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Chromosome-level genome of the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae) provides a resource for evolutionary studies on moths

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28 **Short title:** Chromosome-level genome of *Carposina sasakii*

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Abstract

The peach fruit moth (PFM), *Carposina sasakii* Matsumura, is a major phytophagous orchard pest widely distributed across Northeast Asia. Here, we report the chromosome-level genome for the PFM, representing the first genome for the family Carposinidae, from the lepidopteran superfamily Copromorphaidea. The genome was assembled into 404.83 Mb sequences using PacBio long-read and Illumina short-read sequences, including 275 contigs, with a contig N50 length of 2.62 Mb. All contigs were assembled into 31 linkage groups assisted by the Hi-C technique, including 30 autosomes and a Z chromosome. BUSCO analysis showed that 98.3% of genes were complete and 0.4% of genes were fragmented, while 1.3% of genes were missing in the assembled genome. In total, 21,697 protein-coding genes were predicted, of which 84.80% were functionally annotated. Because of the importance of diapause triggered by photoperiod in PFM, five circadian genes in the PFM as well as in the other related species were annotated, and potential genes related to diapause and photoperiodic reaction were also identified from transcriptome sequencing. In addition, manual annotation of detoxification gene families was undertaken and showed a higher number of glutathione S-transferase (GST) gene in PFM than in most other lepidopterans, in contrast to a lower number of uridine diphosphate (UDP)-glycosyltransferase (UGT) gene, carboxyl/cholinesterases (CCE) gene and cytochrome P450 monooxygenase (P450) gene, suggesting different detoxication pathways in this moth. The high-quality genome provides a resource for comparative evolutionary studies of this moth and its relatives within the context of radiations across Lepidoptera.

Keywords: Insect; *de novo* assembly; annotation; detoxification; diapause

Introduction

The peach fruit moth (PFM), *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae, superfamily Copromorphaidea), is a major phytophagous orchard pest of fruit such as apple, pear, peach, apricot and jujube from the families of Rosaceae and Rhamnaceae (**Fig. 1**). The hatched larvae directly bore into fruit to feed, causing losses in fruit production. PFM is one

of the most severe borers on deciduous fruit in northeast Asia. It is also considered a potential risk to fruit production in most parts of the world, although PFM is currently restricted to northeast Asia and far east Russia (Kwon et al., 2018; Wang et al., 2017).

One possible reason for the currently restricted distribution of PFM is its sensitivity to environmental factors. PFM has evolved diapause to cope with cold winter conditions and to synchronize its phenology with host plants (Toshima et al., 1961). Both long-day and short-day photoperiods induce diapause in the last instar of PFM larvae, resulting in a diapausing cocoon (Hua et al., 1998; Huang et al., 1976). The life cycle of PFM can be univoltine or bivoltine, depending on photoperiods encountered and environmental factors like humidity (Chiba & Kobayashi, 1985; Kim et al., 2000; Sato & Ishitani, 1976). Temperature also affects the occurrence of PFM through effects on the development rate and the emergence of the overwintering generation from diapause (Kim et al., 2001; Zhang et al., 2016).

The effects of environmental factors as well as photoperiod on the life history of PFM provides an opportunity to investigate the genomic basis of adaptation to temperate environments in the Copromorphoidea superfamily and across Lepidoptera more generally. Candidate genes involved in climatic adaptation could then also be investigated at the geographic level, given that a combination of mtDNA and microsatellite variation indicates strong genetic differentiation among geographical populations of the PFM across its native range in China (Wang et al., 2017). Although adaptation to climate changes has been found in lepidopteran species (Fält-Nardman et al., 2016; Quezada García et al., 2015; van Asch et al., 2013; Yamanaka et al., 2008), genes underlying climate adaptation remain to be identified (Hoffmann, 2017). Analysis of genomic variation among populations may help to identify putative loci and processes under selection (Hoffmann & Sgro, 2011) and predict the evolutionary adaptation under climate changes (Hoffmann & Sgro, 2011).

The highly variable life history of PFM on different host plants may reflect different host-associated biotypes as supported by an analysis of esterase isozyme patterns (Hua & Hua, 1995) and random amplified polymorphic DNA (RAPD) (Xu & Hua, 2004), although this

is not yet been confirmed by direct studies on population differentiation in PFM (Kwon et al., 2017; Wang et al., 2015). Previous studies have shown that detoxification and chemosensory genes are associated with host adaptation of insects (Heckel, 2018; Rane et al., 2019; Wan et al., 2019). Understanding the genomic features of PFM may provide useful points to investigate host plant adaptation and reveal the genetic basis related to the potential invasiveness of PFM as well as other fruit boring pests (Kirk et al., 2013).

Except for genetic variations, the insect can adapt to the varied environment by plasticity, which often involves the expression change of related genes (Sgro et al., 2016). Transcriptome analysis provides an efficient way to identify the differentially expressed genes. Although the transcripts can be assembled *de novo*, a reference genome-guided analysis may help to make the analysis more precise.

Well-assembled genomes are increasingly becoming available as resources for tracing evolutionary adaptation across the Lepidoptera. Already there are substantial genomic resources for many moths (Chen et al., 2019; Cheng et al., 2017; Kanost et al., 2016; Lange et al., 2018; Ma et al., 2020; Pearce et al., 2017; Wan et al., 2019; Xia et al., 2004; Xiang et al., 2018; Xiao et al., 2020; You et al., 2013; Zhang et al., 2020) and butterflies (Ahola et al., 2014; Cong et al., 2015; Dasmahapatra et al., 2012; Lu et al., 2019; Nishikawa et al., 2015; Zhan et al., 2011) which are being used in comparative analyses to link genomic changes to phenotypes like the detoxification of compounds encountered in hosts (Rane et al., 2019). Available genomes provide abundant reference points for investigating evolution across the Lepidoptera, although most species sequences so far are from the Papilionoidea, Noctuoidea, Bombycoidea and Pyraloidea, with less genomic information available for the Carposinidae (Copromorphoidea) despite the importance of this group as agricultural pests.

In the present study, we report on a chromosome-level genome of PFM, which was *de novo* assembled based on sequences obtained from the PacBio and Illumina platforms and assembled at the chromosome level with the Hi-C technique. We compare features of the PFM genome with those of eleven other moths, focusing particularly on detoxification and chemosensory gene families important in host adaptation and pesticide resistance,

contributing to ecological niches occupied by species (Rane et al 2019). As an initial study using the newly assembled genome, we investigate transcriptomic changes induced by long-day and short-day photoperiods that induce diapause in PFM larvae, and we identify genes involved in these responses which are critical to climatic adaptation by PFM.

Materials and methods

Sample collection and rearing

We established a laboratory strain of PFM from 30 larvae collected from an apple orchard in the Beijing area of China in July 2018. This strain was maintained for five generations on apple (*Malus pumila* Mill) in the laboratory under 25 ± 1 °C, a relative humidity of $75 \pm 5\%$, and a photoperiod of 15L : 9D (ND, normal-day condition). Eggs were laid on filter paper and moved to ripe apples before hatching. Larvae developed in the apples and the last (fifth) instar larvae left the fruit to pupate on prepared sawdust. Samples used in genome sequencing and RNA-seq were from this strain.

In order to induce diapause in larvae, we moved batches of newly hatched larvae to long-day and short-day photoperiodic conditions before they bored into apple for feeding. The long-day condition (LD) was set to a photoperiod of 22 L : 2 D, while the short-day condition (SD) was set to a photoperiod of 8 L : 16 D (Hua et al., 1998; Huang et al., 1976). Both treatments were conducted under 25 ± 1 °C, and a relative humidity of $75 \pm 5\%$. The last instar larvae leaving the fruit were collected and stored in RNAlater at -80 °C (Sigma-Aldrich, St. Louis, USA) for subsequent RNA-seq library construction.

Genome sequencing

We extracted genomic DNA from 12 male pupae using MagAttract HMW DNA kit (Qiagen, Hilden, Germany) for Illumina library and PacBio library. The paired-end Illumina library with insert sizes of about 500 bp, was constructed using VAHTS™ Universal DNA Library Prep Kit for Illumina® V2 (Vazyme, Nanning, China) and sequenced on an Illumina Novaseq platform to obtain 150-bp paired-end reads. The raw reads generated were filtered by the software Trimmomatic v0.38 (Bolger et al., 2014). After filtering, we obtained 31.02 Gb of short clean

reads (coverage: 77.24X). The sequencing data was used to estimate the genome size, heterozygosity and rate of duplication and polish *de novo* assemblies.

