Exon 3 splicing and mutagenesis identify residues influencing cell surface density of heterologously-expressed silkworm (*Bombyx mori*) glutamate-gated chloride channels

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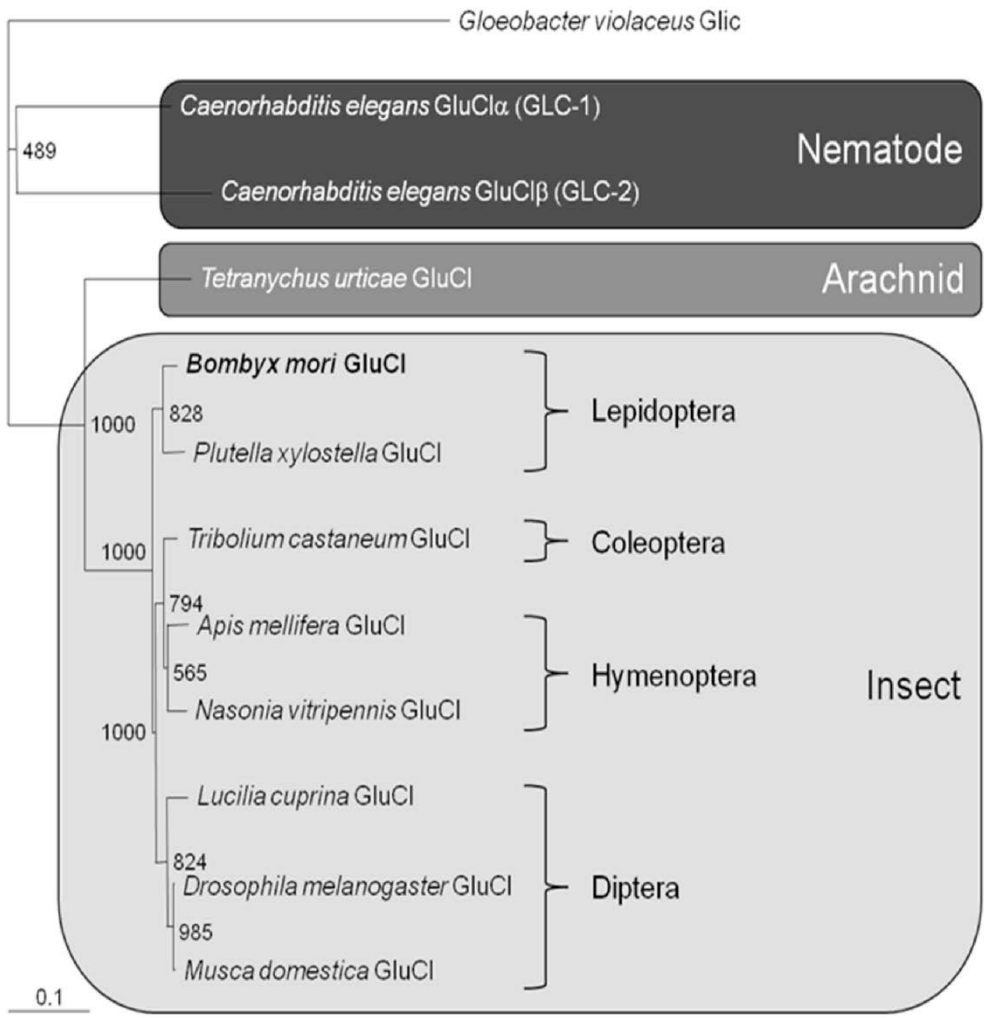

Supplemental Figure 1. Tree showing relationship of *Bombyx mori* GluCl (highlighted in bold) with other GluCls. *Phylogenetic analysis of BmGluCl*–The neighbor-joining method and bootstrap resampling, available with the ClustalX program, were used to construct a phylogenetic tree, which was then displayed using the TreeView application. Identity values between full length subunit peptide sequences were calculated using the GeneDoc program (http://www.psc.edu/biomed/genedoc). Numbers at each branch signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The tree is rooted by the bacterial protein Glic, an ancestral ligand-gated ion channel. The accession numbers of protein sequences used to construct the tree are: *Apis mellifera* GluCl (GB11639), *Bombyx mori* GluCl (Exon 3a variant, KC342243), *Caenorhabditis elegans* GluClα (NP_507090) and GluClβ (NP_491470, *Drosophila melanogaster* GluCl (AAG40735), *Gloeobacter violaceus* Glic (2XQ8.A), *Lucilia cuprina* (AAC31949), *Musca domestica* GluCl (BAD16657), *Nasonia vitripennis* GluCl (NP_001171232), *Plutella xylostella* GluCl (ACT09139), *Tetranychus urticae* GluCl (BAJ41378) and *Tribolium castaneum* GluCl (ABU63599.1).

