC)14A11
Bcaμ4	—	329	2.00e-88	W	71763–72057	(CA)8	(CA)3CT(CA)3(CT)3(CA)2CT(CA)12
CAUD002	—	279	2.00e-73	4	3480635–3480850	A6	(CAG)6
CAUD004	16	246	2.00e-63	26	3883–4114	No	(AC)20
CAUD007	—	285	5.00e-75	1	6540332–6540614	(CAG)8	(CAG)7
CAUD021	—	356	2.00e-96	Un	922693–922909	(CA)4TG(CA)5	(CA)9
CAUD022	—	237	2.00e-60	7	10431–10887	(GCA)6	(GCA)8
CAUD023	1	439	1.00e-121	1	7350218–7350526	No	(AC)17
CAUD024	1	200	1.00e-49	Un	409–567	(TA)10	(TTTC)33 … T19
CAUD038	9	506	1.00e-141	9	4549520–4549914	T6(TC)6TAT9	(TTTC)23CT19
CAUD039	1	283	2.00e-74	1	8839103–8839460	(CA)5	(AC)13
CAUD040	12	117	1.00e-24	Un	145–238	No	(CTTT)19T10
CAUD044	10	333	1.00e-89	10	650993–651330	A15 or T9	(CTTT)11
CAUD047	—	296	1.00e-78	10	1286569–1286749	(CCT)5(CCG)4	(CCG)5CCA(CCG)5
CAUD048	11	269	2.00e-70	11	316437–316504	T(1-8C)17	(TTTC)16 … T24
CAUD049	2	296	2.00e-78	2	219095–219154	(TC)11T9	(TTTC)8TTCC(TTTC)17
CAUD059	5	191	1.00e-46	5	20526472–20526803	A7	(CA)9A(AC)3
CAUD063	—	321	5.00e-86	5	808837–809120	No	(AC)12(AT)6
CAUD066	1	177	1.00e-42	1	2264060–2264169	(AC)13CCGGCCGA13CA2C2	(AC)9 … A22 … (AG)6
CAUD069	1	198	5.00e-86	1	3920500–3920635	No	(TTCC)3TTTC(TTCC)5(TTTC)9CTTC(TTTC)18
CAUD079	—	191	1.00e-46	1	6974939-6975139	(AC)3T(CA)3	(CA)9
CAUD080	—	196	1.00e-48	10	249405–249530	(GCAN)5 … (GCN)5	(CAG)3(CAA)5(CAG)6CAA(CAG)3
CAUD087	—	306	2.00e-81	19	1154613–1154873	(AC)4CA(AC)5	(CA)4CCA(AC)5
CAUD089	2	289	3.00e-79	2	7198807–7199001	No	(CA)6A(AC)9
CAUD091	3	246	1.00e-63	3	5848561–5848703	(AC)3AG(AC)2AT(AC)4	(CA)11
CAUD102	8	167	7.00e-40	Z	4543–4765	(CA)11	(CA)14
CAUD108	18	154	8.00e-36	4	2082703–2082812	No	(GCA)6

(*continued*)

TABLE 2

(Continued)

Locus	Linkage group	Score (bits)	*E*-value	Chromosome	Physical position in chicken genome	Repeat 1 in the chicken	Repeat 2 in the duck
CAUD109	4	135	6.00e-30	4	10405875–10405968	(AAAC)2AACAAAC	(AC)16A20
CAUD110	—	254	8.00e-66	12	3671488–3671806	(CAG)6	(GCA)6
CAUD111	5	142	4.00e-32	5	313716–313858	(AC)3	(AC)13
CAUD120	7	425	1.00e-117	19	8482431–8482721	A9(TC)2(AC)3	(CA)9
CAUD123	10	192	3.00e-47	10	1780792–1780974	(CA)4TG(CA)5	(AC)8
CAUD128	17	400	1.00e-109	3	1072670–1072880	(GC)4(AC)13	(AC)7(GC)2(AC)5GC(AC)7
CAUD132	12	104	1.00e-20	27	91179–1241	No	(AGC)5OR(AGC)8
CAUD135	—	344	5.00e-93	3	2318733–2318974	A5(AC)3	(AC)6 … (AC)9
CAUD138	2	306	2.00e-81	2	7414342–7414518	(CAG)4(CAA)3(CAG)2CAA (CAG)2(CAA)2(CAG)4	(NCA)13

