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A first-generation microsatellite linkage map of the ruff

Lindsay L. Farrell1,2, Terry Burke1, Jon Slate1 & David B. Lank2

1Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, U.K.
2Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Keywords
Chromosomes, genetic map, linkage groups, microsatellite, ruff.

Abstract
A linkage map of the ruff (Philomachus pugnax) genome was constructed based on segregation analysis of 58 microsatellite loci from 381 captive-bred individuals spanning fourteen breeding years and comprising 64 families. Twenty-eight of the markers were resolved into seven linkage groups and five single marker loci, homologous to known chicken (Gallus gallus) and zebra finch (Taeniopygia guttata) chromosomes. Linkage groups range from 10.1 to 488.7 cM in length and covered a total map distance of 641.6 cM, corresponding to an estimated 30–35% coverage of the ruff genome, with a mean spacing of 22.9 cM between loci. Through comparative mapping, we are able to assign linkage groups Ppu1, Ppu2, Ppu6, Ppu7, Ppu10, Ppu13, and PpuZ to chromosomes and identify several intrachromosomal rearrangements between the homologs of chicken, zebra finch, and ruff microsatellite loci. This is the first linkage map created in the ruff and is a major step toward providing genomic resources for this enigmatic species. It will provide an essential framework for mapping of phenotypically and behaviorally important loci in the ruff.

Introduction
Uniquely among birds, ruffs (Philomachus pugnax) exhibit three different and distinct permanent alternative male reproductive morphs, with correlated differences in territorial lekking behavior, body size, and the presence or coloration of ornamental breeding plumage. All populations include: (1) dark-plumed territorial “Independents,” (2) white-plumed nonterritorial “Satellites,” and (3) small female mimics called “Faeders” (Hogan-Warburg 1966; Höglund and Lundberg 1989; Van Rhijn 1973; Jukema and Piersma 2006). Status as an independent or satellite has been previously shown to be due to a genetic polymorphism in male mating behavior consistent with a single-locus, two-allele autosomal Mendelian mode of inheritance (Lank et al. 1995). More recently, it has been discovered that a dominant autosomal allele controls development in to female-mimicking faeders (Lank et al. 2013).

With the current evidence for Mendelian genetic determination of behavioural type (Lank et al. 1995) and a strong genetic basis also suspected for plumage characters (Dale et al. 2001), the ruff presents an ideal species for the study of functional genetic variation underlying phenotypic traits. However, genomic resources for the ruff are limited; only nine previously published microsatellite markers were
available (Thuman et al. 2002) until the recent publications of Farrell et al. (2012) and Verkuil et al. (2012). As a step toward developing genomic resources for the ruff and to allow mapping of phenotypic traits, we performed linkage analysis of 58 microsatellites from 381 captive individuals comprising 64 families, and present here the resulting linkage map.

**Methods**

**Mapping population**

The genetic mapping population consisted of 381 individuals belonging to a captive population maintained by DBL over fourteen breeding years at Simon Fraser University, Canada. This population was established from 31 individuals raised from eggs collected on breeding grounds near Oulu, Finland in 1985, to which 63 additional wild birds were added during the years up to 1990. In 2006, two faeders, one satellite male, and one female captured in the Netherlands were added to the captive population. The pedigree used in this project contains individuals from 64 families, with 62 fathers and 93 mothers, with hatch years extending from 1985 for the original parental generation to 2009 for the most recent chicks. Breeding records held by DBL and genotyping of several loci by SB McRae (SBM; East Carolina University) determined parentage prior to this study.

**Microsatellite markers**

In total, 102 microsatellite markers were tested, of which 52 were found to be polymorphic and were developed and characterized (Farrell et al. 2012). Forty-seven of these were selected for linkage mapping and used together with 11 ruff loci previously developed for population genetic studies (Thuman et al. 2002; Verkuil et al. 2012), and 5 other shorebird loci identified from cross-utility testing in the ruff and many other avian species (Saether et al. 2007; St. John et al. 2007). Chromosomal positions were determined parentage prior to this study.

