

**JAV-01965**

Kilvitis, H. J., Schrey, A. W., Ragsdale, A. K., Berrio, A., Phelps, S. M. and Martin, L. B. 2019. DNA methylation predicts immune gene expression in introduced house sparrows (*Passer domesticus*). – J. Avian Biol. 2019: e01965

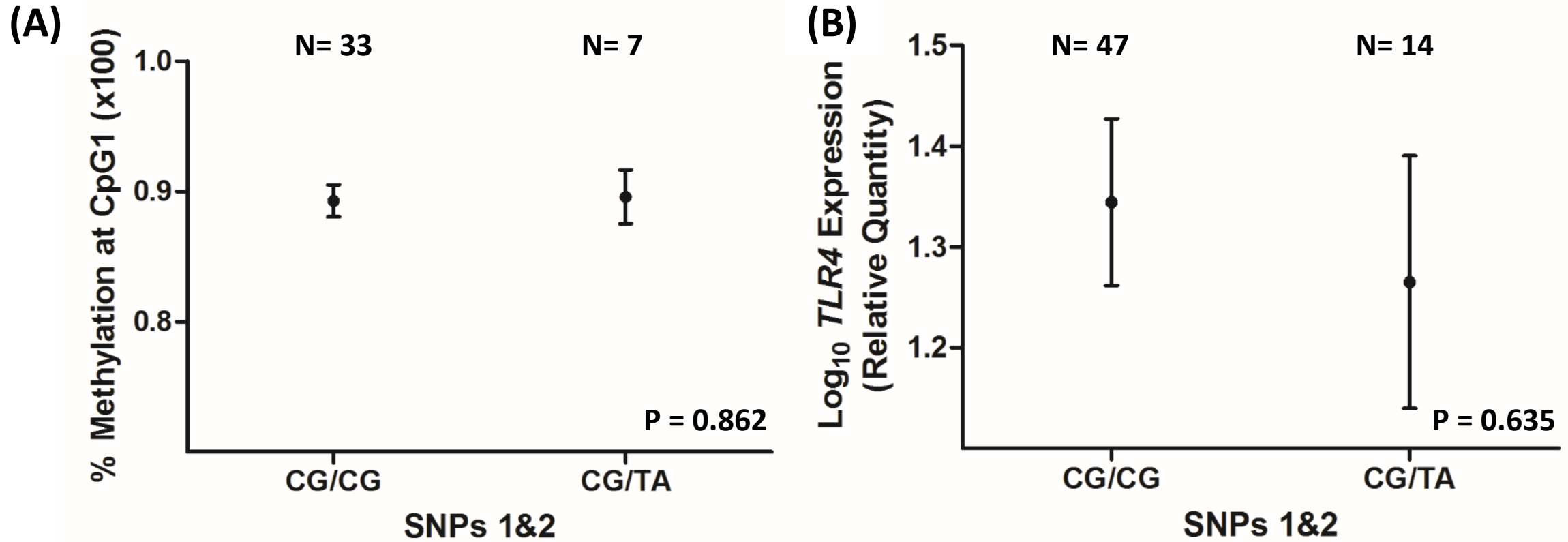
**Supplementary material**

**Supplementary Table A1: qPCR, pre-bisulfite and post-bisulfite sequencing primers (5' to 3') for house sparrows**

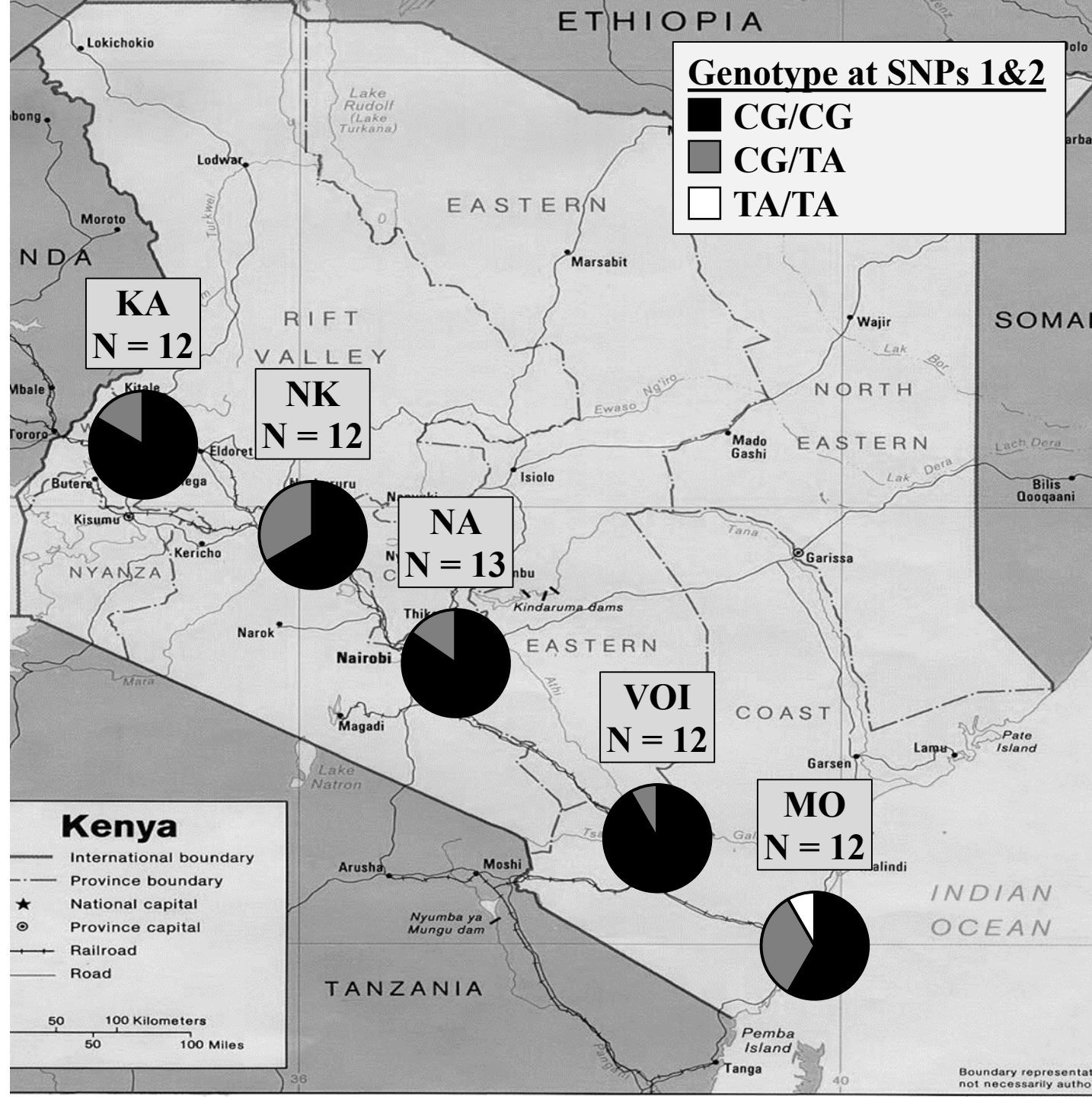
<b>Primer Type</b>	<b>Forward (5' to 3')</b>	<b>Reverse (5' to 3')</b>
qPCR primers	GCTCCTGTGTGTACCTGGAC	ACAACACAACCACTGGGGAG
Pre-bisulfite primers	GGGATTTTGTAGAACTTGCCAAATT	AAAGCTCCCTGCCTTCATTTAGTCTG
Post-bisulfite primers	GGGATTTTGTAGAAATTTGTTAAATTTAAT	AAAACCTCCCTACCTTCATTTAATCTAATAA
Bisulfite sequencing primer	GGGATTTTGTAGAAATTTGTTAAATT	

**Supplementary Table A2: PCR and sequencing primers (5' to 3') for characterizing putative *TLR4* promoter in zebra finches and house sparrows**

<b>Primers</b>	<b>Sequence 5' to 3'</b>	<b>T<sub>m</sub></b>
TLR4 PCR (For)	AATTCCTCCATCTTGTC	52.3 C
TLR4 PCR (Rev)	GTTGTGTTTCATCACTCCTG	51.4 C
TLR4 Seq 1 (For)	ATGTGGATTGCCTCATTCCA	54.4 C
TLR4 Seq 2 (For)	TCTTCCTCTAGACCAATATTCC	50.9 C