×, markers that were not amplified with the resource population; —, markers that were unlinked with anyone. Chromosome refers to the pair of chromosomes in chicken. "un" indicates that the position in the chicken genome is uncertain.

for capturing DNA fragments. The majority (83.69%) of the microsatellite sequences isolated in this study contained one or more purposed repeat motifs. The results suggested that $(CA)_n$, $(CAG)_n$, $(GCC)_n$, or $(TTTC)_n$ repeats are randomly distributed in the duck genome. $(CA)_n$, $(CAG)_n$, and $(TTTC)_n$ repeats have been found in mammalian and avian species (Cheng *et al.* 1995; Band and Ron 1996; McConnell *et al.* 1999). The $(GCC)_n$ repeat, however, which is abundant in the human genome (Kleiderlein *et al.* 1998; Chowdhury and Bansal 2001), is rare in other animal species. In addition to the above microsatellite motif repeats, other repeats were also found in this duck library. This result suggested that the mix-enriched library would be efficient to isolate microsatellite DNA from species for which the content of repeat cores was unknown.

Estimation of genetic map: A comprehensive genetic linkage map would speed the process of molecular studies in ducks, ranging from the construction of a comparative genetic map to gene identification of quantitative difference in growth, meat quantity, and disease susceptibility. Here, a primary genetic map for duck composed of 19 linkage groups, including 115 microsatellite markers with the averaged coverage of 1353.3 cM, was developed in a population with 224 G_2 individuals. Although the average marker spacing of the map is only 15.04 cM, 26.67% of this map still has regions where the distance between two adjacent markers is considerably >20 cM. The objectives of future work on the duck genetic map will be to increase the genome coverage and add more markers in those regions.

With the differences in recombination frequencies being detected on the sex chromosomes or in typing errors, there can be large differences between the male and female maps (Rappold 1993; Kondo *et al.* 2001). We have observed differences in the recombination rates between the sexes (see Table 1), but the differences are smaller than those observed in other species such as humans, pigs, dogs, tiger pufferfish, and zebrafish (Archibald *et al.* 1995; Dib *et al.* 1996; Neff *et al.* 1999; Singer *et al.* 2002; Kai *et al.* 2005), and more similar to those observed in chicken and Pacific oyster (Groenen *et al.* 1998; Hubert and Hedgecock 2004). Although the overall difference in the length of the male map is larger than that of the female map, several of the female linkage groups were larger than their male counterparts.

Conservation of microsatellite DNA sequence between duck and chicken: To construct comparative genetic maps for model species and less-studied species, the isolation of the orthologous markers, especially microsatellite marker, is a prerequisite. In recent years, several attempts have been made to amplify microsatellite loci specific for some model species, such as chicken, pig, and cattle, from genomic DNA of related species. The success rates of the heterologous amplification with microsatellite primers in closely related

