

Figure S1. Detailed characterization of pigments in the head feathers of the black and red morph Gouldian finches. (A) A representative transmission electron micrograph of the cortex of a head feather barb from a black morph imaged at 10,000x magnification. Note the electron-dense melanosomes throughout the barb cortex. Scale bar = 500 nm. (B) A representative transmission electron micrograph of the cortex of a head feather barb from a red morph imaged at 10,000x magnification. Electron-dense melanosomes are almost entirely absent from the barb. (C) Representative normalized UV-Vis absorbance spectra for the four major carotenoids detected in the head feathers of the red morph. Peak numbers refer to the chromatograms presented in Figure 1 of the main text.

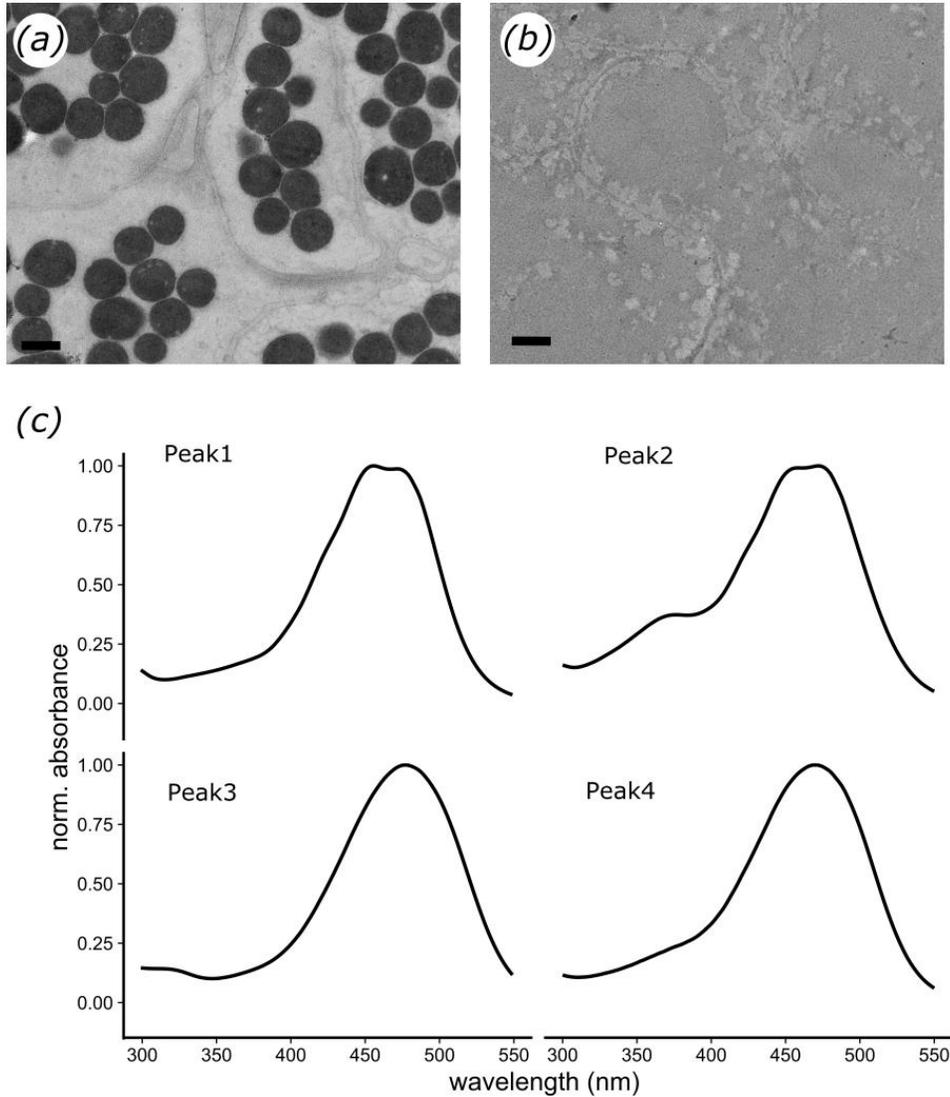


Figure S2. Genetic structure among sampled individuals summarized by means of Principal Component Analysis (PCA).

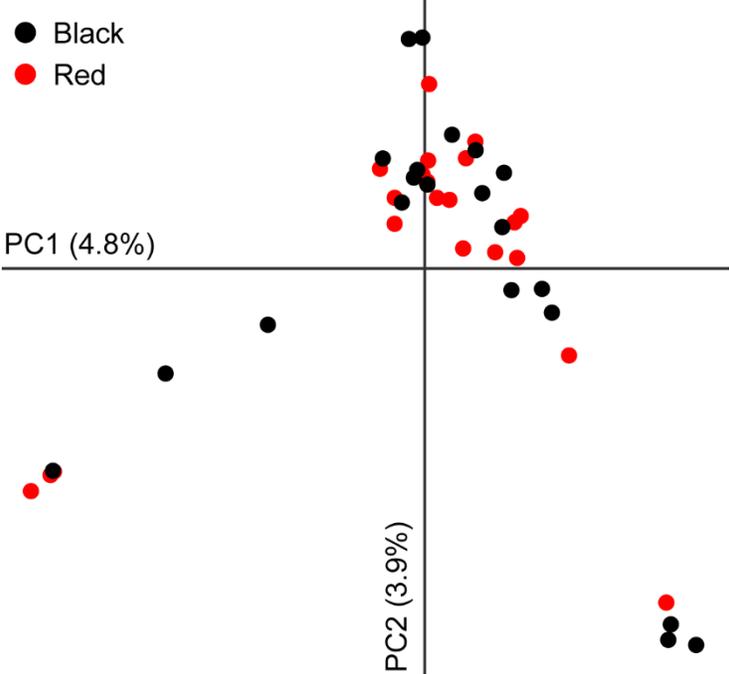


Figure S3. Dot plot summarizing an alignment between the Gouldian finch and zebra finch assemblies in a 4 Mb window around the candidate region on scaffold 11.