TLR4 Seq 3 (For)	AAACCTGGATGTTTGCTCAC	53.5 C
TLR4 Seq 4 (Rev)	CCCATAATTCAAATGCACTGGT	53.7 C
TLR4 Seq 5 (Rev)	GTCTTCGTGGAGATAACTAAGA	51.3 C
TLR4 Seq 6 (Rev)	GCTTCTGTGCCTGGATTAAGGAA	57.3 C



**Supplementary Figure A1:** The effects of genetic variation within the putative *TLR4* promoter (i.e. genotype at SNPs 1&2) on DNA methylation at CpG1 and *TLR4* expression. (A) DNA methylation at CpG1 did not significantly vary among genotypes at SNPs 1&2. (B) *TLR4* expression did not significantly differ among genotypes at SNPs 1&2. Bars are means +/- 1SE.



**Supplementary Figure A2:** Distribution of genotypes at SNPs 1&2 according to distance from Mombasa (dfM) indicates evidence of genetic admixture among populations. Populations are indicated by the following abbreviations and distances relative to Mombasa: MO (Mombasa—0km), VOI (Voi—160km), NA (Nairobi—500km), NK (Nakuru—650km), and KA (Kakamega—885km).

**Supplementary Table A3. Spearman rank correlations of % methylation among CpG sites 1-3**

	<b>CpG1</b>	<b>CpG2</b>	<b>CpG3</b>
<b>CpG1</b>	1.00	0.37	0.17
<b>Cpg2</b>	0.37*	1.00	0.55**
<b>Cpg3</b>	0.17	0.55**	1.00

\*p < 0.05

\*\* p < 0.01