

## File S1

### Supporting Methods

#### (a) Detailed operation of physiology test and behavior assays

In the dropping experiment, 20 *Dazao-stony* and *Dazao* individuals were subjected to a height of 2 m, followed by motion of a free falling body. The numbers of ruptured *Dazao* and *Dazao-stony* individuals were added up to compare their abilities to buffer the shock. To compare the flexibility and nesting ability of internode and intersegmental fold between *Dazao* and *Dazao-stony*, we injected 4 mL air into larvae, and stretch along the anterior-posterior axis of the body. To determine abdomen crooking ability, larvae were inverted to turn the dorsum down and the thorax pressed lightly to prevent writhing to the two sides. Subsequently, inversion towards the abdomen was observed. The number of individuals able to writhe to abdomen, time needed, and the angle between the dorsum and horizontal plane (performed using the digital camera, image J, and angle test software) were recorded. To determine grasping state and duration, larvae were placed on the middle of the mallet (diameter: ~0.9 and ~1.4 cm) in the larval grasping experiment. In the inversion assay analyzing dorsum crooking ability, we fixed the back of larvae on the plane carefully and unclamped them quickly to record the rolling state and time required. We additionally performed a food-inducing crawling experiment. Specifically, 5<sup>th</sup> instar day 5 larvae subjected to 10 h starvation were selected. Larvae were placed ~4 cm away from fresh mulberry leaves (DETHIER 1941; SLIFER 1955), and their crawling behavior and time to reach leaves recorded. In the phenotype analysis, physiology test and behavior assays, healthy *Dazao* and *Dazao-stony* larvae on day 5 of 5<sup>th</sup> instar glutted with mulberry leaf and subsequently starved for 4 h were selected, except for those used in the crawling experiment.

#### **(b) Detailed operation of chitin binding**

The soluble proteins extracted from sediment were dialyzed to eliminate SDS using Tang's method (TANG *et al.* 2010). Ethanol in mixed chitin beads was discarded and chitin resuspended with binding buffer (20 mM HEPES, 15 mM NaCl, pH 7.4). Next, ~100 µg cuticular protein of *Dazao* was mixed with chitin (volume ratio of 1:2, total volume 1 ml) in a 1.5 mL EP tube. The solution was incubated at 16°C for 4 h and shaken at 40 rpm. Following centrifugation (9000g, 4°C), the supernatant (S-incubation) was collected and added to 1.2 mL washing buffer (20 mM HEPES, 1 M NaCl, pH 7.4). Next, the mixture was shaken for 40 s and centrifuged under set conditions (9000g, 4°C). The supernatant was removed and the wash step repeated once. The final supernatant fraction (W2) and remaining chitin sediment were collected. S-incubation, W2 and chitin sediment were mixed with Laemmli sample buffer and heated at 95°C for 10 min, and the supernatant collected and analyzed using 15% SDS-PAGE.

#### **(c) Detailed operation of LC-MS/MS**

The gel was suspended in 200-400 µL decolorizing agent (30% ACN/100 mM NH<sub>4</sub>HCO<sub>3</sub>) until transparent, the supernatant was removed and frozen to dryness. Next, 100 mM DTT was added and incubated for 30 min under 56°C. After removal of the supernatant, 200 mM IAA was added, and the sample incubated in the dark for 20 min. The supernatant was removed and the sample treated with 100 mM NH<sub>4</sub>HCO<sub>3</sub> at room temperature for 15 min. Following removal of the supernatant, ACN was added. After 5 min, the supernatant was blotted and frozen to dryness. Next, the dried sample was incubated overnight with 2.5-10 ng/µL Trypsin solution (37°C, about 20 h) and the enzymatic hydrolysate added to a new EP tube. We further added 100 µL extraction buffer (60%ACN/0.1%TFA) to the gel, followed by ultrasound for 15 min, combined with the enzymatic hydrolysate for freezing to dryness. The capillary performance liquid chromatography method was performed as follows: the chromatographic column was equilibrated with 95% solution A (0.1% formic acid), and the sample

loaded onto Trap column through an automatic sampler. Solution B (84% acetonitrile) flowthrough was as follows: 0-30 min with a linear gradient of 4 to 50%, 30-34 min with a linear gradient of 50 to 100%, and 34-40 min whereby the solution was maintained at 100%. The mass-charge ratios of peptide and peptide fragments were collected using the method for collecting MS2 scans after each full scan. Using BOWWORKS and SEQUEST programs, the raw file was searched in the related database (NCBI, <http://silkworm.swu.edu.cn/silkdb/doc/download.html>). Search steps were referenced to the procedure of Tang et al. (TANG *et al.* 2010).

#### **Literature cited**

- DETHIER, V. G., 1941 The Function of the Antennal Receptors in Lepidopterous Larvae. *Biological Bulletin* **80**: 403-414.
- SLIFER, E. H., 1955 The detection of odors and water vapor by grasshoppers (Orthoptera, Acrididae) and some new evidence concerning the sense organs which may be involved. *Journal of Experimental Zoology* **130**: 301-317.
- TANG, L., J. LIANG, Z. ZHAN, Z. XIANG and N. HE, 2010 Identification of the chitin-binding proteins from the larval proteins of silkworm, *Bombyx mori*. *Insect Biochem Mol Biol* **40**: 228-234.