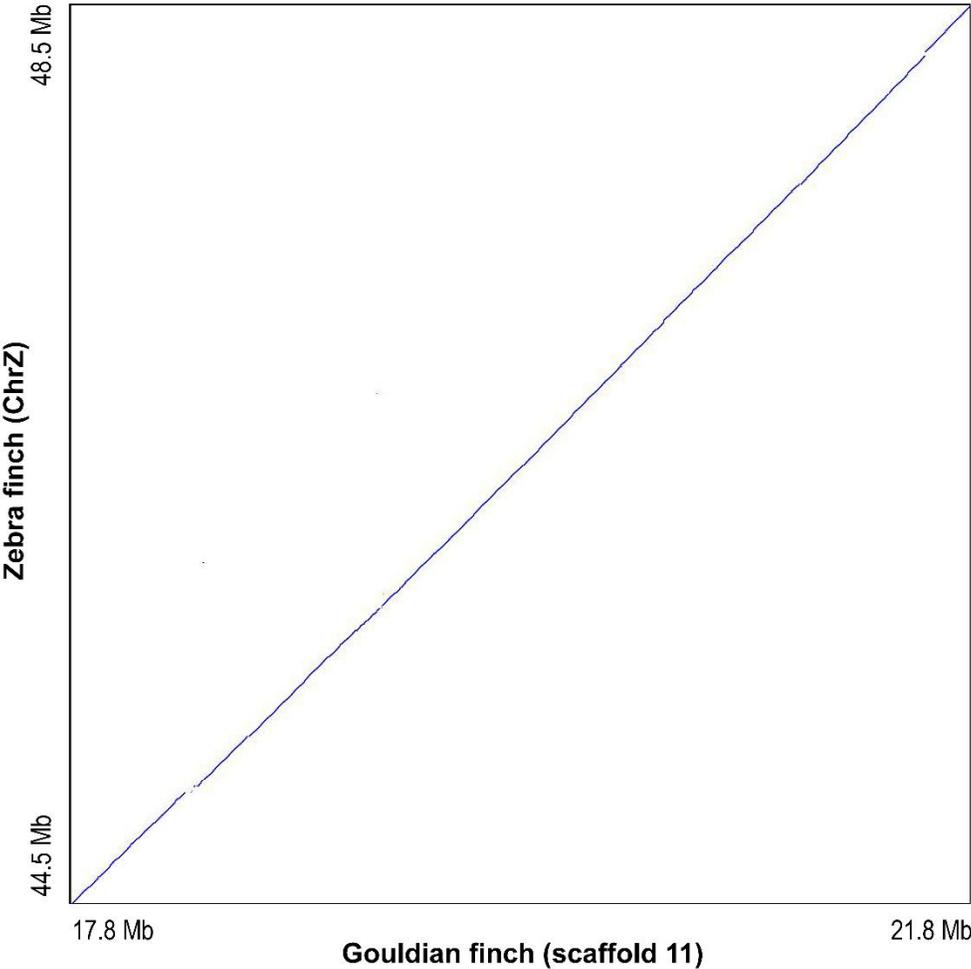


Figure S4. A schematic representation of the *FST* locus in the EryGou1.0 genome assembly with exon-exon splice junctions mapped from the RNA-seq reads. The exons of *FST* isoforms *X1* and *X2* are indicated in blue and the open reading frame indicated in light blue. The locations of the primers used for transcript amplification and qPCR analysis of transcript isoforms are shown in green. The dashed green line indicates where the primer spans an exon-exon junction in the mature transcript. Sashimi plots (in black and red) (Katz *et al.* 2015) show the read depth (bar blot) at each exon and the number of reads spanning each exon-exon junction (numbered arcs) for each of the samples in our RNA-seq analysis of 10 days post-pluck regenerating skin from the masks of black, orange, and red morph Gouldian finches. Red and orange morphs are indicated in red because both carry the same ‘red’ allele on the scaffold 11 locus (see main text).