**DNA extraction and genotyping**

We obtained DNA from blood and frozen tissue that had been collected from individuals and stored in absolute ethanol. Genomic DNA was extracted using an ammonium acetate precipitation method (Nicholls et al. 2000; Richardson et al. 2001). Each 2-μL PCR contained approximately 10 ng genomic DNA, 0.2 μmol/L of each primer, and 1 μL QiaGen Multiplex PCR Mix (QiaGen Inc.). PCR amplification was performed using a DNA Engine Tetrad 2 Thermal Cycler (MJ Research, BioRad, UK) with the profile: 15 min at 95°C, followed by 35 cycles of 94°C for 30 sec, annealing temperature (Table 1) for 90 sec and 72°C for 1 min, then a final step of 60°C for 10 min. PCR products were loaded onto an ABI3730 Genetic Analyzer (Applied Biosystems) using ROX500 size standards, and genotypes were scored with GENEMAPPER v.4.0 software (Applied Biosystems). Observed and expected heterozygosities were calculated using CERVUS v3.0 (Kalinowski et al. 2007; Table 1). Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were assessed using GENEPOP v.4.0 (Rousset 2008). Four loci identified in ruffs (Ppu023, Ppu033, and Ppu012; Farrell et al. 2012), and one primer set from another species (Chmo06; St. John et al. 2007) failed to amplify in the genotyping multiplexes and were excluded from further analysis.

**Pedigree assembly and linkage mapping**

Parentage assignment was performed using genotypic data for all 58 microsatellite markers in 381 individuals (including 8% data replicates) using CERVUS v.3.0. The resulting parentage assignments were compared with the previous pedigree, held by DBL and SBM, for inconsistencies. Grandparent–Parent–Offspring genotypic inconsistencies arising from incorrect parentage assignment or microsatellite genotyping errors were detected through a three-generation Pedigree Program (K. W. Kim, unpublished) and either resolved by rechecking the parentage and past genotyping records held by DBL and SBM, reviewing raw allele peaks on GENEMAPPER v.4.0 or, in any remaining cases of uncertainty, rescored as untyped.

Linkage analysis was performed using a version of CRIMAP v.2.4 (Green et al. 1990), modified by Xuelu Liu (Monsanto) to accommodate large numbers of markers in complicated pedigrees. Prior to input into CRIMAP, CRIGEN was used to simplify the pedigree and omit
Table 1. Summary of genotyping results (58 loci) and predicted genome locations (53 loci) of ruff microsatellite markers.