For long-read sequencing, SMRTbell libraries were constructed with Sequel® Sequencing Kit 3.0 (Pacific Biosciences, Menlo Park, CA, USA). Long DNA fragments of approximately 20 kb were sequenced on a PacBio Sequel sequencer (Pacific Biosciences, Menlo Park, CA, USA). Four SMRT cells were processed and 55.52 Gb subreads (mean subread length: 18.13 kb, subread N50 length: 32.84 kb, coverage: 138.2X) were obtained for contig-level genome assembly.

To assist the chromosome-level assembly, we used the Hi-C (High-throughput chromosome conformation capture) technique to capture genome-wide chromatin interactions (Belaghzal et al., 2017). Twenty 5th instar larvae were ground in 2% formaldehyde for cross-linking of cellular protein. Chromatin was digested with restriction enzyme *MboI* overnight. Then, the DNA ends were flatted, marked with biotin-14-dCTP and ligated with bridge linker. The samples were digested with proteinase K and purified by phenol-chloroform extraction. Biotins on unligated DNA fragments ends were removed with T4 DNA polymerase. Fragments were sheared into 200-600 base pairs using an S220 Focused-ultrasonicator (Covaris, U.S.). Biotin marked DNA fragments were enriched using streptavidin C1 magnetic beads. Illumina library was constructed from the enriched fragments using VAHTS™ Universal DNA Library Prep Kit for Illumina® V2 (Vazyme, Nanning, China) and sequenced on an Illumina Novaseq platform to obtain 150-bp paired-end reads. After removing the low-quality reads, 1,509 million clean reads were retained (coverage: 559.3X).

Genome survey

We used the k-mer method to survey the genome features of the PFM. The k-mer count histogram was obtained from Illumina paired-end sequencing data using Jellyfish v2.99 (Marçais & Kingsford, 2011) with 17, 21, 25 and 35 mers. Genome size, heterozygosity and rate of duplication were estimated by GenomeScope v1.0 (Vurture et al., 2017).

Genome assembly and evaluation

Long reads generated from PacBio sequencing were corrected and assembled using CANU version 1.8 (Koren et al., 2017) with default parameters. The initial assembly was polished using Pilon v1.22 (Walker et al., 2014) with short reads from Illumina paired-end sequencing for three times. Two haplotypes in part of the genome might be assembled as separate primary contigs due to the high degree of heterozygosity (Roach et al., 2018). To correct these possible allelic contigs, we reassigned the polished assembly using the pipeline Purge Haplotigs to identify pairs of contigs that are syntenic and removed one of them (Roach et al., 2018), resulting in a contig-level genome.

Clean reads sequenced from the Hi-C library were aligned to the contig-level genome with an end-to-end algorithm implemented in Bowtie v2.3.5 (Langmead & Salzberg, 2012) according to the HiC-Pro strategy (Langmead & Salzberg, 2012; Servant et al., 2015). The Juicer v1.5 and 3D *de novo* assembly (3D-DNA) pipelines were used to assemble the contigs into a chromosome-level genome (Dudchenko et al., 2017; Durand et al., 2016). The completeness of the genome was evaluated through the analysis of single-copy orthologs (Simao et al., 2015), implemented in Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0.2 (Simao et al., 2015), based on the insecta_odb9 database (1,658 genes). Synteny between PFM and *Cydia pomonella* (Lepidoptera: Tortricidae) (Assembly accession: GCA_003425675.2) (Wan et al., 2019) and *Spodoptera litura* (Assembly accession: GCF_002706865.1) (Cheng et al., 2017) genomes were analyzed using TBtools v0.58 (Chen et al., 2020).

Transcriptome sequencing and assembly

To provide evidence of transcripts for genome structure annotation, we conducted RNA-seq for four developmental stages of egg, larva, pupae, and adults (male and female) reared under normal conditions as described above. To identify the differentially expressed genes between normal (ND) and diapausing larvae, we constructed another two RNA-seq libraries for the long-day (LD) and short-day (SD) induced 5th instar larvae. In total, 7 RNA-seq libraries were constructed, including one for eggs, three for larvae, one for pupae, one for male adults and one for female adults of PFM. One individual collected from each

stage/treatment was used (except for eggs, for which about 100 eggs were used) for RNA-seq and one library was constructed for each treatment. All libraries were prepared using VAHTSTM mRNA-seq V2 Library Prep Kit for Illumina according to the manufacturer's instructions (Vazyme, NanJing, China) and sequenced on an Illumina Novaseq platform to obtain 150-bp paired-end reads. After removing the low quality reads with Trimmomatic v0.38 (Bolger et al., 2014), the reads were mapped to the chromosome-level genome using Hisat v2.2.0 (Kim et al., 2019) and assembled with StringTie v2.1.2 (Pertea et al., 2015). FPKM (Fragments Per Kilobase per Million) values of each annotated gene in each RNA-seq were estimated with cufflinks v2.2.1 (Kim et al., 2013).

We analyzed the differentially expressed gene (DEG) among the 5th instar larvae reared at different photoperiods (SD, ND and LD). The ND was used as a control, while the SD and LD were compared to the ND. Due to the lack of biological replicate, we used cufflinks v2.2.1 (Kim et al., 2013) in the assessment of DEGs, which allowed one repeat in the analysis. Because we analyzed the DEGs for the 5th instar larvae, which is the last developmental stage before diapause, we assumed that the fold change of DEGs related to diapause should be large. Thus, we used a fold-change ≥ 2 and q-value ≤ 0.05 as the cutoffs of significant DEGs between samples. For significantly expressed genes, up-regulated or down-regulated genes in both comparisons (ND vs. LD and ND vs. SD) were considered as genes related to diapause, while the FPKM values specifically high in the SD or LD condition were considered as light-induced genes. Gene expression visualization of DEGs was conducted with the *Pheatmap* R package.

Repeat element and non-coding RNA annotation

Repeats and transposable element families in the PFM genome were first detected by RepeatMasker v4.0.7 pipeline (Tarailo-Graovac & Chen, 2009) against the Insecta repeats within RepBase Update (<http://www.girinst.org>) and Dfam database (20170127), with RMBlast v2.10.0 as a search engine. The noncoding RNAs (ncRNA) were annotated by aligning the genomic sequence against RFAM v14.2 (<http://rfam.xfam.org/>) with BLASTN.

The tRNAs and rRNAs were predicted by tRNAscan-SE and RNAmmer (Lagesen et al., 2007; Lowe & Eddy, 1997).

Protein-coding gene annotation and filtering

We annotated protein-coding genes using *ab initio*, RNA-seq-based, and homolog-based methods in the MAKER v2.31.10 genome annotation pipeline (Cantarel et al., 2008). Augustus v3.2.3 (Stanke & Waack, 2003) and SNAP v2013-02-16 (Korf, 2004) were used for the *ab initio* gene prediction. For Augustus, we used the retrained parameters obtained in the above BUSCO analysis of genome assembly by invoking the Augustus retraining option. In the first round of annotation, we ran MAKER by providing transcriptome assemblies of PFM, protein sequences from eight lepidopteran species (*Bombyx mori*, *Trichoplusia ni*, *Ostrinia furnacalis*, *Bombyx mandarina*, *Galleria mellonella*, *Spodoptera litura*, *Helicoverpa armigera*, *Plutella xylostella*) and the Augustus model as evidence. The GFF3 file of first round annotation was used to train parameters of SNAP. In the next three rounds of annotation, GFF3 from the last round, Augustus and SNAP models were used as evidence.

The annotation results from the MAKER pipeline were filtered by using gene expression evidence, functional annotation results and Annotation Edit Distance (AED) value. Genes that had a FPKM value great than 0 in any RNA-seq were considered as real genes and retained in further analysis. Functional domains for proteins were identified using InterproScan 5.34-74.0 (Jones et al., 2014) against Pfam database v32.0 (El-Gebali et al., 2019). The gene models were filtered based on domain content and evidence support following Campbell et al. (2014). Finally, the annotations with AED < 0.75 were removed (Campbell et al., 2014).

Functions of the protein-coding genes and their GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) items were annotated using the software eggNOG-Mapper v1.0.3 (Huerta-Cepas et al., 2017), a tool for fast functional annotation of novel sequences using precomputed eggNOG-based orthology assignments, against the database EggNOG v5.0 (Huerta-Cepas et al., 2019).

