Supporting Information

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Fig. S1. Phylogenetic and gene tree of *csd* and *fem* of corbiculate bees. (A) Phylogeny of the four major groups of corbiculate bees (redrawn after ref. 1). (B) Genealogy of *csd* and *fem* protein sequences using the minimum evolution method. Numbers denote bootstrap values > 80%.



Fig. 52. Putative region of strong purifying selection in Fem of *Apis*. Shown are Fem proteins of *Apis* (represented here by *A. mellifera*) and non-*Apis* species (*M. compressipes* and *B. terrestris*) with domains (RS and P) and the N-term region. Using a sliding window (window length, 30 bp; step size, 10 bp), d_n/d_s ratios were calculated for each window between *A. mellifera/M. compressipes*, *A. mellifera/B. terrestris*, and *B. terrestris/M. compressipes*. One region comprising 18 amino acids was detected that showed the lowest d_n/d_s between *Apis* and non-*Apis* but the highest d_n/d_s for the non-*Apis* comparison, indicative of strong purifying selection in *Apis*.

1. Grimaldi D, Engel MS (2005) Evolution of the Insects (Cambridge Univ Press, Cambridge, UK).

Table S1. Test for heterogeneity of n/s ratio across *fem* within evolutionary time windows in *Apis* and non-*Apis* species

	N term n/s	RS n/s	P n/s	N-term vs. RS	N-term vs. P	RS vs. P
A fem	1/2	0/6	0/2	<i>P</i> > 0.3	<i>P</i> > 0.6	not calculable
fem Apis	4/14	4/8	0/7	<i>P</i> > 0.6	<i>P</i> > 0.2	<i>P</i> > 0.2
MB fem	13/6	6/4	1/1	<i>P</i> > 0.6	<i>P</i> > 0.6	<i>P</i> > 0.6
fem MB	18/15	10/13	3/7	<i>P</i> > 0.5	<i>P</i> > 0.2	<i>P</i> > 0.7

Evolutionary time windows reflect fem evolution before Apis species divergence (A fem), within Apis species (fem Apis), and before (MB fem) and after (fem MB) non-Apis species divergence. Testing for heterogeneity among ratios across the fem N-term region and RS- and P-rich domains was performed using Fisher's exact test.

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