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Association between changes in reproductive activity and D-glucose metabolism in the tephritid fruit fly, *Bactrocera dorsalis* (Hendel)

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Reproduction is an important life process in insects; however, few studies have attempted to demonstrate the association between reproductive activity and energy metabolism. To address this problem, we focused on the reproductive changes in *Bactrocera dorsalis* males. We analyzed *B. dorsalis* male gene expression profiles during mating (DM), 3 h after mating (A3HM) and 12 h after mating (A12HM). Gene annotation and pathway analyses of differentially expressed genes show that galactose metabolism and the starch and sucrose metabolism pathway activities were significantly higher in A12HM group. Moreover, the maltase D gene was the most strongly up-regulated gene. The D-glucose levels were significantly higher in A12HM group. Maltase D expression level was significantly higher in males reared with sucrose. Body weights of the males reared with D-glucose and sucrose were significantly higher than those of the males reared with yeast extract. We observed more mated males from the groups fed sucrose and D-glucose than from those fed yeast extract. The D-glucose levels in individual males were highest at 18:00 h, when flies exhibit the most active mating behavior. This study shows that the maltase D gene and D-glucose are the critical gene and substrate, respectively, in male *B. dorsalis* mating process.

In recent decades, reproductive roles have garnered great attention, and many interesting results have been generated[1–4]. In insects, such as tephritid fruit flies, nutrient metabolism has a critical effect on male sexual performance[5]. For example, nutrient reserves can regulate male participation in leks, as observed in the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann)[6]. In the Mediterranean fruit fly, manuka oil can significantly increase the mating success of both wild males and mass-reared, sterile males, and α-copaene is the key regulator[7]. Furthermore, the male diet can affect male mating success and longevity as well as female remating[8]. Reproductive systems can have important ecological and evolutionary implications. Changes in reproductive roles can affect population density[9] and the intensity of sexual selection[10]. In honey bees (*Apis mellifera*), different brain gene expression patterns are associated with caste and reproductive status[11]. In the red imported fire ant *Solenopsis invicta*, differentially expressed genes are associated with changes in reproductive roles[12]. Moreover, to adapt to the conditions of an invaded site, plant reproductive systems and evolution can be altered during the biological invasion process[13]. However, cryptic strategies often result in social mating systems that do not reflect the genetic mating system. Although studies have found that diet and nutrition are important for male fruit fly mating behavior[5,6,8,14,15], less is known about the underlying molecular mechanisms.

The oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a notorious pest worldwide that damages many commercial fruits[16,17] and a wide range of other agricultural products[18]. By destroying the marketability of fruit products, this pest has caused dramatic financial loss to orchard crops[18]. *B. dorsalis* has received considerable attention, and its mating behavior has been investigated for decades[19,20]. However, these studies have focused on chemical (e.g., pheromones) factors and mating behavior and not on the association between changes in the reproductive activity and energy metabolism.

To identify the *B. dorsalis* male mating molecular mechanism, we developed a large-scale oligonucleotide microarray for *B. dorsalis* and investigated the transcriptome profiles in males. *B. dorsalis* males need to take up nutrition for approximately one week before mating, and at the end of each mating activity, the male must undergo a complementary nutrition stage prior to another mating activity[5,6,15] (Figure 1). We therefore investi-

