A dominant allele controls development into female mimic male and diminutive female ruffs
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Paternity Assignments

Ruffs were bred in outdoor aviaries near Kingston, ON (1985–1993) and Burnaby, BC (1994–2009). In Kingston, parentage was determined by restricting females’ access to individual males, and monitoring their laying and incubation. In Burnaby, parentage of chicks produced in 2002–2009 was determined using microsatellite markers [1], together with knowledge of subdivided aviary locations of females and their access to subsets of males.

Microsatellite genotyping was performed at Sheffield and East Carolina Universities (ECU) for overlapping samples, including all individuals alive from 2006–2009. Methodologies for genotyping and parentage assignments used at Sheffield are described in Farrell et al. [1,2].

At ECU, PCR was conducted in a gradient thermocycler (PTC-200, MJ Research), with at least 10 ng of genomic DNA, 1X PCR buffer (20 mM Tris-HCl, 50 mM KCl, pH8.4), 0.2 mM dNTPs (Invitrogen), 1 pmol forward primer labelled with 6-FAM, HEX (Invitrogen or Bioneer), PET, NED or VIC (Applied Biosystems), 1 pmol reverse primer, and 0.5–1.0 µL genomic DNA template. Multiplex PCR combined reactions for Ruff 5 with Ruff 10, as well as for PGT83 with Tgu06. All other loci were run individually. Products were multiplexed for fragment analysis in three panels (Panel 1: Ruff 1, Ruff 6, Ruff 5 and Ruff 10, Ruff 8, Ruff 50; Panel 2: PGT83 and Tgu06, TG22-001, Chmo06, Chmo21, Snipe B2, Ruff 12; Panel 3: Ppu057 and Phil9; and Phil2 was run singly). Panel 1 loci reaction volumes were 10 µL with 0.5 Units Taq DNA polymerase (recombinant, Invitrogen); Panel 2 and 3 loci and Phil2 were amplified in reaction volumes of 5 µL with 0.25 Units Taq. PCR conditions for Panels 1 and 3 loci and Phil2 included an initial denaturation step at 94°C for 2 min, 25 cycles including denaturation at 94°C for 30 s, annealing
(temperature varied per locus) for 1 min, and extension at 72°C for 30 s, followed by a final extension step at 72°C for 5 min. PCR conditions for Panel 2 loci included an initial denaturation step at 94°C for 2 min, 30 cycles including denaturation at 94°C for 30 s, annealing at 54°C for 40 s, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 5 min. Fragment analysis was performed on an ABI 3130xl Genetic Analyzer with GeneScan 600 LIZ size standard (Applied Biosystems). Following automated analysis using GENEMAPPER v. 4.0 (Applied Biosystems), all size calls were scrutinized, and questionable allele assignments were rerun.

Parentage assignments used the ‘parent pair (sexes known)’ module in CERVUS v3.0 [3]. Genotypic data from 13 autosomal loci for 314 individual ruffs present in the colony in 2006–2009 were used to create simulations in CERVUS for parentage analyses. All but two loci conformed to Hardy–Weinberg equilibrium. The exceptions were *PGT83*, which had only two alleles and a high number of homozygotes for one of these, and *Ruff10*, which had over-representation of homozygotes for one allele and three rare alleles. These loci were included in parentage analyses because they were still informative in at least some families. The limited number of alleles in the captive colony, in conjunction with highly skewed polygynous mating behaviour on the lek, meant that allele distributions were not even. Three variable Z-linked variable microsatellite loci helped eliminate parental candidates for male offspring, significantly increasing the probability of identifying exclusive parental candidates. After double-blind determination of parentage in the lab, each assignment was compared with breeding records indicating which adults were housed in the aviary pen from which the eggs were collected. On occasions when CERVUS assigned more than one plausible pair of parental candidates, we were usually able to exclude all but one pair based on breeding records. Cases with multiple matches
occurred when candidates were related as full siblings or parent–offspring. This allowed us to make final eliminations to arrive at a single mother–father pair for each chick.

**Logistic regression assignment of phenotype for unknown males**

Twenty-seven males died prior to expressing definitive behavioural morph characteristics. Since faeders are smaller than ornamented males (Table 1, [4]), we categorized these 27 unknown males as faeders or not based on body size. We created a logistic regression model based on tarsus, culmen, and minimum mass from 21 identified faeders and 178 ornamented males. The model was highly significant ($LR \chi^2=85.9$, df=3, $p<0.0001$), with partial contributions from all three variables (mass: Wald $\chi^2=10.6$, df=1, $p=0.001$; culmen: Wald $\chi^2=3.2$, $p=0.075$; tarsus: Wald $\chi^2=3.5$, $p=0.063$). The model’s assignments based on body size were 98.1% concordant with the original behavioural observations. Applied to males with unknown behaviour, the model categorized one faeder son as a faeder and four as ornamented (inclusion and exclusion probabilities >0.95). All 22 ornamented males’ sons were categorized as ornamented ($p>0.87$).

**Summary of size distributions of wild samples of female ruffs**

We examined published size distributions of female ruffs for evidence that unusually small females occur in the wild. Six studies provide histograms of female size with a suggestion of a very small left more or negative skew. Table S1 presents summary statistics of these distributions extracted from these histograms, which should be viewed to obtain more detail information.
Table S1. Summary statistics for distributions of body size in female ruff sandpipers.

Morphometric data (wing length in millimeters) based on large samples from populations of migrating ruffs captured at different sites throughout Europe were compiled and tested for deviation from normality. For three of the four distributions of wing length in which skewness and kurtosis could be measured, the distributions were platykurtic. In the fourth case (Gill et al. 1995), the 5mm bin size confounds consideration of shape.

<table>
<thead>
<tr>
<th>Location and time of females caught</th>
<th>Reference</th>
<th>N (females)</th>
<th>Metric</th>
<th>Bin resolution (mm)</th>
<th>Smallest size class (mm)</th>
<th>Skewness</th>
<th>Kurtosis</th>
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<tr>
<td>Wash, UK, winter</td>
<td>[5]: Fig. 1</td>
<td>183</td>
<td>wing length (mm)</td>
<td>5</td>
<td>140-144</td>
<td>-0.22</td>
<td>4.06</td>
</tr>
<tr>
<td>Puck Bay, Poland, southward migrants</td>
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<td>383</td>
<td>wing length (mm)</td>
<td>1</td>
<td>147</td>
<td>0.04</td>
<td>-0.78</td>
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<tr>
<td>Friesland, winter + migrants</td>
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<td>236</td>
<td>wing length (mm)</td>
<td>1</td>
<td>148</td>
<td>-0.22</td>
<td>-0.84</td>
</tr>
<tr>
<td>Finland, southward migrants</td>
<td>[7]: Fig. 2</td>
<td>987</td>
<td>wing length (mm)</td>
<td>1</td>
<td>142</td>
<td>-0.15</td>
<td>-1.36</td>
</tr>
<tr>
<td>Belarus, northward migrants</td>
<td>[8]: Fig. 3</td>
<td>913</td>
<td>wing length (mm), other measures</td>
<td>1</td>
<td>143</td>
<td>visually, possible left skew</td>
<td>na</td>
</tr>
<tr>
<td>Münster, Germany, migrants</td>
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<td>ca. 2037, 1918</td>
<td>discriminant function, tarsus (mm)</td>
<td>n/a, 1</td>
<td>not meaningful for df, 37</td>
<td>visually, possible left skew</td>
<td>na</td>
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</tbody>
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Supplementary Material References