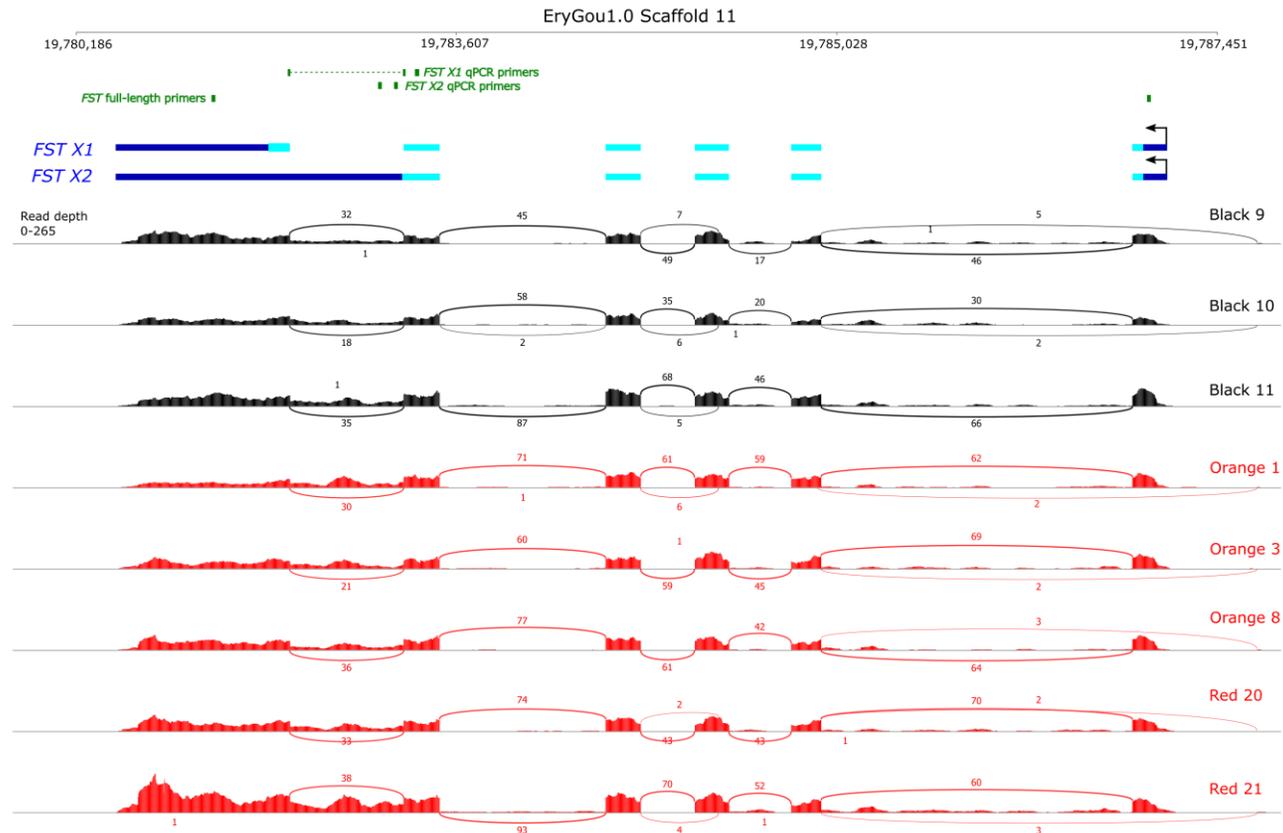


Figure S5. Gouldian finches express two transcript isoforms of *FST* that encode different amino acid sequences at the C-terminus of the protein. (A) PCR amplification of cDNA from 2 days post-pluck regenerating skin of red and black morph Gouldian finches with primers targeting the 5' and 3'-UTR of *FST*. The two amplicons are consistent with the inclusion/exclusion of the intron between exons 5 and 6 in the mature transcript of isoforms X1 and X2, respectively. (B) The predicted amino acid sequence of Gouldian finch transcript isoforms *FST X1* and *FST X2* have 90% and 91% identity with the *FST315* (NP_001288302.1) and *FST288* (NP_001288304.1) isoforms previously characterized in mouse (*Mus musculus*), respectively. Identical amino acids among the proteins are indicated in red. (C) qPCR measurements of the expression of *FST X2* relative to *FST X1* in the regenerating skin at 2 and 4 days post-pluck indicate that there were not significant differences in isoform expression between the red and black morphs (day 2: $t = 0.76$, $df = 4$, $p = 0.49$, day 4: $t = -1.736$, $df = 4$, $p = 0.16$). Each point represents a sample from an individual bird, and bar indicates mean for each morph.