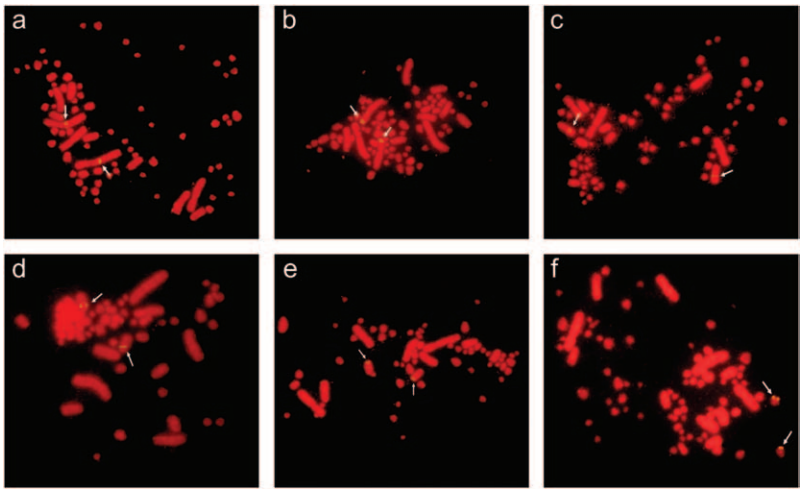

Figure 2.—FISH mapping of six orthologous loci to duck chromosomes with chicken BAC probes: (a) CAUD023, (b) CAUD138, (c) CAUD091, (d) CAUD109, (e) AMU6, and (f) CAUD044.

species ranged from 16.23 to 62.89% (Moore *et al.* 1991; Kemp *et al.* 1995; Fields and Scribner 1997; Baratti *et al.* 2001; Kayang *et al.* 2002; Reed *et al.* 2003; Kim *et al.* 2004). Studies show that it was difficult to screen microsatellite markers for duck by cross-species amplification from chicken loci (Huang *et al.* 2005). In this article, the conservation of microsatellite DNA between duck and chicken was estimated by sequence Blast comparisons at 240 loci. Results showed that only 20.42% microsallite loci were conserved in both species. Of the orthologs, 40.82% (20) with a core repeat >15 bp were suitable for designing primers in the above species. The variance in the flanking sequences of the orthologous loci ranged from 75 to 99%. Thus, <8.3% orthologous polymorphic microsatellite loci could be isolated by heterologous amplification between duck and chicken.

Integration of genetic and cytogenetic map by heterologous hybridization: The full utilization of genetic maps requires knowledge of the correspondence between the genetic and cytogenetic maps. Several methods have been used to correlate genetic and cytogenetic maps (Stephens *et al.* 2004). FISH provides the most direct way of physically mapping DNA sequences on chromosomes. However, FISH mapping of eukaryotic genomes depends heavily on the development of recombinant DNA BAC libraries. Because of a lack of large insert libraries, FISH mapping in less-developed species has usually been performed using heterologous BAC clones (Bonnet *et al.* 2001). In this study, 10 duck loci were assigned to duck macrochromosomes, 10 loci to small macrochromosomes, and 4 loci to microchromosomes using chicken BAC clones. Much research has shown strong chromosome homology between macrochromosomes in different bird species (Masabanda *et al.* 2004). In our research, 19 orthologous loci (CAUD023, CAUD069, AMU182, APH12, CAUD049, CAUD138, CAUD091, CAUD128, CAUD109, AMU6, CAUD111, CAUD022, APH20, CAUD038, AMU52, AMU174, CAUD044, CAUD080, and CAUD123) were assigned to the same macrochromosome or small macrochromosome in duck and chicken, respectively, 1 orthologous locus (CAUD021) was located on duck chromosome 4, but uncertain in the chicken genome. Moreover, 1 orthologous locus (CAUD108) was located on the duck microchromosome, but on chicken chromosome 4. However, the physical mapping of the 3 orthologous loci, which were localized on the microchromosomes in chicken, was uncertain in the duck genome.

Microsatellites are excellent genetic markers because of their high polymorphism and abundant distribution throughout the genome. A microsatellite-based genetic map is an essential tool for linkage mapping of monogenic as well as polygenic traits of interest (Ihara *et al.* 2004). However, only 137 microsatellite markers specific for waterfowl were previously reported and a genetic map for duck has not been developed (Fields and Scribner 1997; Buchholz *et al.* 1998; Maak *et al.* 2000, 2003; Genet *et al.* 2003; Paulus and Tiedemann 2003; Stai and Hughes 2003; Huang *et al.* 2005). The goals of this study were construction of a genetic map and the integration of genetic and cytogenetic maps. The resulting genetic and cytogenetic maps will be helpful in mapping QTL in ducks and in conducting genomic comparisons between chicken and duck. In addition, this map and the novel microsatellite loci will be useful for population genetic studies and in pedigree control and breeding applications in managed or commercially bred populations.

