

Supplementary Information File

Article title:

EGFR gene methylation is not involved in Royalactin controlled phenotypic polymorphism in honey bees

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Table S1

Primer sequences used to generate EGFR bisulfite amplicons used in this study. Cloning adaptors routinely added to the nested PCR primers are underlined.

Primary PCR	EGFR A1 F1	5' -GAGGAAGAATATTGTTTAAAGTTTA
	EGFR A1 R1	5' -AAACACCTACCCTACCAACACTAA
Nested PCR	EGFR A1 F2	5' - <u>GCAGAATTCGATGTGTTAGATGTAGAATTTGGA</u>
	EGFR A1 R2	5' - <u>CGCAAGCTTAATACTTCTAACAATTCTCAAACC</u>
Primary PCR	EGFR A2 F1	5' -TAATTGGGAGGAAATAATTATAGG
	EGFR A2 R1	5' -AACCAATTTCAATTATTTCAATTAT
Nested PCR	EGFR A2 F2	5' - <u>GCAGAATTCGGTTTGAGAATTGTTAGAAGTATT</u>
	EGFR A2 R2	5' - <u>CGCAAGCTTTATATACATTTCCAATACATTTTC</u>

Figure S1

Corrected EGFR gene model showing the region used for methylation analysis in this study. Blue: annotation of *Apis mellifera* LG6 (accession NC_007075.3), nucleotides 3,042,121 to 3,043,054. Exon numbering reflects the presence of additional exons identified by analysing RNAseq data. The 5' region of exon 5 and the adjacent intron sequence, aligning with a 117 bp stretch of Ns in the genome assembly 4.5 (LG6, NC_007075.3, nucleotides 3,042,143 to 3,042,259), were reconstructed from RNAseq and homology data following a failure to identify the original forward primer using the coordinates in the Kamakura paper (Supplementary Information, page 18, paragraph "DNA extraction and bisulfite treatment"). The placement of Kamakura's CpG map based on Figure S34 (yellow) is a guesstimate as no coordinates were supplied with that figure. We aligned the beginning of Exon 1 with the ATG corresponding to the first methionine of AmEGFR protein in Figure S21. This protein, as well as the NCBI EGFR RefSeq model (XP_006560089.1), is truncated by circa 170 amino acids compared with the *Drosophila* EGFR orthologue (FlyBase EGFR-PA) as shown in the figure. The Kamakura first region bisulfite fragment (orange) is aligned using bisulfite primers coordinates after correction. EGFR amplicons A1 and A2 are marked in green.