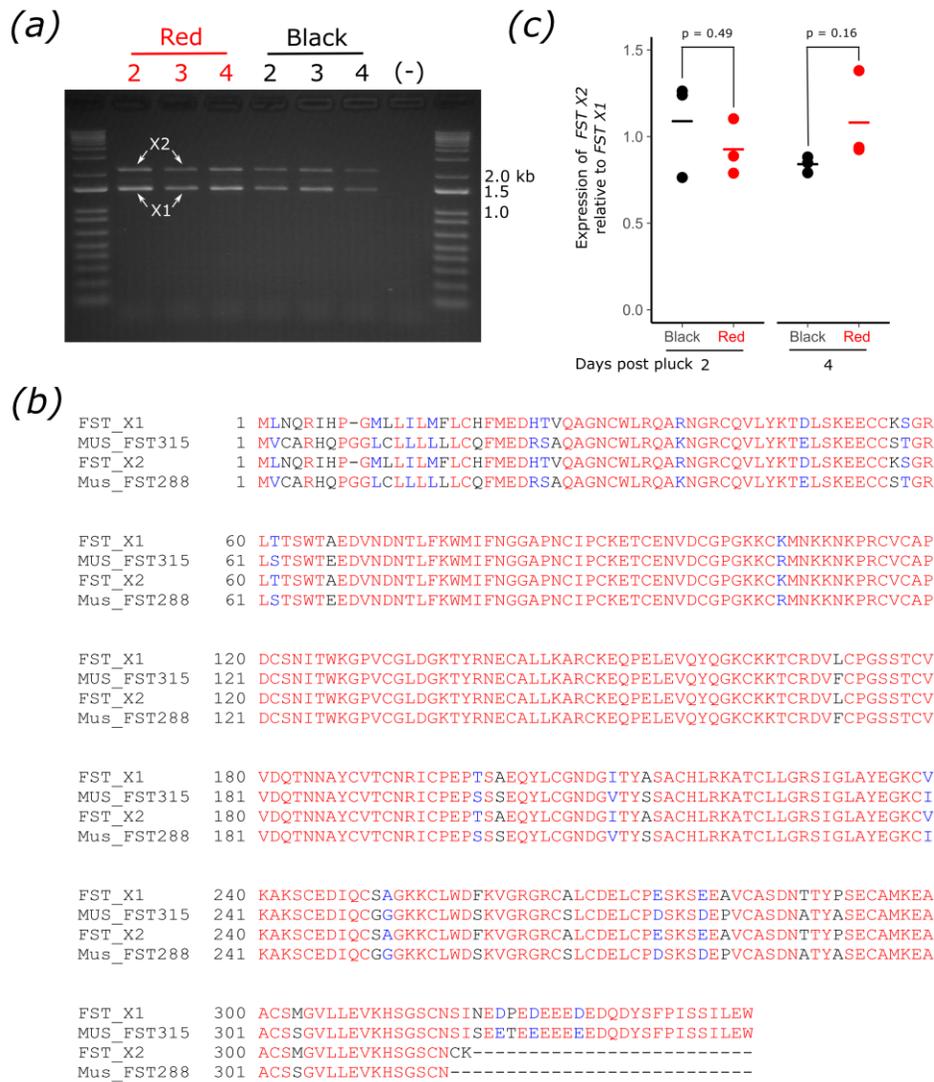


Figure S6. A schematic representation of the *MOCS2* locus in the EryGou1.0 genome assembly with exon-exon splice junctions mapped from the RNA-seq reads. The exons of *MOCS2* isoforms *X1* and *X2* are indicated in blue with the open reading frames (ORFs) of *MOCS2* subunits A and B highlighted in red and purple respectively. The locations of the primers used for qPCR analysis are noted in green. Sashimi plots (in black and red) (Katz *et al.* 2015) show the read depth (bar blot) at each exon and the number of reads spanning each exon-exon junction (numbered arcs) for each of the samples in the RNA-seq analysis of 10 days post-pluck regenerating skin from the masks of black, orange, and red morph Gouldian finches. Red and orange morphs are indicated in red because both carry the same ‘red’ allele on the scaffold 11 locus (see main text).

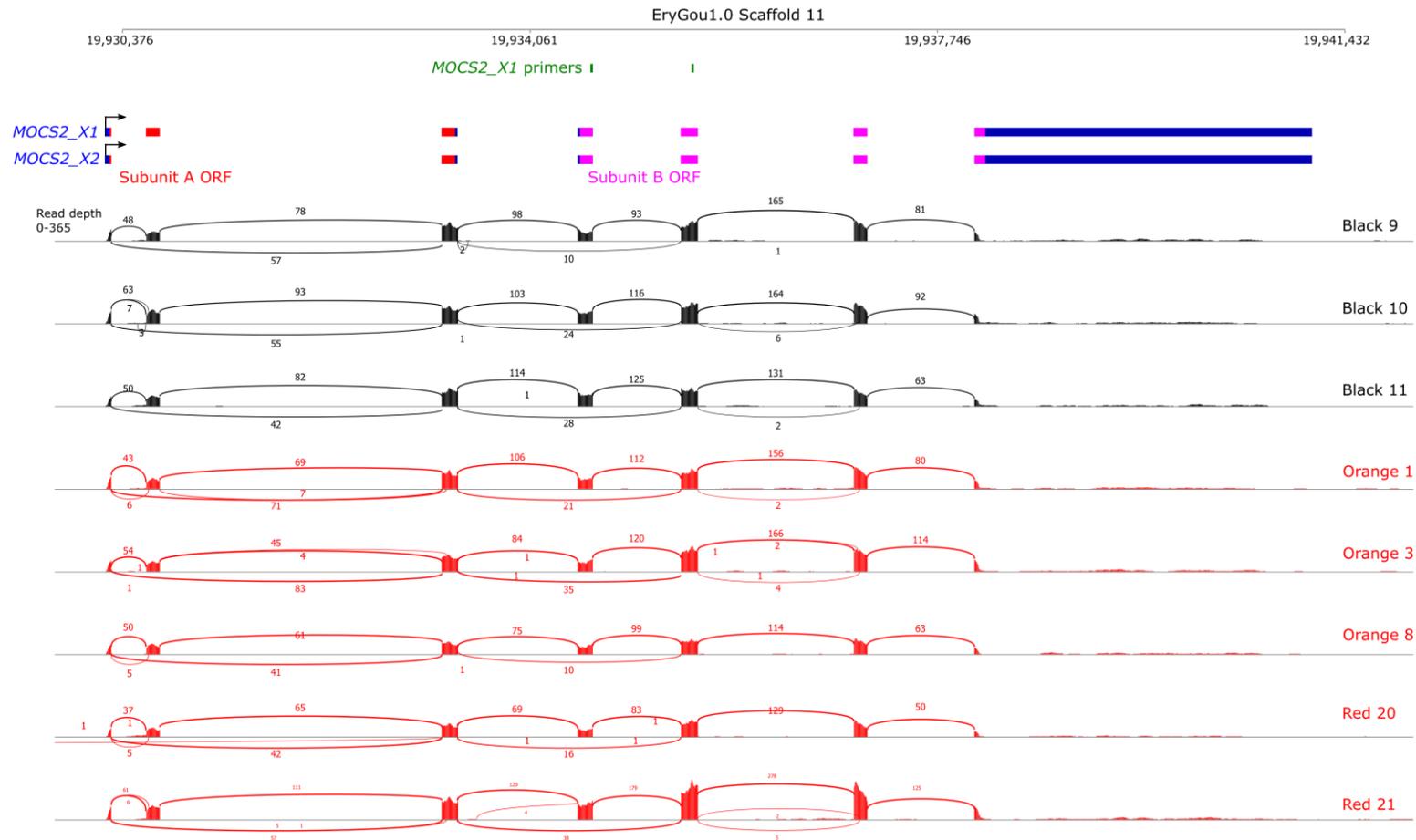


Figure S7. The frequency of reads mapping to alternative exon-exon splice junctions of *MOCS2* do not differ between the head color morphs. (A) The proportion of reads spanning exons 1 to 3 relative to those spanning exons 2 to 3 of *MOCS2* did not differ significantly between black and red morphs ($t = -1.21$, $df = 6$, $p = 0.35$). **(B)** The proportion of reads spanning exons 3 to 5 relative to those spanning exons 4 to 5 of *MOCS2* did not differ significantly between black and red morphs ($t = -0.36$, $df = 6$, $p = 0.73$).