| Locus reference | Fluorolabel | PCR MP set | CH chr | ZF chr | E-value in Chicken locus | E-value in Zebra finch | Repeat motif | Primer Sequence 5'-3' | Allele size range (bp) | H0 | H1 | Est. null allele freq. |
|-----------------|-------------|------------|--------|--------|--------------------------|------------------------|--------------|----------------------|-----------------------|----|----|----------------------|
| Ppu001          | HEX         | 7          | 1      | 1A     | 5.90E-64                 | 1.90E-13               | (TAGA)$_{12}$ | F: ACCAGGCTCTCTCCCTCTGGA | 287-253               | 0.59 | 0.64 | 0.0519               |
| Ppu003          | HEX         | 9          | 1      | 1      | 3.60E-61                 | 3.30E-27               | (CTAT)$_{11}$ | R: TGAAATCTACATTTTTTGGGGATGTA | 296-365               | 0.59 | 0.56 | -0.0268             |
| Ppu005          | 6-FAM       | 4          | 8      | 1      | 8.00E-108                | 8.00E-73               | (TC)$_{5}$    | F: GGAGCAATGTAGACTACCTAAAGGACTG | 217-233               | 0.39 | 0.57 | 0.2050*              |
| Ppu006          | 6-FAM       | 1          | 5      | 1A     | 3.00E-53                 | 3.00E-53               | (GT)$_{9}$    | F: TGGAGATGAGAAGAGTCTGTTG | 245-254               | 0.46 | 0.49 | 0.0319               |
| Ppu007          | 6-FAM       | 9          | 3      | 1A     | 4.00E-27                 | 6.10E-70               | (TG)$_{5}$    | F: GCCAGATGCAACAAGCTCAGCTGC | 295-456               | 0.51 | 0.53 | 0.0171               |
| Ppu008          | 6-FAM       | 7          | 2      | 1A     | 4.50E-52                 | 8.10E-21               | (CA)$_{9}$    | F: GGAGCTCCTTCACCACTTCG | 295-301               | 0.22 | 0.22 | 0.0076               |
| Ppu009          | HEX         | 7          | 4      | 1A     | 4.00E-66                 | 4.00E-66               | (AC)$_{12}$   | F: TCTTTATGATGTATTGAGGGTTGGG | 419-472               | 0.73 | 0.88 | 0.0892               |
| Ppu011          | HEX         | 1          | 3      | 1A     | 3.70E-46                 | 3.70E-46               | (CA)$_{5}$    | F: AATTAGCGGTGCAAAAGATGC | 215-224               | 0.48 | 0.45 | -0.0423             |
| Ppu013          | HEX         | 8          | 3      | 1A     | 1.40E-61                 | 1.40E-61               | (AG)$_{5}$    | F: ACATGTTGCTCTATTCCATTGGC | 222-229               | 0.53 | 0.49 | -0.0435             |
| Ppu014          | HEX         | 7          | 24     | 1A     | 3.80E-63                 | 3.80E-63               | (GT)$_{9}$    | F: CAACCCCATCTTCGCGGTTT | 207-220               | 0.51 | 0.45 | -0.0639             |
| Ppu015          | HEX         | 4          | 2      | 1A     | 2.00E-30                 | 2.00E-30               | (CA)$_{5}$    | F: TGAGGTGATGACTACCTTTG | 242-247               | 0.48 | 0.64 | 0.1588               |
| Ppu016          | HEX         | 12         | 1      | 1A     | 1.10E-22                 | 1.10E-22               | (TC)$_{6}$    | F: TCAAGCGACTGGGACTAGATGG | 212-229               | 0.66 | 0.66 | -0.0037             |
| Ppu017          | HEX         | 11         | 4      | 1A     | 4.30E-11                 | 4.30E-11               | (TT)$_{7}$    | F: GTGGGCTGGACTGC | 227-229               | 0.02 | 0.48 | 0.9122*              |
| Ppu018          | HEX         | 9          | 2      | 1A     | 4.90E-38                 | 4.90E-38               | (AGAT)$_{3}$ | R: TGGAGCTCAATTTTGGCTG | 242-274               | 0.79 | 0.79 | 0.0014               |
| Ppu019          | HEX         | 2          | 3      | 1A     | 7.60E-10                 | 7.60E-10               | (CA)$_{11}$   | R: TAAACCCAGTGAGCTC | 145-162               | 0.77 | 0.78 | 0.0128               |
| Locus   | Locus reference | Fluorolabel | PCR primer sequence 5'→3' Primer Sequence 3'→5'| Allele size range (bp) | H0 | H1 | Est. null allele freq. |
|---------|----------------|-------------|-----------------------------------------------|------------------------|----|----|-----------------------|
| Ppu020  | Farrell et al. (2012) | HEX 11 11 | F: TCTGGTCCTCTGCTTGGGAAC R: GCCGTTATTCGGGCTAGC | 241–249 | 0.50 | 0.48 | −0.0126 |
| Ppu021  | Farrell et al. (2012) | 6-FAM 6 1 | F: AAACGCTGGAAGCTTAAGCAAT R: AGAAGGATGGCTACGAGTGG | 284–329 | 0.72 | 0.75 | 0.00085 |
| Ppu022  | Farrell et al. (2012) | 6-FAM 13 2 | F: TGAATGCAATGATTAGTGAGTGGGGGAG GAGG CGAAGTACAGAAC | 264–302 | 0.86 | 0.85 | −0.0055 |
| Ppu024  | Farrell et al. (2012) | HEX 6 13 | F: GGAACACTCTCCCACTCAACAG R: GAGGGATGCTAGGGTGG | 122–161 | 0.79 | 0.85 | 0.00307 |
| Ppu025  | Farrell et al. (2012) | 6-FAM 13 1 | F: GTACGAGCTGGCTACACGAC R: GCATACAAATGCAACCTCAG | 332–352 | 0.86 | 0.85 | −0.0102 |