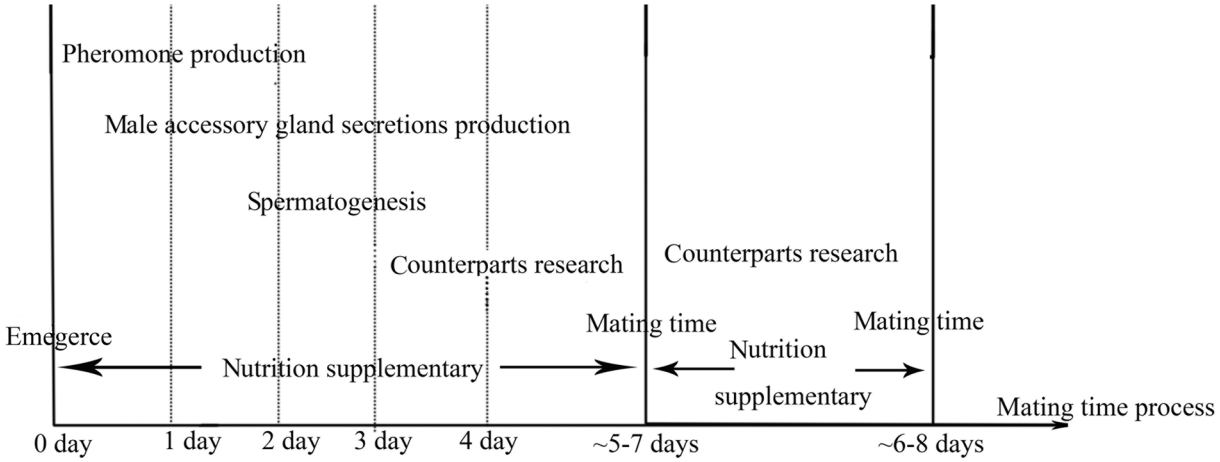

Figure 1 | **Fruit fly mating process timeline**[5,6,15].

Table 1 | Length distribution for the assembled sequences

	Min length	Mean length	Median length	Max length	N50	N90	Total nucleotides
Transcripts	201	1,218	654	11,919	2,272	477	70,667,419
Unigenes	201	929	436	11,919	1,849	328	30,941,876

N50: the length of the transcripts from shortest to longest; the length of the transcript for which the accumulated value is greater than 50% of the total length.
N90: the length of the transcripts from shortest to longest; the length of the transcript for which the accumulated value is greater than 90% of the total length.

gated how mating competitiveness differences gradually progress over time and identified pathways, genes and substrates that can be used to determine the molecular and genetic bases involved in mating competitiveness changes in *B. dorsalis* males. Furthermore, we identified reproductive changes through mating competition tests and metabolic changes during different reproductive statuses.

Results

Evaluation of transcriptome data. The error rate for the base positions in the reference transcriptome was less than 1%, which was considered an acceptable threshold (see Supplementary Figure S1 online). Moreover, the base content distribution shows that the G and C or A and T levels were equal, and horizontal lines were observed during the sequencing process, except for several bases at the beginning positions (see Supplementary Figure S1 online). After quality control, 26,589,907 clean reads out of 27,364,337 raw reads were obtained for pair-end sequencing. We generated 58,009 transcripts and 33,314 unigenes for which the N50 was 1,849 (see Supplementary Figure S2 online, Table 1).

Table 2 | Unigene annotation in the databases

	Number of unigenes	Percentage (%)
Annotated in NR	15,395	46.21
Annotated in NT	4,316	12.95
Annotated in KO	3,864	11.59
Annotated in SwissProt	11,067	33.22
Annotated in PFAM	11,908	35.74
Annotated in GO	13,185	39.57
Annotated in KOG	7,640	22.93
Annotated in all databases	1,057	3.17
Annotated in at least one database	17,615	52.87
Total unigenes	33,314	100

NR: NCBI non-redundant protein sequences; NT: NCBI nucleotide sequences; KO: KEGG orthology; SwissProt: a manually annotated and reviewed protein sequence database; PFAM: protein family; GO: Gene Ontology; and KOG: clusters of orthologous groups of proteins.

Functional annotation of the transcriptome. Through BLAST searches in the seven indicated databases, 33,314 unigenes were successfully annotated; most of the unigenes (15,395) that were annotated were from the non-redundant (NR) database, whereas the fewest (3,864) were from KEGG orthology (KO) (Table 2). In the Gene Ontology (GO) database, the top three GO terms were cellular process, binding process and metabolic process, for which 8,302, 7,620 and 7,171 genes were annotated, respectively (see Supplementary Figure S3 online). In clusters of orthologous groups of proteins (KOG), 7,640 annotated genes were assigned to 26 groups; the (R) general functional prediction only; (T) signal transduction; and (O) post-translational modification, protein turnover and chaperone groups contained the most annotated genes (1,530, 1,163 and 694 genes, respectively) (see Supplementary Figure S4 online). In KEGG, 3,864 genes were annotated with pathways; most of the genes were annotated with the signal transduction; translation; and folding, sorting and degradation categories (622, 497 and 356 genes, respectively) (see Supplementary Figure S5 online). The transcriptome data of *B. dorsalis* males was submitted to TSA database in NCBI (submission ID: SUB741296).

