

ELECTRONIC SUPPLEMENTARY MATERIAL

Rosvall, Bergeon Burns, Jayaratna, Ketterson. Divergence along the gonadal steroidogenic pathway: implications for hormone-mediated phenotypic evolution.

Supplementary quantitative PCR methods:

We used published primer sets whenever possible (CYP17, p450scc), and we otherwise designed primers based on zebra finch sequences (StAR, 3 β HSD, RPL13A) for which we confirmed high sequence identity in the junco transcriptome (Peterson et al. 2012). Each qPCR well contained 2.5 μ L of cDNA (diluted 1:10 from stock), 12.5 μ L of PerfeCta SYBR green low ROX, and forward and reverse primers (0.3 μ M) in a total volume of 25 μ L. Our thermal profile was: 10 min at 95°C; 40 cycles of 95°C for 30s, 60°C for 1 min, and 70°C for 30s; and a dissociation phase that assessed product specificity (95°C for 1 min, 55°C for 30s, and 95°C for 1 min). Dissociation curves demonstrated highly specific amplification for each GOI. We used a 5-point standard curve to assess amplification efficiencies (range: 97.7 to 107.5%) and corrected for this slight variation from perfect efficiency in MxPro.

Bibliography for supplementary methods:

Peterson, M. P., Whittaker, D. J., Ambreth, S., Sureshchandra, S., Buechlein, A., Podicheti, R., Choi, J.-H., Lai, Z., Mockatis, K., Colbourne, J., Tang, H. & Ketterson, E. D. 2012. De novo transcriptome sequencing in a songbird, the dark-eyed junco (*Junco hyemalis*): genomic tools for an ecological model system. *BMC Genomics*, 13, 305.

ESM Table 1. Primer sequences, amplicon size, and GenBank accession numbers for source sequences.

StAR	
Forward	TGGGCCAGCACATGCTAGTTAAGCAA
Reverse	AGCAGGGCTTCCTTCAGGAACCAAGTAT
Amplicon size	194 bp
GenBank accession no.	NM_001076686.2
Amplification efficiency	103.5%
p450scc	
Forward	GACCGCGAGAAGATGCTGAAA
Reverse	TCTCCTTGATGGTGGCCTTGAG
Amplicon size	55 bp
GenBank accession no.	NM_001127374.1
Amplification efficiency	97.7%
CYP17	
Forward	CATCAACCTCTGGTCTGTGCAC
Reverse	AAGCGGCCAGGATTGAACT
Amplicon size	72 bp
GenBank accession no.	AY313844.1
Amplification efficiency	105.9%
3βHSD	
Forward	ATGAGCTACGCTGACCTGAA
Reverse	CAGCAGCAGCGAGAAGTAATAG
Amplicon size	108 bp
GenBank accession no.	NM_001048264.1
Amplification efficiency	107.1%
Ribosomal protein L13A	
Forward	GCACCACCATGCGCTTCCGCTGT
Reverse	TTCCACTTCCAGCCCCAGCCG
Amplicon size	148
GenBank accession no.	DQ216005.1
Amplification efficiency	107.5%

ESM Table 2. Bivariate correlation coefficients among expression values of all 4 genes. *p<0.05, **p<0.01, ***p<0.0001. Values above the diagonal (shaded) are from field samples (n=34); values below the diagonal (unshaded) are from captivity (n=45).

	<i>StAR</i>	<i>p450scc</i>	<i>CYP17</i>	<i>3βHSD</i>
<i>StAR</i>	-	0.3398*	0.3238	0.6173***
<i>p450scc</i>	-0.1334	-	0.7288***	0.3249
<i>CYP17</i>	-0.1579	0.5429***	-	0.4068*
<i>3βHSD</i>	0.3581*	-0.0298	0.0716	-