| Ppu027  | Farrell et al. (2012) | 6-FAM 12 7 | F: TGGACGGTACGCTTAGTGAGGTTG | 281–379 | 0.61 | 0.67 | 0.0055 |
| Ppu028  | Farrell et al. (2012) | HEX 2 1 | F: CCTGCTGAGCTTAGTTAATCTGTGTTG | 185–197 | 0.65 | 0.62 | −0.0181 |
| Ppu029  | Farrell et al. (2012) | HEX 11 10 | F: AGGGATTTGTGGGAAATAGG | 164–170 | 0.07 | 0.21 | 0.492* |
| Ppu030  | Farrell et al. (2012) | HEX 11 2 | F: CAGCGTTCACACTTTCTCCTC R: CTCGCTTCATAATTGTGAGG | 130–140 | 0.53 | 0.57 | 0.0318 |
| Ppu031  | Farrell et al. (2012) | 6-FAM 5 13 | F: TGAATCTATTTATGATTATTTGTG | 319–326 | 0.32 | 0.30 | −0.0040 |
| Ppu032  | Farrell et al. (2012) | 6-FAM 7 2 | F: TCGGAGCTCTGCTGTTAAGGAC R: TGAGAAGCTGGTTAAGGAC | 248–266 | 0.66 | 0.83 | 0.1094 |
| Ppu034  | Farrell et al. (2012) | HEX 3 10 | F: TTTGGAATGGCAAGTTGTTG | 126–135 | 0.15 | 0.16 | 0.0176 |
| Ppu036  | Farrell et al. (2012) | 6-FAM 1 10 | F: AGGCCCGGAGTTTTAGAGTGG | 200–209 | 0.47 | 0.48 | 0.00114 |
| Ppu037  | Farrell et al. (2012) | HEX 13 26 | F: TCCATATTTATGACGCCGAGAAC | 234–236 | 0.34 | 0.32 | −0.0316 |
| Ppu038  | Farrell et al. (2012) | HEX 12 2 | F: CATTGACTACCCATCGAATCT TCTTGG | 274–282 | 0.20 | 0.19 | 0.00171 |
| Ppu039  | Farrell et al. (2012) | 6-FAM 13 1 | F: GCAATCGTGCCACTCCCAAC R: CTCGCTATCAATGTAAAGTGA | 186–194 | 0.52 | 0.51 | −0.0147 |
| Locus     | Locus reference | Fluorolabel | PCR set | CH chr | Z chr | Chicken locus | Zebra finch locus | Ruff1 ^a | Repeat motif | E-value in Chicken | E-value in Zebra finch | Repeat motif | \( T_a \) (°C) | Primer Sequence 5’–3’ | Allele size range (bp) | \( H_0 \) | \( H_6 \) | Est. null allele freq. |
|-----------|----------------|-------------|---------|--------|--------|---------------|------------------|---------|-------------|---------------------|--------------------------|-------------|-----------|----------------------|------------------------|--------|--------|----------------------|
| Ppu040    | Farrell et al. (2012) | HEX | 9      | 5      | 5      | 2524315 | 1.20E–59 | (TG)\(_9\) | 291 | 5  | 56 | F: CTCTCGGCTGCTGTTGCTGTG | 203–213 | 0.38 | 0.36 | –0.0205 |
| Ppu041    | Farrell et al. (2012) | HEX | 7      | 11     | 11     | 321716 | 1.90E–58 | (TG)\(_9\) | 226 | 3  | 56 | F: CTCTCGGCTGCTGTTGCTGTG | 171–174 | 0.28 | 0.31 | 0.0499 |
| Ppu046    | Farrell et al. (2012) | 6-FAM | 2      | 4      | 4      | 41459422 | 1.90E–138 | (TG)\(_10\) | 332 | 5  | 61 | F: CTCTGCTATTTATGTCGTTT | 173–182 | 0.64 | 0.61 | –0.0224 |
| Ppu047    | Farrell et al. (2012) | HEX | 8      | 6      | 6      | 28674879 | 1.60E–61 | (TC)\(_10\) | 292 | 4  | 57 | F: TGGACACGAGTTGCAAA | 288–294 | 0.58 | 0.55 | –0.0189 |
| Ppu048    | Farrell et al. (2012) | HEX | 2      | 1      | 1      | 4758084 | 3.20E–77 | (CT)\(_10\) | 373 | 6  | 61 | F: TGGACACGAGTTGCAAA | 222–232 | 0.52 | 0.47 | –0.0552 |
| Ppu049    | Farrell et al. (2012) | 6-FAM | 1      | 1      | 1      | 163634502 | 3.00E–08 | (GA)\(_12\) | 167 | 21 | 61 | F: TGGACACGAGTTGCAAA | 226–411 | 0.63 | 0.92 | 0.1866 |
| Ppu054    | Farrell et al. (2012) | 6-FAM | 3      | 8      | 8      | 11832348 | 2.00E–25 | (GT)\(_5\) | 375 | 3  | 61 | F: TGGACACGAGTTGCAAA | 283–289 | 0.01 | 0.01 | –0.0006 |
| Ppu055    | Farrell et al. (2012) | 6-FAM | 2      | 1      | 1      | 196624265 | 8.00E–13 | (AGAA) | 62 | 3  | 61 | F: TGGACACGAGTTGCAAA | 269–278 | 0.11 | 0.35 | 0.5762* |
| Ppu056    | Farrell et al. (2012) | HEX | 5      | 22     | 22     | 690245 | 1.00E–22 | (AA)\(_9\) | 356 | 7  | 59 | F: TGGACACGAGTTGCAAA | 168–205 | 0.70 | 0.62 | –0.0675 |
| Ppu057    | Farrell et al. (2012) | HEX | 6      | 6      | 6      | 20079681 | 3.70E–21 | (GA)\(_8\) | 292 | 16 | 61 | F: TGGACACGAGTTGCAAA | 328–381 | 0.91 | 0.89 | –0.0139 |
| Ppu058    | Farrell et al. (2012) | 6-FAM | 2      | 6      | 6      | 18076829 | 8.50E–28 | (GT)\(_14\) | 272 | 7  | 63 | F: TGGACACGAGTTGCAAA | 221–233 | 0.12 | 0.81 | 0.7399* |
| Ppu059    | Farrell et al. (2012) | HEX | 6      | 1      | 1      | 121754800 | 2.20E–51 | (GT)\(_8\) | 154 | 6  | 62 | F: TGGACACGAGTTGCAAA | 261–264 | 0.21 | 0.66 | 0.5285* |
| Ruff1     | Thuman et al. (2002) | NED | 4      | –      | –      | 15987120 | 2.00E–25 | (GT)\(_5\) | 375 | 3  | 61 | F: TGGACACGAGTTGCAAA | 180–204 | 0.56 | 0.66 | 0.0548 |
| Ruff4     | Thuman et al. (2002) | NED | 7      | –      | –      | 11832348 | 7.50E–48 | (AA)\(_9\) | 224 | 2  | 56 | F: TGGACACGAGTTGCAAA | 238–242 | 0.15 | 0.23 | 0.2220 |
| Ruff5     | Thuman et al. (2002) | HEX | 9      | –      | –      | 71772280 | 6.50E–05 | (AA)\(_9\) | 298 | 6  | 56 | F: TGGACACGAGTTGCAAA | 127–165 | 0.26 | 0.62 | 0.4086* |
| Ruff6     | Thuman et al. (2002) | 6-FAM | 8      | –      | –      | 279113501 | 2.60E–34 | (AA)\(_9\) | 279 | 11 | 57 | F: TGGACACGAGTTGCAAA | 149–186 | 0.82 | 0.82 | –0.0056 |
Table 1. Continued.