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LITERATURE CITED

Archibald, A. L., C. S. Haley, J. F. Brown, S. Couperwhite, H. A. McQueen *et al.*, 1995 The PiGMaP consortium linkage map of the pig (Sus scrofa). Mamm. Genome **6:** 157–175.

Band, M., and M. Ron, 1996 Creation of a SINE enriched library for the isolation of polymorphic (AGC)n microsatellite markers in the bovine genome. Anim. Genet. **27:** 243–248.

Baratti, M., A. Alberti, M. Groenen, T. Veenendaal and F. D. Fulgheri, 2001 Polymorphic microsatellites developed by cross-species amplifications in common pheasant breeds. Anim. Genet. **32:** 222–225.

Bonnet, A., S. Thevenon, F. Claro, M. Gautier and H. Hayes, 2001 Cytogenetic comparison between Vietnamese sika deer and cattle: R-banded karyotypes and FISH mapping. Chromosome Res. **9:** 673–687.

Buchholz, W. G., J. M. Pearce, B. J. Pierson and K. T. Scribner, 1998 Dinucleotide repeat polymorphisms in waterfowl (family Anatidae): characterization of a sex-linked (Z-specific) and 14 autosomal loci. Anim. Genet. **29:** 323–325.

Burt, D. W., D. R. Morrice, A. Sewalem, J. Smith, I. R. Paton *et al.*, 2003 Preliminary linkage map of the turkey (Meleagris gallopavo) based on microsatellite markers. Anim. Genet. **34:** 399–409.

Carleton, K. L., J. T. Streelman, B. Y. Lee, N. Garnhart, M. Kidd *et al.*, 2002 Rapid isolation of CA microsatellites from the tilapia genome. Anim. Genet. **33:** 140–144.

Cathey, J. C., L. M. Smith, R. J. Baker and J. A. DeWoody, 1996 Trinucleotide repeat polymorphism at the alpha-enolase/tau-crystallin locus in ducks. Anim. Genet. **27:** 130–131.

Chakraborty, R., M. Kimmer, D. N. Stivers, L. J. Davison and R. Deka, 1997 Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. Proc. Natl. Acad. Sci. USA **94:** 1041–1046.

Cheng, H. H., I. Levin, R. L. Vallejo, H. Khatib, J. B. Dodgson *et al.*, 1995 Development of a genetic map of the chicken with markers of high utility. Poult. Sci. **74:** 1855–1874.

Chowdhury, S., and M. Bansal, 2001 Modelling studies on neurodegenerative disease-causing triplet repeat sequences d(GGC/GCC)n and d(CAG/CTG)n. J. Biosci. **26:** 649–665.

Cooper, A., J. Rhymer, H. F. James, S. L. Olson, C. E. McIntosh *et al.*, 1996 Ancient DNA and island endemics. Nature **381:** 484.

Coppieters, W., C. Zijlstra, A. Van de Weghe, A. A. Bosma, L. Peelman *et al.*, 1994 A porcine minisatellite located on chromosome 14q29. Mamm. Genome **5:** 591–593.

Crittenden, L. B., L. Provencher, I. Levin, H. Abplanalp, R. W. Briles *et al.*, 1993 Characterization of a Red Jungle Fowl by White Leghorn back cross reference population for molecular mapping of the chicken genome. Poult. Sci. **72:** 334–348.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot *et al.*, 1996 A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature **380:** 152–154.

Donne-Gousse, C., V. Laudet and C. Hanni, 2002 A molecular phylogeny of anseriformes based on mitochondrial DNA analysis. Mol. Phylogenet. Evol. **23:** 339–356.

Fields, R. L., and K. T. Scribner, 1997 Isolation and characterization of novel waterfowl microsatellite loci: cross-species comparisons and research applications. Mol. Ecol. **6:** 199–202.