Orthology identification and phylogenetic inference

Protein-coding genes from another 11 species of Lepidoptera as well as two species of Coleoptera and two species of Diptera were obtained from the NCBI genomes database for comparative analysis (**Table 1**). Orthologs were identified using OrthoFinder version 2.2.7 (Emms & Kelly, 2015) under default parameters. The phylogenetic tree was inferred in the OrthoFinder pipeline with an approximately-maximum-likelihood method implemented in FastTree v2.1.10 (Price et al., 2009) based on a concatenated multiple sequence alignment (MSA) of single-copy genes. The most likely category for each site was set using a Bayesian approach with a gamma prior. Amino acid sequences were aligned in MAFFT v7.450 (Katoh & Standley, 2013) with the G-INS-I algorithm.

Manual annotation of circadian genes

We further manually annotated well-studied circadian genes: *period* (PER), *timeless* (TIM), *Clock* (CLK), *cycle* (CYC) and cryptochrome (CRY), using BLAST v2.2.31 (Altschul et al., 1990). Reference protein sequences of insect circadian genes were obtained from the Uniprot database. Conserved domains within proteins were annotated against the conserved domain database (Lu et al., 2020). Circadian genes of the other 15 insect species were annotated in the same way. For a common domain of three genes (CLK, PER and CYC), a neighbor-joining tree was constructed using MEGA7 (Kumar et al., 2016) with 500 bootstrap replicates.

Manual annotation of detoxification and chemosensory gene families

We manually annotated five detoxification gene families and four chemosensory gene families, including cytochrome P450 monooxygenase (P450s), glutathione S-transferase (GSTs), carboxyl/cholinesterases (CCEs), uridine diphosphate (UDP)-glycosyltransferases (UGTs), ATP-binding cassette (ABC) transporters, gustatory receptors (GR), ionotropic receptors (IR), odorant-binding proteins (OBP), olfactory receptors (OR) genes. We used the bioinformatic pipeline BITACORA (Vizueta et al., 2019) to conduct HMMER v3.3 (Finn et al., 2011) and BLAST v2.2.31 (Altschul et al., 1990) analyses under a full mode. Hits were filtered with a default cutoff E-value of 10e-5. The HMMs of P450 were downloaded from Pfam v32.0 (El-Gebali et al., 2018), while other HMMs of detoxification gene families were

created by HMMER v3.3 (Finn et al., 2011). Orthologs from *Bombyx mori* and *D. melanogaster* were used as evidence. The annotated genes were further filtered manually based on gene length and the presence of conserved domains. The longest transcripts of alternative splicing was kept. Genes with a length shorter than 80 amino acids were removed. Orthologs were aligned with the G-INS-I algorithm implemented in MAFFT v7.450 (Kato & Standley, 2013). A neighbor-joining tree was constructed for each gene family using MEGA7 (Kumar et al., 2016) with 500 bootstrap replicates.

Results and discussion

Features of the assembled genome

The genome size of PFM is estimated to be 338.52-352.59 Mb through k-mer analysis depend on the k-mers used ($k = 17, 21, 25, 35$). The k-mer distributions showed double peaks, indicating that this genome has a high rate of duplication and heterozygosity. The estimated heterozygosity ranges from 1.06% to 1.15% and the rate of duplication range from 1.95% to 2.06% (**Fig. 2a**). The high heterozygosity of the PFM genome might be caused by the pooling of the samples for short-read sequencing. The heterozygosity of PFM is similar to the *Thrips palmi* (1.01% to 1.32%) (Guo et al., 2020) and the caddisfly *Stenopsyche tienmushanensis* (1.05%-1.10%) (Luo et al., 2018), which were also sequenced from pooling DNA of multiple individuals. The heterozygosity of PFM is higher than beet armyworm *Spodoptera exigua* (0.59%) (Zhang et al., 2019), the invading fall webworm (0.75% and 0.83%) (Wu et al., 2018) and *Cydia pomonella* (0.6%) (**Wan et al., 2019**), which were estimated from one individual or multiple individuals of sibling mating lines.

At the contig level, we assembled the PFM genome into 404.83 Mb sequences, including 275 contigs, with a contig N50 length of 2.62 Mb. Based on contig interaction frequency calculated from the pairs aligned to the contigs, the 275 contigs were clustered into 31 linkage groups (**Fig. 2b**). The longest contig group was 19.1 Mb while the shortest one was 6.30 Mb, with an N50 of 14.39 Mb. BUSCO analysis showed that 98.3% (single-copied gene: 97.4%, duplicated gene: 0.9%) of 1,658 genes were identified as

complete, 0.40% of genes were fragmented, while 1.3% of genes were missing in the assembled genome. The genome comprised 36.88% GC base pairs.

Synteny analysis showed that the PFM, *S. litura* and *C. pomonella* genome have a highly conserved gene order (**Fig. 2c**). Chromosome 01 of PFM was identified as the Z chromosome based on its high synteny to the Z chromosome of *S. litura* (**Fig. 2c**). Chromosome 04 of PFM shows high synteny to chromosome 02 of *S. litura*, which fused with Z chromosome in *C. pomonella*, forming the neo-Z chromosome (chromosome 01) (Wan et al., 2019). In conclusion, PFM has similar chromosomes as *S. litura*, including 30 autosomes and a Z chromosome. The female-specific W chromosome of PFM was not determined in this genome, since male pupae were used for sequencing. Identification of W chromosome is challenged by its high degenerate, being gene-poor and repeat-rich in Lepidoptera (Bergero & Charlesworth, 2009; Wan et al., 2019). The chromosome-level assembly of the PFM genome provides resources for understanding chromosome evolution in the Lepidoptera (**Ahola et al., 2014**).

Genome annotation

We identified 27,598 protein-coding genes in the 1st round of MAKER annotation. BUSCO analysis revealed 91.5% of the evaluated single-copy genes were identified as complete. After three rounds of MAKER annotation, the number of genes increased to 50,676, while the proportion of complete single-copy genes was up to 95.0%. After filtering based on gene expression analysis, functional domains and AED values, 21,695 genes remained. BUSCO analysis showed that 94.8% (single-copied gene: 90.5%, duplicated gene: 4.3%) of the evaluated single-copy genes were identified as complete, 3.6% of the genes were fragmented, and 1.6% of the genes were missing in the annotated gene set. In total, 18,397 genes (84.80%) were functionally annotated, of which 5,303 (24.44%) and 3,829 (17.65%) genes were annotated to GO terms and KEGG KOs respectively. We predicted 53 rRNAs, 11,076 tRNAs, 20 small nuclear RNAs, and 48 micro RNAs in the PFM genome based on Rfam databases.

In total, 35.70 Mb (8.95%) of the genome was identified to be repeat DNA. Overall, 179,142 transposable elements (TEs, 35.14 Mb), including 139,294 retroelements (16,818 short interspersed nuclear elements (SINEs), 107,497 long interspersed nuclear elements

(LINEs) and 14,979 long terminal repeats (LTR)) and 39,848 DNA transposons were identified (Table 1, Table S1). The PFM genome shows the lowest repeat DNA among seven chromosome-level assembled genomes from the Lepidoptera (Table 1).

Orthology and phylogenetic relationships of lepidopterans

OrthoFinder assigned 320,821 genes (93.41% of total) to 15,076 orthogroups for the 16 species compared. Fifty percent of the assigned genes were in orthogroups with 28 or more genes (G50 was 28) and were contained in the largest 3,174 orthogroups (O50 was 3,174).

There were 947 single-copy genes with 364,262 reliable sites retained for phylogenetic inference. The topology is congruent with previously inferred phylogenetic relationships of Lepidoptera, in which no representative of the Copromorphaidea was included (Wan et al., 2019). Current molecular phylogenetic studies have not resolved the phylogenetic relationship between Copromorphaidea and Papilionoidea (Mitter et al., 2017). Our result supports the notion that PFM from the Copromorphaidea forms a sister-group relationship to the butterfly *D. plexippus* (Papilionoidea), rather than a sister group between Copromorphaidea/Papilionoidea and Pyraloidea + (Noctuoidea + Bombycoidea) (Fig. 3).

We investigated orthogroups shared by PFM and four species of Lepidoptera, representing different clades of the phylogenetic tree of Lepidoptera (Fig. S1). There were 7,827 orthogroups (60.5% of 12,938 orthogroups) shared by all five lepidopteran species and 1,549 orthogroups shared by four species except for *C. pomonella*. We identified 357 orthogroups specific to PFM, fewer than that of *B. mori* (406), but higher than other three lepidopteran species (Fig. S1).

