Supplemental Methods

RNA sequencing

In this study, we employed the laboratory strain of P. puparum (Ppup-ZJU) for sampling purposes. We utilized several RNA sequencing technologies, namely PacBio HiFi long-read sequencing, strand-specific Illumina RNA-Seq, CAGE-Seq, and PAS-Seq. For full-length RNA sequencing using PacBio HiFi, we collected venom gland (100 tissues) and carcass samples of P. puparum. To extract the total RNA from all the samples, we employed the TRIzol-based method (Invitrogen). The integrity of the RNA was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies) and agarose gel electrophoresis. Subsequently, we evaluated the mRNAs using the Nanodrop (Thermo Fisher Scientific) and Qubit (Thermo Fisher Scientific), and only high-quality preparations (OD260/280 = 1.8-2.2, OD260/230 ≥ 2.0, RIN ≥ 8) were utilized for constructing the sequencing library. We reverse transcribed approximately 300 ng RNA into cDNA and amplified it using the NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module and Iso-Seq Express Oligo Kit. The cDNAs were purified using ProNex Beads and then used to construct the library using the SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). This involved repairing any damage, performing end repair, A-tailing, and ligating the sequencing adapters. The SMRTbell template was annealed to the sequencing primer, bound to the polymerase and subsequently sequenced on the PacBio Sequel II platform using the Sequel II Binding Kit 2.0 (Pacific Biosciences).

For Illumina RNA sequencing, we collected a total of six samples from venom gland (100 tissues per sample) and carcass of P. puparum. Each sample was prepared as three biological replicates. The total RNA from all samples was extracted using the TRIzol-based method (Invitrogen) and sequenced individually on the Illumina HiSeq X Ten platform. The sequencing libraries were constructed as strand-specific, paired-end libraries with an insert size of 300 bp.

For CAGE-Seq, we extracted total RNA from the venom gland (100 tissues) and carcass of P. puparum using the TRIzol-based method (Invitrogen). For each sample, 1 μg of total RNA was treated with T4 polynucleotide kinase (NEB) at 37℃ for 30 minutes and purified using Gnome size selector beads (Gnome). Subsequently, the RNA was digested

with Terminator 5´-Phosphate-Dependent Exonuclease (Ambion) at 30℃ for 30 minutes and purified again using Gnome size selector beads (Gnome). The capped mRNA was subjected to reverse transcription using an RT primer, followed by synthesis of DNA using a Terminal-Tagging oligo. Next, the cDNAs were treated with Exonuclease I (Epicentere), purified, and amplified using PCR primers (Illumina). The PCR products within the 200-500 bp range were purified and quantified. Paired-end sequencing of the libraries was performed on a NextSeq 500 system with a read length of 151 nt.

For PAS-Seq, we extracted total RNA from the venom gland (100 tissues) and carcass of P. puparum using the TRIzol-based method (Invitrogen). In each sample, we captured mRNAs using mRNA Capture Beads kit (Vazyme, N401-01). The captured mRNAs were then fragmented and utilized for directional RNA-Seq library preparation employing the KAPA Stranded mRNA-Seq Kit for Illumina Platforms (KK8544). The fragmented mRNAs were converted into double-stranded cDNA. Following end repair and A-tailing, the DNAs were ligated to a Diluted Roche Adaptor (KK8726). After the ligation product was purified and size fractioned to a range of 300-500 bp, the ligated products were amplified, purified, and quantified. The libraries were subjected to paired-end sequencing with a read length of 151 nt, which was performed on a NovaSeq 6000 system.

Venom proteome

The venom proteome analysis was conducted following the previously described protocol (Ye et al. 2022). Approximately 100 venom reservoirs, which are the organs for venom storage, were isolated from *P. puparum*. The reservoirs were pierced and washed for three times in sterile PBS. After centrifugation at 12,000 g for 10 minutes, the supernatant was collected and digested into peptides using trypsin. The digested peptides were desalted using C18 Cartridges (Sigma-Aldrich) and reconstituted in 40 µL of 0.1% (v/v) formic acid. LC-MS/MS analysis was performed on a timsTOF Pro mass spectrometer (Bruker) coupled to Nanoelute (Bruker Daltonics). The peptides were loaded onto a homemade C18-reversed phase analytical column (25 cm long, 75 µm inner diameter, 1.9 µm, C18) in buffer A (0.1% Formic acid) and separated using a linear gradient of buffer B (99.9% acetonitrile and 0.1% Formic acid) at a flow rate of 300 nL/min. The mass spectrometer

operated in positive ion mode, collecting ion mobility MS spectra within a mass range of m/z 100-1700 and 1/k0 of 0.75-1.35. It then performed 10 cycles of PASEF MS/MS with a target intensity of 1.5k and a threshold of 2500. Active exclusion was enabled with a release time of 0.4 minutes. The raw data were processed using MaxQuant v2.0.3.1 (Tyanova et al. 2016) and the annotated protein sequences of *P. puparum* were used as the search database.

References

Tyanova S, Temu T, Cox J. 2016. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nat Protoc* **11**: 2301-2319. doi: 10.1038/nprot.2016.136

Ye X, Yang Y, Zhao C, Xiao S, Sun YH, He C, Xiong S, Zhao X, Zhang B, Lin H, et al. 2022. Genomic signatures associated with maintenance of genome stability and venom turnover in two parasitoid wasps. *Nat Commun* **13**: 6417. doi: 10.1038/s41467-022-34202-y