| Locus | Locus reference | Fluorolabel | PCR MP set | CH chr | ZF chr | Chicken locus | Zebra finch locus | E-value in Chicken | Repeat motif | nA | T0 (°C) | Primer Sequence 5′–3′ | Allele size range (bp) | H0 | H0 | Est. null allele freq. |
|-------|-----------------|-------------|------------|--------|--------|--------------|------------------|------------------|--------------|-----|--------|----------------------|---------------------|----|----|---------------------|
| Ruff8 | Thuman et al. (2002) | 6-FAM | 7 | Z | – | – | – | – | (CTAT)10 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| Ruff12 | Thuman et al. (2002) | 6-FAM | 8 | Z | – | – | – | – | (CTACC) | 255 | 14 | 57 | R: TGCTGTCAATTTCATCTCTGTTG | 206–263 | 0.83 | 0.87 | 0.0225 |
| Ruff50 | Thuman et al. (2002) | HEX | 5 | 18 | 7536672 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| Phil2 | Verkuil et al. (2012) | 6-FAM | 2 | 1 | 114546517 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| Phil9 | Verkuil et al. (2012) | NED | 10 | 2 | 990418 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| Chmo21 | St. John et al. (2007) | NED | 5 | 6 | 30023165 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| RGB18 | Kupper et al. (2008) | NED | 3 | 9 | 15167245 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| SnipeB2 | Saether et al. (2007) | NED | 9 | 1 | 85466778 | – | – | – | – | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| PGT83 | Blomqvist et al. (2010) | 6-FAM | 12 | 12 | 4732069 | 94732069 | 9.03E–02 | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| TG22-001 | Dawson et al. (2010) | HEX | 6 | 12 | 529247 | 12019183 | 9.03E–02 | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |
| TGO5-053 | Dawson et al. (2010) | 6-FAM | 9 | 5 | 55948193 | 61276203 | 9.03E–02 | (AC)7 | 229 | 11 | 56 | F: ATCTTGCAAGGAAATCAAAATGTTG | 92–151 | 0.41 | 0.83 | 0.3337* |