Evolution of circadian genes

Five core genes of the circadian clock were annotated in the PFM genome and the other reference insect species. The PER gene was not found in currently assembled genomes of *Cydia pomonella* and *Anoplophora glabripennis* (Fig. 4). Two types of CRY gene were annotated in 16 species, mammalian-type cryptochrome (CRY-m) and *Drosophila* type cryptochrome (CRY-d). CRY-d is a UV- and blue-light photoreceptor and is not considered as a core component of the circadian clock in *D. melanogaster* (Goto, 2013). In circadian clock model established from *Danaus plexippus* (L.), CRY-m function as a transcriptional repressor instead as a photoreceptor (Yuan et al., 2007; Zhu et al., 2008), and it was incorporated into the core component of the circadian clock as a negative regulator, like PER and TIM. For

most of the 16 insects, two types of CRY genes were found, while only CRY-m was found in two Coleoptera species, and only CRY-d was found in *D. melanogaster*. In the wasp *Nasonia vitripennis*, only CRY-m was found and TIM does not exist in the genome (Schurko et al., 2010). In the honey bee *Apis mellifera*, TIM was lost (Rubin et al., 2006). In the PFM, FPKM values of CRY-m were higher than CRY-d in each stage, indicating that CRY-m may be a major element in the circadian clock of PFM. These considerable differences in the gene composition of the circadian clock indicate a diverse circadian clock model among insects.

Domains of circadian genes were conserved among the 16 species (Fig. 4). The PAS domains are widely present in photoreceptors and circadian proteins of many eukaryotes (Taylor & Zhulin, 1999). For insects investigated in this study, *CLK*, *CYC* and *PER* genes have two PAS domains. In previous studies, the two PAS domains were defined as PAS-A and PAS-B. In this study, PAS-A is equivalent to PAS and PAS-fold (Fig. 4 and Fig. 5). Besides PAS domains, *CLK* and *CYC* genes have other common domains bHLH before two PAS domains (Fig. 4). Both *CRY-d* and *CRY-m* gene have a PhrB domain, and *TIM* gene has a TIMELESS domain (Fig. 4). We constructed phylogenetic relationships among the PAS domains for the 16 species. The phylogenetic tree of PAS domains revealed six clades, corresponding to two PAS domains of three genes (*CLK*, *CYC* and *PER*) (Fig. 5).

Gene expression in diapause and non-diapause PFM

Compared with larvae that developed under a normal day photoperiod, 11 genes were significantly up-regulated and 9 genes were down-regulated in larvae that developed under long-day or short-day photoperiods (Table S2, Fig. S2). Genes highly expressed in pre-diapause larvae (SD and LD photoperiod) included genes encoding CUSOD2 (CS_07203), an enzyme that destroys radicals, and that plays an important role in diapause and cold tolerance of insect (Bi et al., 2014; He et al., 2013; Isobe et al., 2006; Kim et al., 2010; Sim & Denlinger, 2011; Zhao & Shi, 2009). We identified a cytochrome P450 gene (CS_20496) showing weak expression in pre-diapause larvae and high expression in the other stages, which was also found in diapausing larvae of the wild silk moth, *Antheraea yamamai* (Yang et al., 2008).

We identified 44 genes specifically up-regulated under a long-day photoperiod, and 14 genes specifically up-regulated under a short-day photoperiod (Table S2, Fig. S2). Four

genes (CS_04235, CS_05017, CS_15183, CS_01854) related to digestion of proteins were up-regulated in larvae developing under a long-day photoperiod. This is congruent with previous reports suggesting that the photoperiod had significant effects on digestive enzyme activity (Espinosa-Chaurand et al., 2017; Ramzanzadeh et al., 2016; Shan et al., 2008; Subala & Shivakumar, 2017). The functional link of many of these genes to diapause is not really clear. The circadian genes, which are important in diapause in another moth (Kozak et al., 2019), did not show significant changes for larvae under different photoperiods.

Evolution of detoxification and chemoreceptor genes

We manually identified 95 P450s, 76 GSTs, 63 CCEs, 27 UGTs, and 93 ABCs in the PFM genome (**Table 2; Fig. S3**). PFM had the lowest number of UGT genes and CCE genes, along with two other moths located at basal lineages of the Lepidoptera. The number of P450 genes in PFM are the second lowest, only slightly higher than *Bombyx mandarina*. The number of ABCs genes in PFM are at an intermediate level. We found that the PFM had the highest number of GST genes. These results suggest that PFM may have a unique way of detoxication with reduced importance of UGT and P450 when compared to the other moths. This may have implications for pesticide responses in PFM give that these detoxification genes can respond in different ways to various pesticides in moths (Hu et al., 2019).

The chemosensory system plays an important role in locating food, shelter, mates, and oviposition sites (Wan et al., 2019). We manually annotated several well-studied gene families of the chemosensory system; we identified 41 GRs, 49 IRs, 56 OBPs, and 176 ORs in the PFM genome (**Table 2; Fig. S4**). PFM had the lowest number of GR genes and the second-lowest number of IR genes, OBP genes, and OR genes following the *C. pomonella* when compared to the other lepidopterans. Both PFM and *C. pomonella* have a lower number of chemoreceptor genes. The PFM and *C. pomonella* mainly feed on apple and pear, with a narrow host range. Females of these two moths lay eggs on the surface of their host fruits, and the hatched larvae bore into the fruit directly without long-time searching behavior (van der Geest & Evenhuis, 1991). The narrow host range and feeding behavior of larva may explain the lower number of chemoreceptor genes in their genomes.

Conclusions

We assembled the chromosome-level genome for the PFM using PacBio long-read and Hi-C technology. This is the first assembled genome for the superfamily Copromorpha. This novel genomic resource allowed us to explore possible genes in PFM associated with adaptation to environmental factors. We identified five core genes relating to the circadian rhythm in PFM and annotated models for each gene. Using the genome as a reference, we investigated DEGs related to the diapause of OFM, which may point to candidate genes. Given the expression of long-day and short-day diapause by PFM, this moth species will be a useful model to further investigate adaptive shifts involving diapause, particularly by combining genomic information with intraspecific comparisons across geographic gradients (Ragland et al., 2019). The assembled genome provides a resource for further comparative studies of moths and butterflies, particularly with respect to life cycle evolution and parallel evolution in detoxification and chemosensory functions.

Data Availability Statement

The Whole Genome assembly has been deposited in the Genome repository of NCBI (accession numbers: CP053148-CP053178) under BioProject PRJNA627116. Raw reads obtained for genome assembly were deposited in the Sequence Read Archive (SRA) repository (accession numbers: SRR12328811 and SRR12336732). Scripts used for assembling and annotating the genome and sequences of manually annotated gene families were deposited in the Dryad repository (<https://doi.org/10.5061/dryad.m0cfxpp1j>).

Author contributions

Shu-Jun Wei conceived and designed the study; Jin-Cui Chen and Ya-Jun Gong conducted the collection and rearing of the insect; Li-Jun Cao conducted the molecular works; Li-Jun Cao, Wei Song, Lei-Yue, Shao-Kun Guo, and Shu-Jun Wei analyzed the data; Li-Jun Cao, Shu-Jun Wei, and Ary Hoffmann discussed the results and wrote the manuscript.

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Reference

- Ahola, V., Lehtonen, R., Somervuo, P., Salmela, L., Koskinen, P., Rastas, P., . . . Hanski, I. (2014). The Glanville fritillary genome retains an ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. *Nature Communications*, 5, 4737. doi:10.1038/ncomms5737
- Altschul, S., Gish, W., Miller, W., Myers, E., & Lipman, D. (1990). Basic local alignment search tool. *Journal of molecular biology*, 215(3), 403-410.
- Belaghzal, H., Dekker, J., & Gibcus, J. H. (2017). Hi-C 2.0: An optimized Hi-C procedure for high-resolution genome-wide mapping of chromosome conformation. *Methods*, 123, 56-65.
- Bergero, R., & Charlesworth, D. (2009). The evolution of restricted recombination in sex chromosomes. *Trends in Ecology and Evolution*, 24(2), 94-102. doi:10.1016/j.tree.2008.09.010
- Bi, Z., Yang, X., Yu, W., Shu, J., & Zhang, Y. (2014). Diapause-associated protein3 functions as Cu/Zn superoxide dismutase in the Chinese oak silkworm (*Antheraea pernyi*). *PLoS ONE*, 9(3), e90435. doi:10.1371/journal.pone.0090435
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120. doi:10.1093/bioinformatics/btu170
- Campbell, M. S., Holt, C., Moore, B., & Yandell, M. (2014). Genome Annotation and Curation Using MAKER and MAKER-P. *Current Protocols in Bioinformatics*, 48(1), 4.11.11-14.11.39. doi:10.1002/0471250953.bi0411s48
- Cantarel, B. L., Korf, I., Robb, S. M., Parra, G., Ross, E., Moore, B., . . . Yandell, M. (2008). MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18(1), 188-196. doi:10.1101/gr.6743907