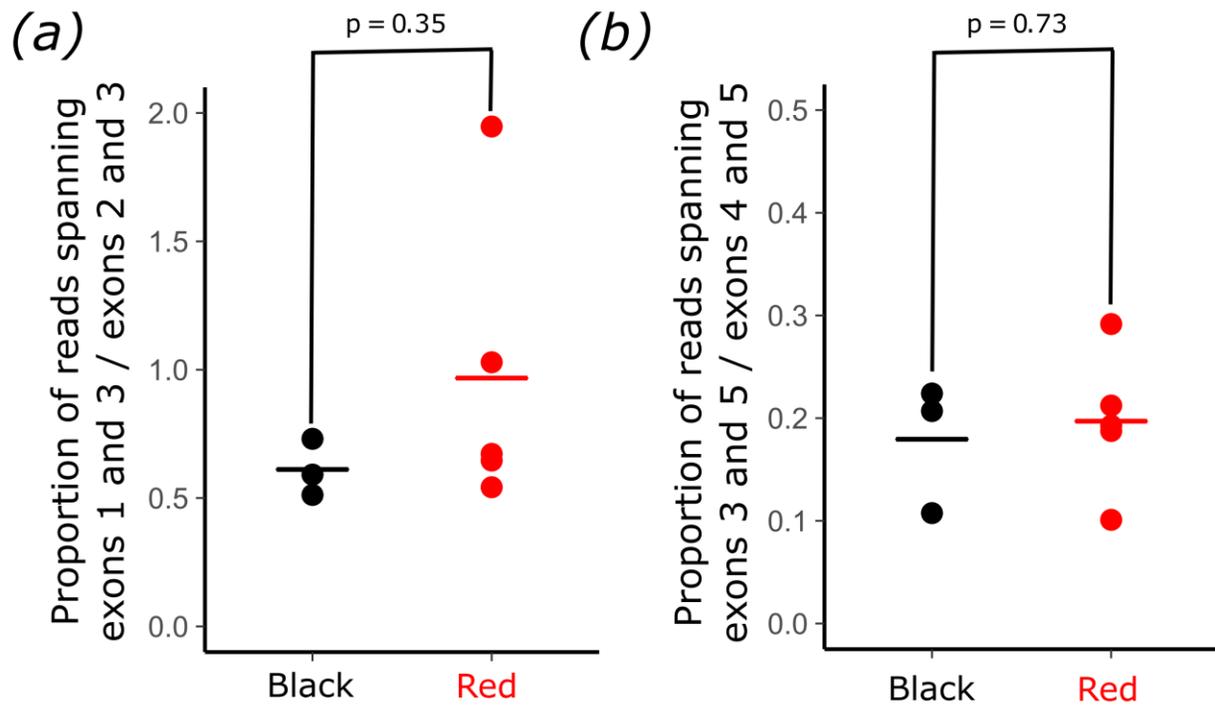


Figure S8. *FST* and *MOCS2* expression in the testis of black and red morphs. (A) qPCR measurements of *FST X1* and *X2* expression relative to *GAPDH* in the testis. Each point represents a sample from an individual bird, and bar indicates mean for each morph. (B) qPCR measurements of *MOCS2* expression relative to *GAPDH* in the testis.

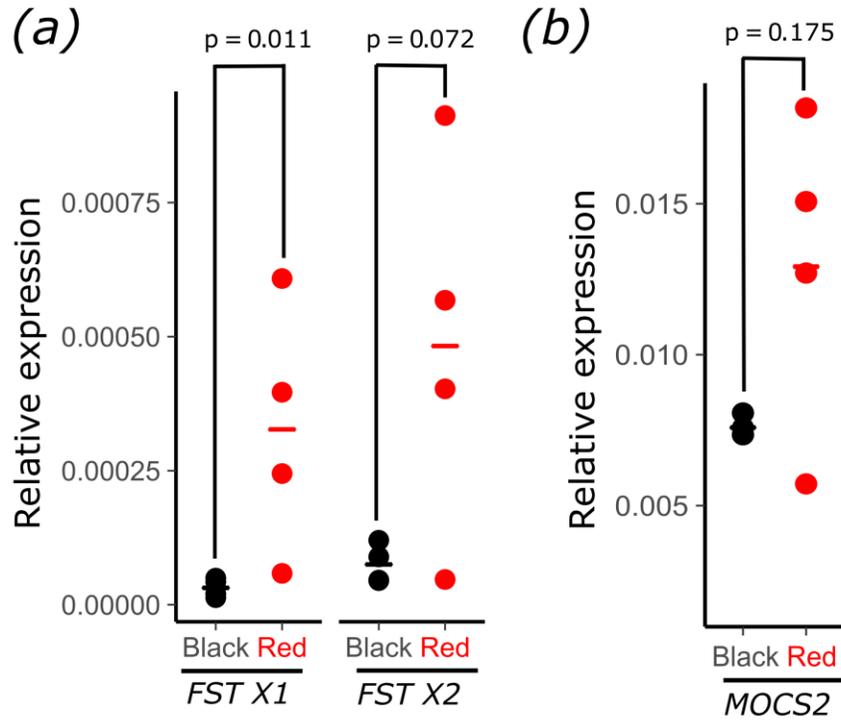


Figure S9. Quantile–quantile (QQ) plot for the genome-wide association analysis. The genomic inflation factor (λ) is indicated. The red box highlights the section of the QQ-plot where we observed an inflation of the P -values we obtained compared to the expected distribution. 75.3% of the SNPs contained within this box are located in the candidate region of scaffold 11 (± 20 kb).

