Supplementary Table 1

Variation in steroid concentrations. Generalized linear model of variation in steroid concentrations with respect to morph and date.

	Total Testosterone (T + DHT)				Androstenedione (A4)			
Fixed Effects	Estimate	SE	$F_{1,97}$	P	Estimate	SE	$F_{1,97}$	P
Day of year	-0.0610	0.0360	0.55	0.460	0.18720	0.187200	6.77	0.010
Day^2	0.0002	0.0001	0.94	0.330	-0.00075	0.000121	8.37	< 0.010
Morph (Ind – Sat)	-9.3090	2.9420	10.01	< 0.010	13.10050	3.174700	17.03	< 0.001
Day of year x morph	0.1580	0.0480	10.88	≤ 0.001	-0.23770	0.052580	20.44	< 0.001
Day^2 x morph	-0.0010	0.0000	10.33	< 0.010	0.00090	0.000200	19.65	< 0.001
Random effects								
Assay Method	0.000	0.000			0.00	0.00		
Year	0.000	0.000			0.00	0.00		
Bird	0.083	0.093			0.00	0.00		

Supplementary Table 2

Overview of the genomic sequencing data. Library, platform and mapping information for high-throughput DNA and RNA sequencing.

Sample ID	Library[1]	Platform[2]	Insert Size (bp)	Read length (bp)	No. of reads	No. clean reads	No. mapped reads[3]	Mapped reads[3] (%)
Genomic sequencing libraries								
MB1386	DNA-PE	IH2500	200	150	109,109,178	104,567,414	111,491,450	99.63
MB1387	DNA-PE	IH2500	200	150	100,539,926	96,173,726	102,660,447	99.60
MB1388	DNA-PE	IH2500	400	150	104,292,358	99,418,692	106,495,792	99.49
MB1389	DNA-PE	IH2500	400	150	120,418,946	115,125,042	123,202,076	99.47
MB1390	DNA-PE	IH2500	600	150	216,079,192	204,670,708	216,932,244	99.44
MB1391	DNA-PE	IH2500	600	150	141,204,802	132,483,520	141,596,792	99.43
MB2923	DNA-MP	IH2500	3,000	150	83,268,564	49,026,400	49,195,255	98.70
MB2924	DNA-MP	IH2500	5,000	150	235,755,936	139,447,886	140,078,428	98.69
PacBio	PacBio	PBII		~5,713	1,853,351	1,612,195[4]	1,481,807[5]	91.91[5]
Genomic re-sequencing libraries								
MB8684	DNA-PE	IH2500	550	150	805,311,920		795,419,399	98.77
MB8685	DNA-PE	IH2500	550	150	624,247,439		616,286,971	98.72
MB8686	DNA-PE	IH2500	550	150	931,537,826		918,758,871	98.63
MB8687	DNA-PE	IH2500	550	150	763,312,379		753,655,429	98.73
MB8688	DNA-PE	IH2500	550	150	700,990,546		692,791,230	98.83
RNAseq libraries								
Egg	RNA-PE	IH2500		150	47,200,308	24,790,198	22,575,206	91.07
Chick heart	RNA-PE	IH2500		150	73,554,376	38,225,478	34,481,114	90.20
Chick lung	RNA-PE	IH2500		150	49,638,762	25,928,198	23,166,138	89.35
Chick brain	RNA-PE	IH2500		150	36,948,692	19,326,273	17,598,275	91.06
Female heart	RNA-PE	IH2500		150	52,972,520	27,625,579	25,031,195	90.61
Female brain	RNA-PE	IH2500		150	48,012,484	25,162,177	22,875,920	90.91
Male testes	RNA-PE	IH2500		150	53,455,840	27,866,438	25,507,436	91.53
Male brain	RNA-PE	IH2500		150	49,618,940	25,888,872	23,889,673	92.28
Male liver	RNA-PE	IH2500		150	47,489,458	24,849,173	22,383,425	90.08

[1] DNA-MP: genomic paired-end; DNA-MP: genomic mate pair, RNA-PE: stranded mRNA paired-end.

[2] IH2500: Illumina HiSeq 2500; PBII: PacBio RS II platform

[3] Calculated by samtools flagstat.

[4] Reads > 1000 kb.

[5] Uniquely mapping reads calculated directly.

Supplementary Table 3

Genome assembly statistics. Table showing summary of genome assembly of the ruff genome.

	Contigs	Scaffolds
Total number	41,014	18,799
Total length (bp)	1,154,149,914	1,165,202,088
Longest (bp)	3,674,146	9,022,850
> 1k bp	28,293	12,079
> 10k bp	10,410	4,346
> 100k bp	2,505	1,753
> 1M bp	112	238
Mean length (bp)	28,140	61,982
Median length (bp)	2,244	1,725
N50 length (bp)	265,412	878,409
L50 count	975	292
%N	0.04	0.99

Supplementary Table 4

Genome annotation statistics. Table with summary of the gene annotation for the final assembly.

	No. predicted genes
Trinity highly expressed unique transcripts	30089
Maker pass1 total mRNA	54598
Maker pass1 mRNA AED[*] < 1	17499
Augustus	29596

[*] AED: Annotation Edit Distance

Supplementary Table 5

Genome assembly statistics. Table with CEGMA completeness report for the genome assembly.

CEGMA	No. Proteins	% Complete	Total[*]	Aver[†]	% Orthol[‡]
Complete	140	56.5	172	1.2	17.1
Partial	220	88.7	354	1.6	45.9

[*] Total number of detected copies of the genes,
[†] Average number of copies per gene,
[‡] Average percentage of orthology

Supplementary Table 7

Candidate genes within the inversion. Candidate genes of known function that might contribute to phenotypic differences among alternative reproductive morphs in ruffs.

Gene ID	**Location**	**Length**[1]	**Protein**	**Identity**[2]	**Function**[3]
9645 9646 9647 14258	Contig 2063 866370-88102, Contig 1270 554–6565	1060	Zinc finger protein *ZFPM1*	75%	transcription factor, expressed in gonads
9689	Contig 1270 691183–698352	480	Growth arrest-specific protein *GAS8*	91%	sperm motility
9685[4]	Contig 1270 663189–673684	1345	Transcription factor *TCF25*	90%	transcription factor
9686	Contig 1270 676152–677096	314	Melanocortin 1 receptor *MC1R*	96%	pigmentation regulation
18874	Contig 3357 542795–590127	1260	1-phosphatidylinositol -bisphosphate phosphodiesterase γ-2 *PLCG2*	98%	steroid hormone metabolism, feather array formation
18875	Contig 3357 597364–600788	535	Short-chain dehydrogenase reductase *SDR42E1*	94%	steroid hormone metabolism
18876	Contig 3357 618128–629243	385	Estradiol 17-beta dehydrogenase *HSD17B2*	90%	steroid hormone metabolism
9705	Contig 1270 798473–809553	148	Cytochrome b5 *CYB5B*	87%	steroid hormone metabolism
20688	Contig 4111 44279–59313	322	Palmitoyltransferase *ZDHHC7*	98%	steroid receptor regulation

[1] Amino-acid sequence length predicted from reference genome.

[2] Mean similarity to identified blast homologues.

[3] Relevant function regarding morph differences.

[4] Gene 9685 is included as a transcription factor of unknown function because it lies in a region with a high density of Satellite-specific differences in close proximity to *MC1R*.