-
- Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., & Xia, R. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant*, doi.org/10.1016/j.molp.2020.1006.1009. doi:10.1101/289660 %J bioRxiv
- Chen, W., Yang, X., Tetreau, G., Song, X., Coutu, C., Hegedus, D., . . . Wang, P. (2019). A high-quality chromosome-level genome assembly of a generalist herbivore, *Trichoplusia ni*. *Molecular Ecology Resources*, 19(2), 485-496. doi:10.1111/1755-0998.12966
- Cheng, T., Wu, J., Wu, Y., Chilukuri, R. V., Huang, L., Yamamoto, K., . . . Mita, K. (2017). Genomic adaptation to polyphagy and insecticides in a major East Asian noctuid pest. *Nature Ecology and Evolution*, 1(11), 1747-1756. doi:10.1038/s41559-017-0314-4
- Chiba, T., & Kobayashi, M. (1985). Seasonal prevalence of the peach fruit moth, *Carposina niponensis* Walsingham, in the apple orchards in Iwate Prefecture. *Bulletin of the Iwate Horticultural Experiment Station*, 6, 1-14.
- Cong, Q., Borek, D., Otwinowski, Z., & Grishin, N. V. (2015). Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. *Cell Rep*, 10(6), 910-919. doi:10.1016/j.celrep.2015.01.026
- Dasmahapatra, K. K., Walters, J. R., Briscoe, A. D., Davey, J. W., Whibley, A., Nadeau, N. J., . . . Consortium, H. G. (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*, 487(7405), 94-98. doi:10.1038/nature11041
- Dudchenko, O., Batra, S. S., Omer, A. D., Nyquist, S. K., Hoeger, M., Durand, N. C., . . . Aiden, A. P. (2017). De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science*, 356(6333), 92-95.
- Durand, N. C., Shamim, M. S., Machol, I., Rao, S. S., Huntley, M. H., Lander, E. S., & Aiden, E. L. (2016). Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. *Cell Systems*, 3(1), 95-98. doi:10.1016/j.cels.2016.07.002

510 El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., . . . Smart, A. (2018).
511 The Pfam protein families database in 2019. *Nucleic Acids Research*, 47(D1),
512 D427-D432.

513 El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., . . . Finn, R. D.
514 (2019). The Pfam protein families database in 2019. *Nucleic Acids Research*, 47(D1),
515 D427-D432. doi:10.1093/nar/gky995

516 Emms, D. M., & Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole genome
517 comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*,
518 16(157), 1-14. doi:10.1186/s13059-015-0721-2

519 Espinosa-Chaurand, D., Vega-Villasante, F., Carrillo-Farnes, O., & Nolasco-Soria, H. (2017).
520 Effect of circadian rhythm, photoperiod, and molt cycle on digestive enzymatic
521 activity of *Macrobrachium tenellum* juveniles. *Aquaculture*, 479, 225-232.
522 doi:10.1016/j.aquaculture.2017.05.029

523 Fält-Nardman, J., Klemola, T., Roth, M., Ruohomäki, K., & Saikkonen, K. (2016). Northern
524 geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures
525 than their southern conspecifics: Implications of climate change. *Europe Journal of*
526 *Entomology*, 113(1), 337-343.

527 Finn, R. D., Clements, J., & Eddy, S. R. (2011). HMMER web server: interactive sequence
528 similarity searching. *Nucleic Acids Research*, 39(suppl_2), W29-W37.

529 Goto, S. G. (2013). Roles of circadian clock genes in insect photoperiodism. *Entomological*
530 *Science*, 16(1), 1-16. doi:10.1111/ens.12000

531 Guo, S. K., Cao, L. J., Song, W., Shi, P., Gao, Y. F., Gong, Y. J., . . . Wei, S. J. (2020).
532 Chromosome-level assembly of the melon thrips genome yields insights into
533 evolution of a sap-sucking lifestyle and pesticide resistance. *Molecular Ecology*
534 *Resources*, 10.1111/1755-0998.13189. doi:10.1111/1755-0998.13189

535 He, C., Meng, Q.-K., Yang, X.-B., & Hua, L. (2013). Carbohydrate metabolism and antioxidant
536 defense during diapause development in larvae of oriental fruit moth (*Grapholita*

537 *molesta*) at low temperature. *International Journal of Agriculture and Biology*, 15(1),
538 101-106.

539 Heckel, D. G. (2018). Insect detoxification and sequestration strategies. *Annual Plant*
540 *Reviews*, 47, 77-114.

541 Hoffmann, A. A. (2017). Rapid adaptation of invertebrate pests to climatic stress? *Current*
542 *Opinion in Insect Science*, 21, 7-13.

543 Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*,
544 470(7335), 479.

545 Hu, B., Zhang, S. H., Ren, M. M., Tian, X. R., Wei, Q., Mburu, D. K., & Su, J. Y. (2019). The
546 expression of *Spodoptera exigua* P450 and UGT genes: tissue specificity and
547 response to insecticides. *Insect Science*, 26(2), 199-216.
548 doi:10.1111/1744-7917.12538

549 Hua, B.-Z., Zeng, X.-H., & Zhang, H. (1998). Diapause of *Carposina sasakii* Matsumura
550 (Lepidoptera Carposinidae) on various host plants. *Acta Universitatis Agriculturae*
551 *Boreali-occidentalis*, 26(5), 25-29.

552 Hua, L., & Hua, B. Z. (1995). Preliminary study on the host-biotypes of peach fruit borer.
553 *Acta Phytophylacica Sinica*, 22(2), 165-170.

554 Huang, K. X., Wang, Y. Z., Ye, Z. X., Zhang, N. X., & Zhang, L. Y. (1976). Influence of
555 photoperiod and temperature on diapause of the peach fruit moth *Carposina sasakii*
556 Matsumura. *Acta Entomologica Sinica*, 19(2), 149-156.

557 Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., &
558 Bork, P. (2017). Fast genome-wide functional annotation through orthology
559 assignment by eggNOG-Mapper. *Molecular Biology and Evolution*, 34(8), 2115-2122.
560 doi:10.1093/molbev/msx148 %J Molecular Biology and Evolution

561 Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernandez-Plaza, A., Forslund, S. K., Cook, H., . . .
562 Bork, P. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically
563 annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic*
564 *Acids Research*, 47(D1), D309-D314. doi:10.1093/nar/gky1085

565 Isobe, M., Kai, H., Kurahashi, T., Suwan, S., Pitchayawasin-Thapphasaraphong, S., Franz,
566 T., . . . Nishida, H. (2006). The molecular mechanism of the termination of insect
567 diapause, part 1: A timer protein, TIME-EA4, in the diapause eggs of the silkworm
568 *Bombyx mori* is a metallo-glycoprotein. *Chembiochem*, 7(10), 1590-1598.
569 doi:10.1002/cbic.200600138

570 Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., . . . Hunter, S. (2014).
571 InterProScan 5: genome-scale protein function classification. *Bioinformatics*, 30(9),
572 1236-1240. doi:10.1093/bioinformatics/btu031

573 Kanost, M. R., Arrese, E. L., Cao, X., Chen, Y. R., Chellapilla, S., Goldsmith, M. R., . . . Blissard,
574 G. W. (2016). Multifaceted biological insights from a draft genome sequence of the
575 tobacco hornworm moth, *Manduca sexta*. *Insect Biochemistry and Molecular Biology*,
576 76, 118-147. doi:10.1016/j.ibmb.2016.07.005

577 Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7:
578 improvements in performance and usability. *Molecular Biology and Evolution*, 30(4),
579 772-780. doi:10.1093/molbev/mst010

580 Kim, D.-S., Lee, J.-H., & Yiem, M.-S. (2000). Spring emergence pattern of *Carposina sasakii*
581 (Lepidoptera: Carposinidae) in apple orchards in Korea and its forecasting models
582 based on degree-days. *Environmental Entomology*, 29(6), 1188-1198.
583 doi:10.1603/0046-225x-29.6.1188

584 Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome
585 alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*,
586 37(8), 907-915. doi:10.1038/s41587-019-0201-4

587 Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TopHat2:
588 accurate alignment of transcriptomes in the presence of insertions, deletions and
589 gene fusions. *Genome Biology*, 14(4), R36. doi:10.1186/gb-2013-14-4-r36

590 Kim, D. S., Lee, J. H., & Yiem, M. S. (2001). Temperature-dependent development of
591 *Carposina sasakii* (Lepidoptera : Carposinidae) and its stage emergence models.
592 *Environmental Entomology*, 30(2), 298-305.