Garner, T. W., 2002 Genome size and microsatellites: the effect of nuclear size on amplification potential. Genome **45:** 212–215.

Genet, C., A. Vignal and C. Larzul, 2003 Isolation and characterisation of microsatellite genetic markers from Peking and Muscovy ducks. Br. Poult. Sci. **44:** 794–795.

Gibbs, M., D. A. Dawson, C. McCamley, A. F. Wardle, J. A. Armour *et al.*, 1997 Chicken microsatellite markers isolated from libraries enriched for simple tandem repeats. Anim. Genet. **28:** 401–417.

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

Groenen, M. A., R. P. Crooijmans, A. Veenendaal, H. H. Cheng, M. Siwek *et al.*, 1998 A comprehensive microsatellite linkage map of the chicken genome. Genomics **49:** 265–274.

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

Harry, D. E., Z. David, J. M. Paul and M. R. Kent, 2003 A first-generation map of the turkey. Genome **46:** 914–924.

Huang, Y. H., G. J. Xu, Z. Q. Du, X. X. Hu, L. Zhang *et al.*, 2004 Genetic analysis of cattle cloned from somatic cells and gene transferred somatic cells. Prog. Biochem. Biophys. **31:** 699–704.

Huang, Y. H., J. F. Tu, X. B. Cheng, B. Tang, X. X. Hu *et al.*, 2005 Characterization of 35 novel microsatellite DNA markers from the duck (Anas platyrhynchos) genome and cross-amplification in other birds. Genet. Sel. Evol. **37:** 455–472.

Hubert, S., and D. Hedgecock, 2004 Linkage maps of microsatellite DNA markers for the Pacific oyster *Crassostrea gigas*. Genetics **168:** 351–362.

Ihara, N., A. Takasuga, K. Mizoshita, H. Takeda, M. Sugimoto *et al.*, 2004 A comprehensive genetic map of the cattle genome based on 3802 microsatellites. Genome Res. **14:** 1987–1998.

Jernej, J., and J. Branka, 2001 High throughout isolation of microsatellites in Hop (Humulus lupulus L.). Plant Mol. Rep. **19:** 217–226.

Johnson, K. P., and M. D. Sorenson, 1998 Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (tribe: Anatini). Mol. Phylogenet. Evol. **10:** 82–94.

Johnson, K. P., and M. D. Sorenson, 1999 Phylogeny and biogeography of dabbling ducks (Genus: Anas): a comparison of molecular and morphological evidence. Auk **116:** 792–805.

Kai, W., K. Kikuchi, M. Fujita, H. Suetake, A. Fujiwara *et al.*, 2005 A genetic linkage map for the tiger pufferfish, *Takifugu rubripes*. Genetics **171:** 227–238.

Kayang, B. B., M. Inoue-Murayama, T. Hoshi, K. Matsuo, H. Takahashi *et al.*, 2002 Microsatellite loci in Japanese quail and cross-species amplification in chicken and guinea fowl. Genet. Sel. Evol. **34:** 233–253.

Kemp, S. J., O. Hishida, J. Wambugu, A. Rink, M. L. Longeri *et al.*, 1995 A panel of polymorphic bovine, ovine and caprine microsatellite markers. Anim. Genet. **26:** 299–306.

Kim, K. S., M. S. Min, J. H. An and H. Lee, 2004 Cross-species amplification of Bovidae microsatellites and low diversity of the endangered Korean goral. J. Hered. **95:** 521–525.

Kleiderlein, J. J., P. E. Nisson, J. Jessee, W. B. Li, K. G. Becker *et al.*, 1998 CCG repeats in cDNAs from human brain. Hum. Genet. **103:** 666–673.

Knutson, T. P., L. D. Chaves, M. K. Hall and K. M. Reed, 2004 One hundred fifty-four genetic markers for the turkey (Meleagris gallopavo). Genome **47:** 1015–1028.

Kondo, M., E. Nagao, H. Mitani and A. Shima, 2001 Differences in recombination frequencies during female and male meioses of the sex chromosomes of the medaka, Oryzias latipes. Genet. Res. **78:** 23–30.