Supplemental Table 1. Exon specific primers for real time PCR analyses

Target sequence	Forward (5’ to 3’)	Reverse (5’ to 3’)
Exon 3a	CGGCAAGATAAACTTCAGG	CATTTTAATATCACTAATTGTCG
Exon 3b	CGGCAAGATAAACTTCAGG	CATTGTGACGTCATCGATC
Exon 3c	CGGCAAGATAAACTTCAGG	CATTTTGTAATCATCTATTTTGC
Exon 3Δ	CAACGGAACTGATGAATACTC	CCTTCCTAAATTGTTAAACTTCAG
Exon 9	CAAAAGCCATCGACGTCTG	CTTAGTGTCCACGCCACCGC
Exon 9 partially Δ	CAAAAGCCATCGACGTCTG	CTGCCGCATCATAGCGAAG

Supplemental Table 2. Primers used to chimeras of exon 3a and 3c variants

Target sequence	Forward (5’ to 3’)	Reverse (5’ to 3’)
Block I Exon 3a to Exon 3c	GGCGATGCGCCAACGTTAGTTCGCGTCAACAT	CAGATACATGTTGACGCGAACTAACGTTGGC
	GTATCTGAGAAGCATAACGACAATTAG	GCATCGCCAGTTCCGTTGATGCCTGACG
Block I Exon 3c to Exon 3a	GATGGCCCAGCCGTCGTTAATATAAACCTGTT	CAAACAGGTTTATATTAACGACGGCTGGGCC
	TGTGCGATCCATCAGCAAAATAGATG	ATCAGTTCCGTTGATGCCTGACGGTC
Block II Exon 3a to Exon 3c	CGTTAATATAAACCTGTTTGTGAGAAGCATAA	CTCTGAACGTGAGTTGCACAGAGTATTCCAT
	GCAAAATAGATGATTACAAAATG	TTTGTAATCATCTATTTTGCTG
Block II Exon 3c to Exon 3a	GTTCGCGTCAACATGTATCTGCGATCCATCAC	CTGAACGTGAGTTGCACAGAGTATTCCATTT
	GACAATTAGTGATATTAAAATG	TAATATCACTAATTGTCG

Supplemental Table 3. Primers used to mutate cDNAs in Block II of exon 3

Exon 3a		Exon 3c	
Name	Sequence (5’ to 3’)	Name	Sequence (5’ to 3’)
A-1 (Wild type)	AATTGTCGTTATGCTTCTCACAAACAGGTT	C-1 (Wild type)	TATTTTGCTGATGGATCGCAGATACATGTT
A-2 (T77S)	AATTGTGCTTATGCTTCTCACAAACAGGTT	C-2 (S78T)	TATTTTCGTGATGGATCGCAGATACATGTT
A-3 (T78K)	AATTTTCGTTATGCTTCTCACAAACAGGTT	C-3 (K79T)	TATTGTGCTGATGGATCGCAGATACATGTT
A-4 (T77S-T78K)	AATTTTCGTTATGCTTCTCACAAACAGGTT	C-A (Wild type)	GATGATTACAAAATGGAATACTCTGTGCAA
A-A (Wild type)	AGTGATATTAAAATGGAATACTCTGTGCAA	C-B (D81S)	AGTGATTACAAAATGGAATACTCTGTGCAA
A-B (S80D)	GATGATATTAAAATGGAATACTCTGTGCAA	C-C (Y83I)	GATGATATTAAAATGGAATACTCTGTGCAA
A-C (I82Y)	AGTGATTACAAAATGGAATACTCTGTGCAA		
A-D (S80D-I82Y)	GATGATTACAAAATGGAATACTCTGTGCAA		

A-2 (T77S) and A-D (S80D-I82Y) primers were used to prepare the T77S;S80D;I82Y mutant of exon 3a variant, while C-2 (S78T) and C-A (Wild type) primers were used to prepare the S78T mutant of exon 3c variant. In a similar way, the other mutants were prepared with appropriate primers shown above.