-
- 593 Kim, Y. I., Kim, H. J., Kwon, Y. M., Kang, Y. J., Lee, I. H., Jin, B. R., . . . Seo, S. J. (2010).
594 Modulation of MnSOD protein in response to different experimental stimulation in
595 *Hyphantria cunea*. *Comparative Biochemistry and Physiology B-Biochemistry &*
596 *Molecular Biology*
597 157(4), 343-350. doi:10.1016/j.cbpb.2010.08.003
- 598 Kirk, H., Dorn, S., & Mazzi, D. (2013). Molecular genetics and genomics generate new
599 insights into invertebrate pest invasions. *Evolutionary Applications*, 6(5), 842-856.
- 600 Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., & Phillippy, A. M. (2017).
601 Canu: scalable and accurate long-read assembly via adaptivek-mer weighting and
602 repeat separation. *Genome Research*, 27(5), 722-736. doi:10.1101/gr.215087.116
- 603 Korf, I. (2004). Gene finding in novel genomes. *BMC Bioinformatics*, 5, 59.
604 doi:10.1186/1471-2105-5-59
- 605 Kozak, G. M., Wadsworth, C. B., Kahne, S. C., Bogdanowicz, S. M., Harrison, R. G., Coates, B.
606 S., & Dopman, E. B. (2019). Genomic basis of circannual rhythm in the european corn
607 borer moth. *Current biology*, 29(20), 3501-3509 e3505.
608 doi:10.1016/j.cub.2019.08.053
- 609 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics
610 Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7),
611 1870-1874. doi:10.1093/molbev/msw054
- 612 Kwon, D., Kwon, H., Kim, D., & Yang, C. (2018). Larval species composition and genetic
613 structures of *Carposina sasakii*, *Grapholita dimorpha*, and *Grapholita molesta* from
614 Korea. *Bulletin of entomological research*, 108(2), 241-252.
- 615 Kwon, D. H., Kim, D. H., Kim, H. H., Lee, S. H., & Yang, C. Y. (2017). Genetic diversity and
616 structure in apple-infesting pests of *Carposina sasakii*, *Grapholita dimorpha* and
617 *Grapholita molesta* in Korea. *Journal of Asia-Pacific Entomology*, 20(1), 13-16.
618 doi:10.1016/j.aspen.2016.11.002

-
- 619 Lagesen, K., Hallin, P., Rodland, E. A., Staerfeldt, H. H., Rognes, T., & Ussery, D. W. (2007).
620 RNAMmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids*
621 *Research*, 35(9), 3100-3108. doi:10.1093/nar/gkm160
- 622 Lange, A., Beier, S., Huson, D. H., Parusel, R., Iglauder, F., & Frick, J. S. (2018). Genome
623 Sequence of *Galleria mellonella* (Greater Wax Moth). *Genome Announcement*, 6(2),
624 e01220-01217. doi:10.1128/genomeA.01220-17
- 625 Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature*
626 *Methods*, 9(4), 357-359. doi:10.1038/nmeth.1923
- 627 Lowe, T. M., & Eddy, S. R. (1997). tRNAscan-SE: a program for improved detection of
628 transfer RNA genes in genomic sequence. *Nucleic Acids Research*, 25(5), 955-964.
629 doi:10.1093/nar/25.5.955
- 630 Lu, S., Wang, J., Chitsaz, F., Derbyshire, M. K., Geer, R. C., Gonzales, N. R., . . .
631 Marchler-Bauer, A. (2020). CDD/SPARCLE: the conserved domain database in 2020.
632 *Nucleic Acids Research*, 48(D1), D265-D268. doi:10.1093/nar/gkz991
- 633 Lu, S., Yang, J., Dai, X., Xie, F., He, J., Dong, Z., . . . Li, X. (2019). Chromosomal-level reference
634 genome of Chinese peacock butterfly (*Papilio bianor*) based on third-generation DNA
635 sequencing and Hi-C analysis. *Gigascience*, 8(11), 1-10.
636 doi:10.1093/gigascience/giz128
- 637 Luo, S., Tang, M., Frandsen, P. B., Stewart, R. J., & Zhou, X. (2018). The genome of an
638 underwater architect, the caddisfly *Stenopsyche tienmushanensis* Hwang (Insecta:
639 Trichoptera). *GigaScience*, 7(12), 1-12. doi:10.1093/gigascience/giy143
- 640 Ma, W., Zhao, X., Yin, C., Jiang, F., Du, X., Chen, T., . . . Lin, Y. (2020). A chromosome-level
641 genome assembly reveals the genetic basis of cold tolerance in a notorious rice
642 insect pest, *Chilo suppressalis*. *Molecular Ecology Resources*, 20(1), 268-282.
643 doi:10.1111/1755-0998.13078
- 644 Marçais, G., & Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting
645 of occurrences of k-mers. *Bioinformatics (Oxford, England)*, 27(6), 764-770.
646 doi:10.1093/bioinformatics/btr011

-
- 647 Mitter, C., Davis, D. R., & Cummings, M. P. (2017). Phylogeny and evolution of Lepidoptera.
648 *Annual Review of Entomology*, 62, 265-283.
649 doi:10.1146/annurev-ento-031616-035125
- 650 Nishikawa, H., Iijima, T., Kajitani, R., Yamaguchi, J., Ando, T., Suzuki, Y., . . . Fujiwara, H.
651 (2015). A genetic mechanism for female-limited Batesian mimicry in *Papilio* butterfly.
652 *Nature Genetics*, 47(4), 405-409. doi:10.1038/ng.3241
- 653 Pearce, S. L., Clarke, D. F., East, P. D., Elfekih, S., Gordon, K. H. J., Jermin, L. S., . . . Wu, Y. D.
654 (2017). Genomic innovations, transcriptional plasticity and gene loss underlying the
655 evolution and divergence of two highly polyphagous and invasive *Helicoverpa* pest
656 species. *BMC Biology*, 15(1), 63. doi:10.1186/s12915-017-0402-6
- 657 Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L.
658 (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq
659 reads. *Nature Biotechnology*, 33(3), 290-295. doi:10.1038/nbt.3122
- 660 Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: computing large minimum
661 evolution trees with profiles instead of a distance matrix. *Molecular Biology and*
662 *Evolution*, 26(7), 1641-1650. doi:10.1093/molbev/msp077
- 663 Quezada García, R., Seehausen, M. L., & Bause, É. (2015). Adaptation of an outbreaking
664 insect defoliator to chronic nutritional stress. *Journal of Evolutionary Biology*, 28(2),
665 347-355. doi:10.1111/jeb.12571
- 666 Ragland, G. J., Armbruster, P. A., & Meuti, M. E. (2019). Evolutionary and functional genetics
667 of insect diapause: a call for greater integration. *Current Opinion in Insect Science*
668 36, 74-81. doi:10.1016/j.cois.2019.08.003
- 669 Ramzanzadeh, F., Yeganeh, S., JaniKhalili, K., & Babaei, S. S. (2016). Effects of different
670 photoperiods on digestive enzyme activities in rainbow trout (*Oncorhynchus mykiss*)
671 alevin and fry. *Canadian Journal of Zoology*, 94(6), 435-442.
672 doi:10.1139/cjz-2015-0180