Liu, H. T., Y. H. Hu, C. T. Wang and L. Y. Lin, 1996 Sequences and comparisons of duck mitochondrial DNA control regions. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **115:** 209–214.

Liu, W., Z. Liu, X. Hu, Y. Zhang, J. Yuan *et al.*, 2003 Construction and characterization of a novel 13.34-fold chicken bacterial artificial chromosome library. Anim. Biotechnol. **14:** 145–153.

Maak, S., K. Neumann, G. von Lengerken and R. Gattermann, 2000 First seven microsatellites developed for the Peking duck (Anas platyrhynchos). Anim. Genet. **31:** 233.

Maak, S., K. Wimmers, S. Weigend and K. Neumann, 2003 Isolation and characterization of 18 microsatellites in the Peking duck (Anas platyrhynchos) and their application in other waterfowl species. Mol. Ecol. Notes **3:** 224–227.

Masabanda, J. S., D. W. Burt, P. C. O'Brien, A. Vignal, V. Fillon *et al.*, 2004 Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. Genetics **166:** 1367–1373.

McConnell, S. K., D. A. Dawson, A. Wardle and T. Burke, 1999 The isolation and mapping of 19 tetranucleotide microsatellite markers in the chicken. Anim. Genet. **30:** 183–189.

Moore, S. S., L. L. Sargeant, T. J. King, J. S. Mattick, M. Georges *et al.*, 1991 The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics **10:** 654–660.

Neff, M. W., K. W. Broman, C. S. Mellersh, K. Ray, G. M. Acland *et al.*, 1999 A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. Genetics **151:** 803–820.

Olson, S. L., 1985 The fossil resords of birds, pp. 79–238 in *Avian Biology*, edited by K. C. Parkes. Academic Press, New York.

Paulus, K. B., and R. Tiedemann, 2003 Ten poymorphic autosomal microsatellite loci for Eider duck Somaterica mollissima and their cross-species applicability among waterfowl species (Anatidae). Mol. Ecol. Notes **3:** 250–252.

Rappold, G. A., 1993 The pseudoautosomal regions of the human sex chromosomes. Hum. Genet. **92:** 315–324.

Reed, K. M., L. D. Chaves, J. R. Garbe, Y. Da and D. E. Harry, 2003 Allelic variation and genetic linkage of avian microsatellites in a new turkey population for genetic mapping. Cytogenet. Genome Res. **102:** 331–339.

Reed, K. M., J. Holm, M. Morrisson, S. Leroux and A. Vignal, 2005a Assignment of non-informative turkey genetic markers through comparative approaches. Cytogenet. Genome Res. **109:** 527–532.

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

Singer, A., H. Perlman, Y. Yan, C. Walker, G. Corley-Smith *et al.*, 2002 Sex-specific recombination rates in zebrafish (*Danio rerio*). Genetics **160:** 649–657.

Sraml, M., L. Christidis, S. Easteal, P. Horn and C. Collet, 1996 Molecular relationships within Australian waterfowl (Anseriformes). Aust. J. Zool. **45:** 47–58.

Stai, S. M., and C. R. Hughes, 2003 Characterization of microsatellite loci in wild and domestic Muscovy ducks (Cairina moschata). Anim. Genet. **34:** 387–389.

Stephens, J. L., S. E. Brown, N. L. Lapitan and D. L. Knudson, 2004 Physical mapping of barley genes using an ultrasensitive fluorescence in situ hybridization technique. Genome **47:** 179–189.

Tang, B., Y. H. Huang, L. Lin, X. X. Hu, J. D. Feng *et al.*, 2003 Isolation and characterization of 70 novel microsatellite markers from ostrich (Struthio camelus) genome. Genome **46:** 833–840.

Tuinen, M. V., and E. A. Hadly, 2004 Error in estimation of rate and time inferred from the early amniote fossil record and avian molecular clocks. J. Mol. Evol. **59:** 267–276.

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

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