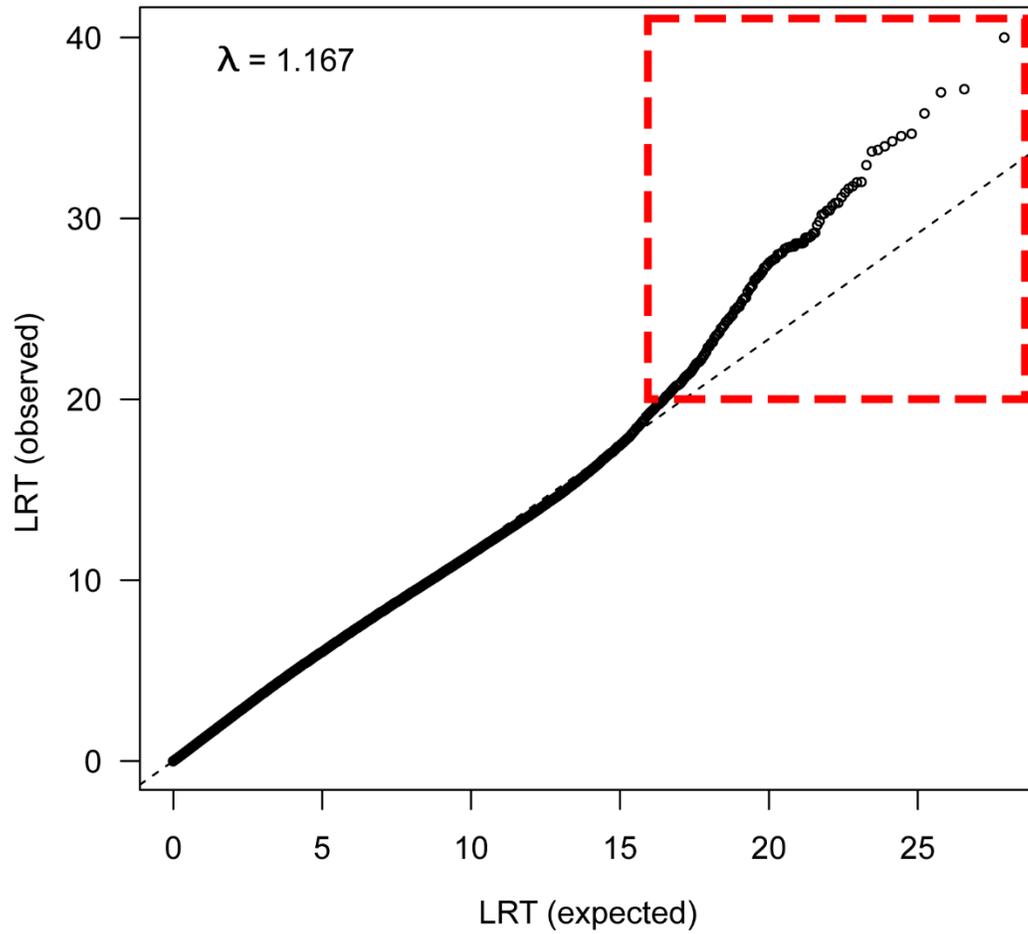


Table S1. Whole genome resequencing details and read mapping statistics

| Head phenotype | Sample code | Number of reads | Percentage of reads mapping ^a | Percentage of reads MQ ≥ 20 ^b | Percentage of positions ≥ 1 reads ^c | Raw coverage ^d |
|------------------------|-------------|-----------------|--|---|---|---------------------------|
| Black | Black01* | 30,690,308 | 97.88 (94.20) | 86.11 | 71.43 | 3.15X |
| | Black02* | 8,280,819 | 98.92 (94.78) | 85.87 | 38.94 | 0.85X |
| | Black03* | 107,284,227 | 97.23 (92.09) | 78.10 | 57.18 | 11.02X |
| | Black04* | 19,574,205 | 98.54 (95.06) | 86.03 | 55.82 | 2.01X |
| | Black05* | 6,628,599 | 96.87 (93.60) | 86.56 | 36.33 | 0.68X |
| | Black06* | 17,273,466 | 99.27 (96.20) | 89.15 | 52.11 | 1.77X |
| | Black07* | 22,336,406 | 99.09 (96.32) | 90.44 | 64.61 | 2.29X |
| | Black08* | 3,840,089 | 99.49 (95.92) | 88.20 | 24.33 | 0.39X |
| | Black09* | 8,227,208 | 99.38 (95.08) | 85.50 | 37.21 | 0.85X |
| | Black10* | 10,449,863 | 99.38 (94.77) | 85.08 | 41.01 | 1.07X |
| | Black11* | 5,501,051 | 99.47 (96.00) | 88.64 | 32.53 | 0.57X |
| | Black12* | 5,019,680 | 99.41 (94.40) | 83.74 | 24.34 | 0.52X |
| | Black13* | 4,975,563 | 99.44 (94.73) | 85.05 | 26.08 | 0.51X |
| | Black14 | 4,095,880 | 99.48 (95.45) | 86.23 | 23.83 | 0.42X |
| | Black15 | 5,078,737 | 99.39 (95.66) | 87.15 | 29.14 | 0.52X |
| | Black16 | 35,399,863 | 99.44 (95.01) | 84.37 | 66.02 | 3.64X |
| | Black17 | 5,175,981 | 99.47 (95.83) | 87.88 | 30.83 | 0.53X |
| | Black18 | 5,535,424 | 99.29 (95.19) | 85.99 | 29.40 | 0.57X |
| | Black19 | 134,376,825 | 99.29 (94.24) | 79.75 | 66.64 | 13.80X |
| | Black20 | 21,616,437 | 99.36 (94.24) | 79.51 | 35.68 | 2.22X |
| | Black21 | 9,658,503 | 99.54 (95.87) | 88.58 | 46.58 | 0.99X |
| Total _{Black} | 21 | 471,019,134 | | | | 48.38X |
| Red | Red01* | 3,431,082 | 98.84 (95.12) | 87.98 | 22.90 | 0.35X |
| | Red02* | 3,780,715 | 98.50 (95.35) | 88.90 | 25.74 | 0.39X |
| | Red03* | 2,862,057 | 98.95 (95.65) | 89.36 | 20.32 | 0.29X |
| | Red04* | 4,251,054 | 95.30 (91.73) | 84.09 | 26.16 | 0.44X |
| | Red05* | 3,324,372 | 97.45 (93.83) | 86.68 | 22.13 | 0.34X |
| | Red06* | 24,192,758 | 97.70 (94.08) | 86.67 | 66.10 | 2.48X |
| | Red07* | 16,398,889 | 99.10 (95.17) | 87.38 | 54.84 | 1.68X |
| | Red08* | 2,485,522 | 99.13 (96.39) | 90.56 | 18.71 | 0.26X |
| | Red09* | 2,710,196 | 99.55 (95.23) | 86.36 | 17.31 | 0.28X |
| | Red10* | 3,315,750 | 99.44 (95.04) | 85.48 | 19.80 | 0.34X |
| | Red11* | 3,399,163 | 99.52 (95.24) | 86.29 | 21.00 | 0.35X |
| | Red12* | 3,509,070 | 99.46 (95.73) | 87.37 | 22.32 | 0.36X |
| | Red13 | 7,161,142 | 99.49 (95.12) | 86.02 | 35.37 | 0.74X |
| | Red14 | 4,058,569 | 99.45 (95.05) | 85.42 | 23.09 | 0.42X |
| | Red15 | 23,972,387 | 99.41 (94.47) | 82.03 | 45.79 | 2.46X |