-
- Rane, R. V., Ghodke, A. B., Hoffmann, A. A., Edwards, O. R., Walsh, T. K., & Oakeshott, J. G. (2019). Detoxifying enzyme complements and host use phenotypes in 160 insect species. *Current Opinion in Insect Science*, 31, 131-138.
- Roach, M. J., Schmidt, S. A., & Borneman, A. R. (2018). Purge Haplotigs: allelic contig reassignment for third-gen diploid genome assemblies. *BMC Bioinformatics*, 19(1), 460. doi:10.1186/s12859-018-2485-7
- Rubin, E. B., Shemesh, Y., Cohen, M., Elgavish, S., Robertson, H. M., & Bloch, G. (2006). Molecular and phylogenetic analyses reveal mammalian-like clockwork in the honey bee (*Apis mellifera*) and shed new light on the molecular evolution of the circadian clock. *Genome Res*, 16(11), 1352-1365. doi:10.1101/gr.5094806
- Sato, N., & Ishitani, M. (1976). Life-cycle of the peach fruit moth, *Carposina niponensis* Walsingham. *Bulletin of the Aomori Field Crops and Horticultural Experiment Station*, 1, 1-16.
- Schurko, A. M., Mazur, D. J., & Logsdon, J. M. (2010). Inventory and phylogenomic distribution of meiotic genes in *Nasonia vitripennis* and among diverse arthropods. *Insect Molecular Biology*, 19, 165-180. doi:10.1111/j.1365-2583.2009.00948.x
- Servant, N., Varoquaux, N., Lajoie, B. R., Viara, E., Chen, C. J., Vert, J. P., . . . Barillot, E. (2015). HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. *Genome Biology*, 16, 259. doi:10.1186/s13059-015-0831-x
- Sgro, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu Rev Entomol*, 61, 433-451. doi:10.1146/annurev-ento-010715-023859
- Shan, X. J., Xiao, Z. Z., Huang, W., & Dou, S. Z. (2008). Effects of photoperiod on growth, mortality and digestive enzymes in miiuy croaker larvae and juveniles. *Aquaculture*, 281(1-4), 70-76. doi:10.1016/j.aquaculture.2008.05.034
- Sim, C., & Denlinger, D. L. (2011). Catalase and superoxide dismutase-2 enhance survival and protect ovaries during overwintering diapause in the mosquito *Culex pipiens*. *Journal of Insect Physiology*, 57(5), 628-634. doi:10.1016/j.jinsphys.2011.01.012

-
- Simao, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31(19), 3210-3212. doi:10.1093/bioinformatics/btv351
- Song, S. V., Downes, S., Parker, T., Oakeshott, J. G., & Robin, C. (2015). High nucleotide diversity and limited linkage disequilibrium in *Helicoverpa armigera* facilitates the detection of a selective sweep. *Heredity (Edinb)*, 115(5), 460-470. doi:10.1038/hdy.2015.53
- Stanke, M., & Waack, S. (2003). Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics*, 19 Suppl 2, ii215-225. doi:10.1093/bioinformatics/btg1080
- Subala, S. P., & Shivakumar, M. S. (2017). Circadian variation affects the biology and digestive profiles of a nocturnal insect *Spodoptera litura* (Insecta: Lepidoptera). *Biological Rhythm Research*, 48(2), 207-226. doi:10.1080/09291016.2016.1251928
- Tarailo-Graovac, M., & Chen, N. (2009). Using RepeatMasker to identify repetitive elements in genomic sequences. *Current protocols in bioinformatics*, 25(1), unit 4.10.
- Taylor, B. L., & Zhulin, I. B. (1999). PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol Mol Biol Rev*, 63(2), 479-506.
- Toshima, A., Honma, K., Masaki, S. J. J. J. o. A. E., & Zoology. (1961). Factors Influencing the Seasonal Incidence and Breaking of Diapause in *Carposina niponensis* WALSHINGHAM. *Japanese Journal of Applied Entomology and Zoology*, 5(4), 260-269.
- van Asch, M., Salis, L., Holleman, L. J., van Lith, B., & Visser, M. E. (2013). Evolutionary response of the egg hatching date of a herbivorous insect under climate change. *Nature Climate Change*, 3(3), 244.
- van der Geest, L. P. S., & Evenhuis, H. H. (1991). *World Crop Pests 5: Tortricid pests their biology, natural enemies and control* (L. P. S. van der Geest & H. H. Evenhuis Eds. Vol. 5). Amsterdam, The Netherlands: Elsevier.

-
- Vizueta, J., Sánchez-Gracia, A., & Rozas, J. (2019). BITACORA: A comprehensive tool for the identification and annotation of gene families in genome assemblies. *bioRxiv*, 593889. doi:10.1101/593889
- Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., & Schatz, M. C. (2017). GenomeScope: Fast reference-free genome profiling from short reads. *Bioinformatics*, 33(14). doi:10.1093/bioinformatics/btx153
- Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., . . . Earl, A. M. (2014). Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE*, 9(11), e112963. doi:10.1371/journal.pone.0112963
- Wan, F. H., Yin, C. L., Tang, R., Chen, M. H., Wu, Q., Huang, C., . . . Li, F. (2019). A chromosome-level genome assembly of *Cydia pomonella* provides insights into chemical ecology and insecticide resistance. *Nature Communications*, 10, doi.org/10.1038/s41467-41019-12175-41469. doi:ARTN 4237 10.1038/s41467-019-12175-9
- Wang, J., Yu, Y., Li, L. L., Guo, D., Tao, Y. L., & Chu, D. (2015). *Carposina sasakii* (Lepidoptera: Carposinidae) in its native range consists of two sympatric cryptic lineages as revealed by mitochondrial COI gene sequences. *Journal of Insect Science*, 15(1), 1-6. doi:10.1093/jisesa/iev063
- Wang, Y. Z., Li, B. Y., Hoffmann, A. A., Cao, L. J., Gong, Y. J., Song, W., . . . Wei, S. J. (2017). Patterns of genetic variation among geographic and host-plant associated populations of the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae). *BMC Evolutionary Biology*, 17(1), 265. doi:10.1186/s12862-017-1116-7
- Wu, N., Zhang, S., Li, X., Cao, Y., Liu, X., Wang, Q., . . . Zhan, S. (2018). Fall webworm genomes yield insights into rapid adaptation of invasive species. *Nature Ecology and Evolution*, 3(1), 105-115. doi:10.1038/s41559-018-0746-5

754 Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., . . . Biology Analysis, G. (2004). A draft
755 sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science*,
756 306(5703), 1937-1940. doi:10.1126/science.1102210

757 Xiang, H., Liu, X., Li, M., Zhu, Y. n., Wang, L., Cui, Y., . . . Zhan, S. (2018). The evolutionary
758 road from wild moth to domestic silkworm. *Nature Ecology and Evolution*, 2(8),
759 1268-1279. doi:10.1038/s41559-018-0593-4

760 Xiao, H., Ye, X., Xu, H., Mei, Y., Yang, Y., Chen, X., . . . Li, F. (2020). The genetic adaptations of
761 fall armyworm *Spodoptera frugiperda* facilitated its rapid global dispersal and
762 invasion. *Molecular Ecology Resources*, 20(4), 1050-1068.
763 doi:10.1111/1755-0998.13182

764 Xu, Q. G., & Hua, B. Z. (2004). RAPD analysis on the speciation in host races of *Carposina*
765 *sasakii* Matsumura (Lepidoptera: Carposinidae). *Acta Entomologica Sinica*, 47(3),
766 379-383.

767 Yamanaka, T., Tatsuki, S., & Shimada, M. (2008). Adaptation to the new land or effect of
768 global warming? An age-structured model for rapid voltinism change in an alien
769 lepidopteran pest. *Journal of Animal Ecology*, 77(3), 585-596.

770 Yang, P., Tanaka, H., Kuwano, E., & Suzuki, K. (2008). A novel cytochrome P450 gene
771 (CYP4G25) of the silkworm *Antheraea yamamai*: Cloning and expression pattern in
772 pharate first instar larvae in relation to diapause. *Journal of Insect Physiology*, 54(3),
773 636-643. doi:10.1016/j.jinsphys.2008.01.001

774 You, M. S., Yue, Z., He, W. Y., Yang, X. H., Yang, G., Xie, M., . . . Wang, J. (2013). A
775 heterozygous moth genome provides insights into herbivory and detoxification.
776 *Nature Genetics*, 45(2), 220-225.