Summary of genotyping results and predicted genome locations of 58 ruff microsatellite loci. Of the 58 polymorphic loci characterized, 55 could be assigned a location in the chicken genome and 53 in the zebra finch genome. MP, the PCR multiplex set used in genotyping; n, number of individuals amplified and genotyped; A, number of alleles observed; H0, observed heterozygosity; H0, expected heterozygosity (calculated from n, using CERVUS v3.0); *markers with null alleles. Null allele frequencies were calculated using the original genotypes and are based on the excess of homozygous individuals. Excesses of homozygotes are probably due to nonrandom population structure caused by captive breeding that included matings between full sibs and second-order relatives (half-sibs and closer relatives).

The location of each microsatellite sequence was assigned in the chicken (Gallus gallus; v 2.1, May 2006 ENSEMBL release) and zebra finch (Taeniopygia guttata; December 2011 ENSEMBL Release 65) genomes based on sequence similarity (see Dawson et al. 2006, 2007).
any noninformative individuals. A two-point linkage analysis of all markers was then performed based on a LOD score > 3.0. Markers were also assumed to be linked if they were supported by a LOD > 2.0 and an expectation of linkage based on a priori knowledge (Slate et al. 2002), that is, linkage was expected based on BLAST search (Altschul et al. 1997) and assignment of chromosomal location in chicken and zebra finch (Dawson et al. 2006, 2007). Linkage groups were created using AUTOGROUP and markers belonging to the same linkage group were analyzed using the BUILD command. PUK LIKE TOL and PK LIKE TOL values were lowered from 3.0 to 2.0, and then 2.0 to 1.0, and the BUILD command rerun until no further markers were added. Marker order was determined and confirmed by the FLIPS command, where new marker orders were tested against alternative orders to determine whether they fitted the data. Recombination frequencies and positions of all loci in linkage groups were visualized using the CHROMPIC function. During map construction, both sex-averaged and sex-specific maps were built; however, only the sex-averaged maps per linkage group are presented, with map distances based on the Kosambi mapping function.

**Genome coverage**

The mean marker spacing was calculated by dividing the total length of the map by the number of intervals. Average intramarker spacing for each linkage group was calculated by dividing the length of each linkage group by the total number of intervals on that linkage group. Linkage map coverage was calculated by summing the difference in base-pair position in chicken of the first and last interval on each linkage group, and dividing by the total base-pair length of the chicken genome (~1.07 Gb; Ensembl database www.ensembl.org/Gallus_gallus/index.html).

**Results and Discussion**

Based on comparative mapping methods of microsatellite loci homologous to the ruff, chicken, and zebra finch, homologs of 55 of the 58 typed microsatellite loci were assigned predicted chromosomal locations in the chicken genome and 53 were assigned locations in the zebra finch (Table 1). Five ruff microsatellite sequences (Ppu008, Ruff1, Ruff4, Ruff6, and Ruff12) could not be assigned predicted chromosomal locations in either genome based on sequence similarity.
The first-generation linkage map of the ruff consisted of 23 microsatellite markers resolved into 7 linkage groups (Ppu1, Ppu2, Ppu6, Ppu7, Ppu10, Ppu13, and PpuZ) homologous to chicken and zebra finch chromosomes. Each linkage group was numbered according to the homologous chicken and zebra finch chromosome number (with the prefix Ppu; Fig. 1). An additional five loci were not expected to be linked to any other marker, based on predicted genomic locations. This expectation was confirmed by the two-point analysis, and so these were treated as linkage groups with a single marker (Fig. 1). The remaining 30 markers were expected to form linkage groups, but were found to be unlinked to all other markers. The map covers 641.6 cM with an average spacing of 22.9 cM. The size of linkage groups, ignoring those that consisted of a single marker, ranged from 10.1 to 488.7 cM. The number of markers per linkage group varied from 2 to 9. The intermarker interval for each linkage group varied from 5.0 to 54.3 cM, with a mean of 16.7 cM.