| | | | | | |
|----------------------|------------|---------------|-------|-------|--------|
| Red16 | 4,774,143 | 99.54 (95.68) | 87.03 | 27.15 | 0.49X |
| Red17 | 32,907,003 | 99.47 (95.38) | 85.96 | 65.23 | 3.38X |
| Red18 | 6,639,591 | 99.53 (96.21) | 88.18 | 37.00 | 0.68X |
| Red19 | 4,645,876 | 99.53 (95.82) | 86.90 | 26.35 | 0.48X |
| Red20 | 30,568,020 | 99.36 (94.93) | 81.12 | 48.33 | 3.14X |
| Red21 | 7,772,159 | 99.53 (96.24) | 88.84 | 40.92 | 0.80X |
| <hr/> | | | | | |
| Total _{Red} | 21 | 196,159,518 | | | 20.15X |
| <hr/> | | | | | |

*Subset of samples used for F_{ST} and d_{XY} analysis.

Table S2. List of primers used in this study

Genotyping

| | |
|-------------------|-------------------------|
| ErGo_genotyping_F | GTCCTAAATACTGAGCCCCAAA |
| ErGo_genotyping_F | TGTGGCAAGTTGAATCACTTATG |

FST isoform amplification

| | |
|------------------------|----------------------|
| ErGo_FST_full_length_F | CTCTCCCTACTCCCCCTCAC |
| ErGo_FST_full_length_R | CCGGCAAAAAGAATATCCAA |

qPCR

| | |
|------------------|-----------------------------|
| ErGo_FST_X1_F | CCAGCGATAACACAACCTTACCC |
| ErGo_FST_X1_R | GTCTTCATTAATTGAGTTGCAAGATCC |
| ErGo_FST_X2_F | CTTCTGAAGGCGATACGATACC |
| ErGo_FST_X2_R | CCAAAGTGTGTGCGTGAAAG |
| ErGo_MOCS2_101_F | GGGCAGTGTCTCTGTTTCATT |
| ErGo_MOCS2_101_R | AAGGCCATTTCTGCCTAACA |
| ErGo_GAPDH_1F | TAGCCATTCCTCCACCTTTG |
| ErGo_GAPDH_1R | ACACGGTTGCTGTATCCGTATT |

Table S3. RNA-sequencing details and mapping statistics

| Head morph | Sample Code | Number of reads (raw data) | Number of reads after filtering ^a | Percentage of reads mapping to genome ^b |
|------------|-------------|----------------------------|--|--|
| Black | Black01 | 50,461,530 | 47,189,574 | 78.63 (66.95) |
| | Black02 | 45,130,642 | 42,362,154 | 77.92 (66.56) |
| | Black03 | 47,450,142 | 44,622,604 | 81.59 (69.66) |
| Red | Red01 | 45,177,360 | 42,318,174 | 79.32 (67.99) |
| | Red02 | 64,308,606 | 60,348,366 | 73.30 (62.68) |
| Orange | Orange01 | 52,319,958 | 49,083,970 | 82.99 (70.47) |
| | Orange02 | 49,796,176 | 46,763,048 | 80.35 (68.54) |
| | Orange03 | 46,456,990 | 43,464,078 | 80.59 (68.98) |

^aAfter removing adapters with *cutadapt* and trimming with *Trimmomatic*.

^bProperly paired reads are shown in parentheses.

Table S4. Summary statistics of the Gouldian finch genome assembly and annotation

| | EryGou1.0 |
|----------------------------------|------------------|
| Genome assembly | |
| Contig N50 (kb) | 148.96 |
| Scaffold N50 (Mb) | 18.97 |
| Assembly size (Gb) | 1.07 |
| Estimated genome size (Gb) | 1.22 |
| Number of scaffolds (>1kb) | 18,372 |
| Number of scaffolds (>10kb) | 831 |
| Largest scaffold (Mb) | 68.02 |
| Phase block N50 (Mb) | 8.40 |
| Heterozygosity | 1/185 |
| GC content (%) | 41.9 |
| Complete BUSCOs reference (%) | 94.2 |
| Fragmented BUSCOs reference (%) | 3.5 |
| Missing BUSCOs reference (%) | 2.3 |
| Genome annotation | |
| Protein-coding genes | 18,989 |
| tRNAs | 432 |
| Complete BUSCOs annotation (%) | 86.0 |
| Fragmented BUSCOs annotation (%) | 7.8 |
| Missing BUSCOs annotation (%) | 6.2 |