777 Yuan, Q., Metterville, D., Briscoe, A. D., & Reppert, S. M. (2007). Insect cryptochromes: gene
778 duplication and loss define diverse ways to construct insect circadian clocks. *Mol Biol*
779 *Evol*, 24(4), 948-955. doi:10.1093/molbev/msm011

-
- Zhan, S., Merlin, C., Boore, J. L., & Reppert, S. M. (2011). The monarch butterfly genome yields insights into long-distance migration. *Cell*, 147(5), 1171-1185.
doi:10.1016/j.cell.2011.09.052
- Zhang, B., Peng, Y., Zhao, X. J., Hoffmann, A. A., Li, R., & Ma, C. S. (2016). Emergence of the overwintering generation of peach fruit moth (*Carposina sasakii*) depends on diapause and spring soil temperatures. *Journal of Insect Physiology*, 86, 32-39.
doi:10.1016/j.jinsphys.2015.12.007
- Zhang, F., Zhang, J., Yang, Y., & Wu, Y. (2019). A chromosome-level genome assembly for the beet armyworm *Spodoptera exigua* using PacBio and Hi-C sequencing. *bioRxiv*, 2019.2012.2026.889121. doi:10.1101/2019.12.26.889121
- Zhang, S., Shen, S., Peng, J., Zhou, X., Kong, X., Ren, P., . . . Zhang, Z. (2020). Chromosome-level genome assembly of an important pine defoliator, *Dendrolimus punctatus* (Lepidoptera; Lasiocampidae). *Molecular Ecology Resources*, 20(4), 1023-1037.
- Zhao, L., & Shi, L. (2009). Metabolism of hydrogen peroxide in univoltine and polyvoltine strains of silkworm (*Bombyx mori*). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, 152(4), 339-345.
doi:10.1016/j.cbpb.2008.12.014
- Zhu, H. S., Sauman, I., Yuan, Q., Casselman, A., Emery-Le, M., Emery, P., & Reppert, S. M. (2008). Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. *Plos Biology*, 6(1), 138-155.
doi:ARTN e4
10.1371/journal.pbio.0060004

Tables

Table 1 Features of chromosome-level genomes in the Lepidoptera

Features	Csas	Cpom	Tni	Bmor	Slit	Mcin	Hmel
Genome size (Mb)	399.04	772.89	368.2	431.7	438.32	393	269
Karyotype	2n=64	2n=56	2n=54	2n=56	2n=62	2n=62	2n=42
No. contigs	275	2221	26,605	15,018	13,636	49,851	NA
No. scaffolds	NA	1717	6181	7397	3597	8262	3807
No. CHR*	31A+Z+W	27A+Z+W	26A+Z+W	27A+Z	30A+Z	30A+Z	20A+Z
Contig N50 (kb)	2620	862.49	621.9	15.5	68.35	13	51
Scaffold N50 (Mb)	NA	8.92	14.2	3.7	0.915	0.119	0.277
BUSCO genes (%)	98.20%	98.5	97.8	97.7	98.3	91.5	97.4
Repeat (%)	11.33	42.87	20.5	43.6	31.83	28	24.94
G+C (%)	36.96	37.43	35.6	37.3	36.5	33.0	NA
No. genes	23,227	17,184	14,043	14,623	15,317	16,667	12,669

Csas, *Carpasina sasakii*; Cpom, *Cydia pomonella*; Tni, *Trichoplusia ni*; Bmor, *Bombyx mori*; Slit, *Spodoptera litura*; Mcin, *Melitaea cinxia*; Hmel, *Heliconius melpomene*; * A represents auto chromosome; Z and W represent sex chromosomes; NA, not available. Data for all species except for Csas were summarized by Wan et al. (2019).

Table 2 Number of genes in five detoxification families across species of Lepidoptera

Species	P450	GST	CCE	ABC	UGT	GR	IR	OBP	OR	Reference
Pxyl	163	55	85	219	38	68	61	91	225	You et al. (2013)
Csas	95	76	63	93	27	41	49	56	176	This study
Cpom	136**	30**	73**	47**	30**	65**	39**	50**	85**	Wan et al. (2019)
Dple	107	35	73	76	47	74	62	68	253	Zhan et al. (2011)
Tni	143	51	122	71	68	85	74	91	274	Chen et al. (2019)
Slit	182	66	153	223	64	107	62	87	261	Cheng et al. (2017)
Harm	122	57	105	76	54	120	71	79	257	Song et al. (2015)
Bman	94	37	94	64	48	69	56	74	218	Xiang et al. (2018)
Bmor	156	51	149	108	50	85	57	93	243	Xia et al. (2004)
Msex	164	66	137	103	51	89	76	79	261	Kanost et al. (2016)
Gmel	137	44	75	72	58	95	81	64	295	Lange et al. (2018)
Ofur	126	48	115	112	46	93	67	75	270	Ma et al. (2020)

**, data from Wan et al. (2019); The other data were manually identified in our study. Pxyl, *Plutella xylostella*; Csas, *Carposina sasakii*; Cpom, *Cydia pomonella*; Dple, *Danaus plexippus*; Tni, *Trichoplusia ni*; Slit, *Spodoptera litura*; Harm, *Helicoverpa armigera*; Bman, *Bombyx mandarina*; Bmor, *Bombyx mori*; Msex, *Manduca sexta*; Gmel, *Galleria mellonella*; Ofur, *Ostrinia furnacalis*. P450, Cytochrome P450 monooxygenase; GST,

glutathione S-transferase; CCEs, carboxyl/cholinesterases; UGT, uridine diphosphate-glycosyltransferases; ABC, ATP-binding cassette transporters; GR, gustatory receptors; IR, ionotropic receptors; OBP, odorant-binding proteins; OR, olfactory receptors.

Figure legends

Fig. 1 Eggs (A), larva (B), cocoons (C) and adult (D) of the peach fruit moth *Carposina sasakii* (A-D) and the damage symptoms to apple (E-G). The hatched larva bores into apple usually near the calyx with white secreta near the boring hole (E); the damaged apple showing shrinkage (F); damage from larvae boring and developing in the apple (G).

Fig. 2 Genome features of *Carposina sasakii*. (a) GenomeScope analysis of genome size, heterozygosity and duplicate rate using k-mers (K = 17) count histogram, indicating a genome size of 338.52 Mb, a heterozygosity of 1.06%, and a duplication rate of 2.06%; (b) Genome-wide all-by-all Hi-C interaction identified 31 linkage groups; (c) Synteny between *Carposina sasakii* (Csas) and *Cydia pomonella* (Cpom) and *Spodoptera litura* (Slit) genomes reveal highly conserved gene order and chromosomal fusion or split events in the three moths.

Fig. 3 Phylogenetic tree of PFM with 15 insect genomes including 11 other Lepidoptera. The phylogeny was inferred from 947 single-copy genes with 364,262 reliable sites by an approximately-maximum-likelihood method. All nodes received bootstrap support of 100.

Fig. 4 Schematic arrangement of the domains of five circadian genes including *period* (PER), *timeless* (TIM), *Clock* (CLK), *cycle* (CYC) and cryptochrome (CRY-m, CRY-d) in *Carposina sasakii* and other 15 insects. Boxes in different color show different domains, while numbers under boxes show the position of domains on protein sequences. Species and their taxonomic status are shown on the left: Tcas, *Tribolium castaneum*; Agla, *Anoplophora glabripennis*; Agam, *Anopheles gambiae*; Dmel, *Drosophila melanogaster*; Pxy, *Plutella xylostella*; Cpom, *Cydia pomonella*; Csas, *Carposina sasakii*; Dple, *Danaus plexippus*; Tni, *Trichoplusia ni*; Slit, *Spodoptera litura*; Harm, *Helicoverpa armigera*; Bmor, *Bombyx mori*; Bman, *Bombyx mandarina*; Msex, *Manduca sexta*; Gmel, *Galleria mellonella*; Ofur, *Ostrinia furnacalis*.

Fig. 5 Phylogenetic relationships of two PAS domains in three circadian genes: *period* (*PER*), *Clock* (*CLK*) and *cycle* (*CYC*). Each tip is labeled by the name of domain, gene and species. Abbreviations of species are same as in Fig. 4. Six clades shaded in different color reveal two domains of three genes, while one domain of *CLK* gene has two different types among species. Tips in red show the position of *Carposina sasakii*.

Supplemental Information

Table S1 Classification of repeat sequences identified in the *Carposina sasakii* genome

Table S2 Gene expression of diapause and day length related genes across different developmental stages

Fig. S1 Orthogroups shared by five Lepidoptera species of *Carposina sasakii*, *Cydia pomonella*, *Bombyx mori*, *Ostrinia furnacalis* and *Helicoverpa armigera*.

Fig. S2 Gene expression of diapause and day length related genes across different developmental stages based on *Carposina sasakii* transcriptome data

Fig. S3 Phylogenetic relationships of *Carposina sasakii* ATP-binding cassette (ABC) transporter gene families (a), carboxyl/cholinesterases (CCEs) (b), glutathione S-transferase (GSTs) (c), cytochrome P450 (P450) (d) and UDP-glycosyltransferases (UGTs) (e) in comparison with *Bombyx mori* and *Cydia pomonella*.

Fig. S4 Phylogenetic relationships of *Carposina sasakii* gustatory receptors (GR) (a), ionotropic receptors (IR) (b), odorant-binding proteins (OBP) (c), olfactory receptors (OR) (d) in comparison with *Bombyx mori*

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CLK

CYC

PER

men 13288 f4.pdf

CRY-d

CRY-m

TIM

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Domains bHLH-PAS_Dclock bHLH-PAS_cycle_like PAS Period_C TIMELESS Atrophin-1 Herpes_BLLF1 bHLH_SF PAS_fold PAS-B PhrB PAS_3 CITED Med3

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