**Construction of the GFP- and RFP-tagged *Tribolium* Protamine Genes and Germline Transformation**

The sequence of the *Drosophila melanogaster* ProtamineA protein (also known as Mst35Ba-PA) was used to query the translated *T. casteneum* genome sequence using the tblastn search function of the NCBI BLAST resource. A gene named LOC663849 (referred to here as *TcProtamine-1*) was identified with significant match (E value of 4e-08). Genomic DNA was isolated from beetles of the GA-2 wild-type strain, and PCR performed to amplify a 2.0 kb fragment containing this gene, using primers: Tc2 = AGCACATCAAAATCTATAAGATAGAATCGG and Tc4 = CAGTTAGCTTCGGTCCGAAATGATGTAAAC. The product was cloned into pCR2.1 using the TOPO TA cloning kit (Invitrogen) and sequenced. The 2.0 kb fragment was then excised using *Eco*RI and ligated into pBS/2xAsc, a modified pBlueScript-KS+ plasmid (Stratagene) in which two *Asc*I sites, flanking the Multiple Cloning Site, had been created by site directed mutagenesis. A unique *Nde*I site was created at the C-terminus of the Tc Protamine-1 coding region by site directed mutagenesis using mutagenesis primers Tc2-4NdeMUTS = AGGAGCGGCTCCCGAAGCATATGCTACAGCTATTAAATTG and Tc2-4NdeMUTAS = CAATTTAATAGCTGTAGCATATGCTTCGGGAGCCGCTCCT. GFP and RFP tagged fusion genes were made by inserting the eGFP or mCherry coding sequences, in frame with the TcProtamine-1 coding sequence, as *Nde*I cassettes. These cassettes were created by PCR amplification of plasmids pEGFP and pmCherry (Invitrogen).

The Tc Protamine-1-GFP or Tc Protamine-1-mCherry constructs were subcloned into the *Asc*I site of the transformation vector pBac3xP3-EGFPaf (Horn and Wimmer 2000; provided by E. A. Wimmer; Georg August University, Göttingen, Germany) to give pBac{3xP3-EGFP, Tc-Protamine-1-GFP} or pBac{3xP3-EGFP, Tc-Protamine-1-mCherry}, respectively. *Tribolium* germline transformation was carried out essentially as described in Berghammer et al.(1999), using the white-eyed *pearl* mutant strain as host (provided by Dr. Richard Beeman; USDA, Manhattan, KS). The helper plasmid was phspBac (Handler and Harrell 1999; provided by Dr. Alfred Handler; USDA, Gainesville, FL). Four independent Protamine-1-GFP, and one Protamine-1-RFP, transformed lines were obtained. The RFP transformation is homozygous lethal, likely due to the construct's insertion location, so heterozygous individuals bearing the dominant eye-specific GFP marker were selected as pupae prior to each experiment. It is important to note that all sperm from RFP males are marked, but not all progeny inherit the transgene.