Table S5. Summary statistics per chromosome of the Gouldian finch genome assembly obtained by whole-genome alignments between Gouldian finch and zebra finch

| Chr | Chromosome size zebra finch (Mb) | Number of aligned scaffolds from the Gouldian finch assembly ^a | Size largest scaffold (Mb) | Ratio largest scaffold vs. chromosome size | Total length of all scaffolds (Mb) | Ratio of total length of all scaffolds vs. chromosome size |
|-------|----------------------------------|---|----------------------------|--|------------------------------------|--|
| 1 | 118.55 | 42 | 41.96 | 0.35 | 110.86 | 0.935 |
| 2 | 156.41 | 91 | 68.02 | 0.43 | 149.00 | 0.953 |
| 3 | 112.62 | 43 | 64.79 | 0.58 | 110.39 | 0.980 |
| 4 | 69.78 | 35 | 45.96 | 0.66 | 67.85 | 0.972 |
| 5 | 62.37 | 19 | 47.33 | 0.76 | 60.56 | 0.971 |
| 6 | 36.31 | 7 | 17.93 | 0.49 | 34.57 | 0.952 |
| 7 | 39.84 | 11 | 19.78 | 0.50 | 37.44 | 0.940 |
| 8 | 27.99 | 25 | 29.33 | 1.05 | 30.87 | 1.103 |
| 9 | 27.24 | 17 | 9.13 | 0.34 | 25.67 | 0.942 |
| 10 | 20.81 | 25 | 17.13 | 0.82 | 20.60 | 0.990 |
| 11 | 21.40 | 14 | 11.05 | 0.52 | 20.87 | 0.975 |
| 12 | 21.58 | 31 | 8.06 | 0.37 | 20.60 | 0.955 |
| 13 | 16.96 | 7 | 15.28 | 0.90 | 16.16 | 0.953 |
| 14 | 16.42 | 23 | 11.44 | 0.70 | 16.76 | 1.020 |
| 15 | 14.43 | 9 | 8.32 | 0.58 | 14.01 | 0.971 |
| 16 | 0.01 | 0 | NA | NA | NA | NA |
| 17 | 11.65 | 9 | 9.06 | 0.78 | 11.36 | 0.976 |
| 18 | 11.20 | 11 | 7.14 | 0.64 | 11.41 | 1.019 |
| 19 | 11.59 | 20 | 3.29 | 0.28 | 14.49 | 1.250 |
| 20 | 15.65 | 23 | 5.98 | 0.38 | 14.78 | 0.944 |
| 21 | 5.98 | 9 | 1.69 | 0.28 | 5.16 | 0.863 |
| 22 | 3.37 | 25 | 1.23 | 0.36 | 2.89 | 0.857 |
| 23 | 6.20 | 22 | 2.13 | 0.34 | 6.06 | 0.979 |
| 24 | 8.02 | 8 | 3.93 | 0.49 | 7.19 | 0.896 |
| 25 | 1.28 | 14 | 0.39 | 0.31 | 1.24 | 0.972 |
| 26 | 4.91 | 8 | 2.29 | 0.47 | 5.42 | 1.104 |
| 27 | 4.62 | 26 | 0.91 | 0.20 | 4.11 | 0.889 |
| 28 | 4.96 | 44 | 0.85 | 0.17 | 5.67 | 1.142 |
| 1A | 73.66 | 27 | 30.92 | 0.42 | 69.32 | 0.941 |
| 1B | 1.08 | 6 | 0.14 | 0.13 | 0.60 | 0.554 |
| 4A | 20.70 | 11 | 15.07 | 0.73 | 19.88 | 0.960 |
| LG2 | 0.11 | 0 | NA | NA | NA | NA |
| LG5 | 0.02 | 0 | NA | NA | NA | NA |
| LGE22 | 0.88 | 4 | 0.22 | 0.25 | 0.68 | 0.775 |
| Z | 72.86 | 74 | 22.39 | 0.31 | 72.50 | 0.995 |

^aOnly scaffolds with at least one alignment block larger than 5 kb were considered.

Table S6. Genotyping results

| | T/T | C/T | C/C |
|-------|------------|------------|------------|
| Red | 6 | 15 | 0 |
| Black | 0 | 0 | 21 |

SNP is located in position 19,840,592 on scaffold 11 (ChrZ)

The results are identical for the 6 remaining SNPs contained within the amplified fragment

Table S7. Results of the differential gene expression analysis between morphs using RNA-seq

| Gene ID^a | Coordinates | Average log counts per million (CPM) | Fold Change (log)^b | FDR |
|----------------------------|-----------------------------|---|--------------------------------------|------------|
| Un | scaffold_277:10086-11519 | 13.0 | -10.2 | 1.58E-08 |
| Un | scaffold_31:4013335-4022765 | 2.5 | -8.3 | 5.05E-05 |
| <i>RNF115</i> | scaffold_2376:697-7626 | 2.8 | -8.6 | 1.40E-04 |
| <i>DNMT3A</i> | scaffold_259:106765-123498 | 3.1 | -8.9 | 1.40E-04 |
| Un | scaffold_31:3979100-4012940 | 2.0 | -5.8 | 4.69E-04 |
| <i>DCTN1</i> | scaffold_842:1184-13358 | 0.9 | 7.1 | 4.69E-04 |
| <i>L3MBTL1</i> | scaffold_405:1251-14208 | 2.2 | -3.4 | 3.12E-03 |
| Un | scaffold_1096:230-2876 | 1.3 | 6.2 | 1.04E-02 |
| <i>DCTN2</i> | scaffold_613:13919-20561 | 1.4 | -7.0 | 1.04E-02 |
| Un | scaffold_1912:2-9289 | 0.4 | 6.4 | 1.24E-02 |
| Un | scaffold_8253:750-2261 | 0.2 | 6.2 | 1.48E-02 |
| Un | scaffold_3136:855-4466 | 1.3 | 4.6 | 2.31E-02 |
| <i>CCDC130</i> | scaffold_11663:1-1713 | 0.1 | 6.0 | 2.84E-02 |
| <i>HSD17B2</i> | scaffold_23:1958928-1971108 | 4.9 | -6.4 | 2.84E-02 |

^aUn – Uncharacterized gene.

^bPositive fold change values indicate overexpression in black individuals.