Four of the markers that lacked predicted genomic locations were subsequently assigned to chromosomes on the basis of the linkage mapping: Ruff1, Ruff6, Ppu008, and Ruff8 were assigned to chromosomes Ppu1, Ppu13, Ppu7, and Z, respectively. Ruff8 was known to be Z-linked from previous work by Thuman et al. (2002); however, its genomic location on chromosome Z is reported here for the first time. Chromosomes Ppu1A, Ppu3, Ppu4, Ppu5, Ppu8, Ppu11 and Ppu22 were all predicted to contain more than one typed marker; yet, linkage groups could

Figure 2. A comparative map of microsatellite loci in ruff (Ppu; Philomachus pugnax), chicken (Gga; Gallus gallus), and zebra finch (Tgu; Taeniopygia guttata) homologous chromosomes. Distinctions between loci in italics, bold font, and underlined are explained in the legend of Figure 1. Three possible intrachromosomal rearrangements between the homologs of chicken, zebra finch and ruff microsatellite loci are reported here for the first time (chr1: loci Ppu001, Ppu021, and Ppu028; chr2: loci Ppu018 and Ppu022; chr7: loci Ppu023 and Ppu027).
not be formed. There are two possible explanations for the failure to assign the markers to these chromosomes. First, the pedigree may have been insufficiently powerful to map all linked markers, especially if they were relatively far apart on a chromosome. Second, the predicted chromosomal locations may not be an accurate indication of the true locations; in other words, synteny may not be highly conserved between ruffs and other birds. Given that no mapped markers were assigned to locations other than their predicted locations, we believe that the failure to assign markers to these chromosomes is an issue of power rather than poorly conserved synteny.

Following the methods of Backström et al. (2008), we used available physical data on the chicken genome to calculate the proportion of the ruff genome covered by the map. The distance on the chicken genome assembly between the homologs of the most distal markers on each ruff linkage group was estimated, and summing across chromosomes was found to be 270 Mb, or 26% of the total ~1.07 Gb chicken genome (Ensembl database www.ensembl.org/Gallus_gallus/index.html). However, additional sequence is covered by the ruff map if the five chromosomes with single markers and the sequence immediately beyond the first and last markers on each linkage group are included. Assuming the ruff has a similar genome size to the chicken (http://www.genomesize.com/), it may be estimated that our map covers 30–35% of the ruff genome. The proportion of the total genetic (i.e., recombination) length of the ruff genome covered by the map is harder to assess, as the microchromosomes are mostly unmapped. Although microchromosomes are physically short and contribute little to the physical genome size, they each have an obligate crossing-over event during meiosis, which contributes 50 cM to the total map length (Jones and Frankin 2006). Thus, compared with its coverage of the physical genome, the map must cover a lower proportion of the total linkage (recombination) map length of the ruff genome.

Despite the highly conserved synteny generally believed to exist among avian genomes (Griffin et al. 2007), comparative mapping among the homologs of chicken, zebra finch, and ruff microsatellite loci results in three possible intrachromosomal rearrangements being reported for the first time on chromosome 1 (involving loci Ppu001, Ppu021, and Ppu028), chromosome 2 (loci Ppu018 and Ppu022) and chromosome 7 (loci Ppu023 and Ppu027; Fig. 2). These types of rearrangements were once thought to be relatively rare in birds (Stapley et al. 2008). However, with the recent sequencing of the turkey (Meleagris gallopavo) genome, comparative analyses between the turkey, zebra finch (Taeniopygia guttata), and chicken (Gallus gallus) have identified a large number of intrachromosomal rearrangements, reflective of avian genome evolution (Skinner and Griffin 2012). Therefore, these regions are of evolutionary interest in the ruff.

In summary, the map of seven linkage groups and length 641.6 cM covers an estimated 30–35% coverage of the ruff genome. It is the first linkage map of any shorebird species and will be of utility, even at this low density, as previous studies with approximately 30% map coverage have met with some success in the mapping of phenotypic loci (Miwa et al. 2006). Thus, this map has the potential to provide an essential framework for further studies mapping important behavioral and plumage traits in this species.

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Conflict of Interest

None declared.

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