Quality assessment of the sequencing data from males with different reproductive statuses. The base error rate in each sample was less than 0.08%, and the rates for the first six positions were relatively higher than at the other positions. The same pattern was observed for the AT/CG content distribution (see Supplementary Figures S6 and S7 online; Table 3).

Analysis of the gene expression levels in males with different reproductive statuses. The data show that more than 93% of the clean reads were successfully mapped to the reference transcriptome for the nine samples (Table 4). The RPKM density distribution

Table 3 | Data output quality for the examined samples

Sample	Raw Reads	Clean Reads	Clean Bases	Error (%)	Q20 (%)	Q30 (%)	GC (%)
DM1	6,963,988	6,958,232	0.35 G	0.01	98.53	95.75	42.35
DM2	8,204,731	8,197,823	0.41 G	0.01	98.52	95.73	42.17
DM3	8,224,767	8,219,090	0.41 G	0.01	98.63	95.97	41.03
A3HM1	7,222,297	7,216,539	0.36 G	0.01	98.51	95.68	42.4
A3HM2	7,318,818	7,313,492	0.37 G	0.01	98.55	95.8	42.34
A3HM3	7,164,287	7,158,418	0.36 G	0.01	98.56	95.85	41.52
A12HM1	8,657,233	8,644,886	0.43 G	0.01	98.63	96.01	40.84
A12HM2	8,173,367	8,166,843	0.41 G	0.01	98.57	95.83	41.94
A12HM3	8,028,746	8,022,262	0.4 G	0.01	98.54	95.79	42.17

DM1, DM2, and DM3: 3 repeats of DM; A3HM1, A3HM2, and A3HM3: 3 repeats of A3HM; A12HM1, A12HM2, and A12HM3: 3 repeats of A12HM; Q20: the percentage of bases for which the Phred value is greater than 20; and Q30: the percentage of bases for which the Phred value is greater than 30.

patterns were similar among the nine samples; they presented two peaks on both sides of zero (see Supplementary Figure S8 online). A correlation analysis of the gene expression levels showed that the correlations were between 0.826 and 0.861 for the replicate treatments; the lowest value (0.826) was generated for DM2 and DM3, and the highest value (0.861) was generated for A12HM1 and A12HM2 (see Supplementary Figure S9 online).

Analysis of differentially expressed genes in males with different reproductive statuses. The numbers of differentially expressed genes that were generated for A3HM vs. DM, A12HM vs. DM and A12HM vs. A3HM were 15 (4 down-regulated, 11 up-regulated), 115 (34 down-regulated, 81 up-regulated) and 72 (22 down-regulated, 50 up-regulated), respectively (Figure 2a, 2b, and 2c; see Supplementary Datasets 1, 2 and 3). A Venn diagram shows that 39 of the differentially expressed genes were shared between A12HM vs. DM and A12HM vs. A3HM; 6 differentially expressed genes were shared between A3HM vs. DM and A12HM vs. DM; 2 differentially expressed genes were shared between A12HM vs. A3HM and A3HM vs. DM; and 1 differentially expressed gene was shared between A12HM vs. DM, A12HM vs. A3HM and A3HM vs. DM. Maltase D (accession number: KM115582) was the only differentially expressed gene that was shared in all of the groups (Figure 3, Supplementary Table S1).

KEGG analysis of differentially expressed genes. When A12HM was compared with DM and A3HM by analyzing the biochemical and signal transduction pathways, the top two significantly enriched pathways were the starch and sucrose metabolism and galactose metabolism pathways (Figure 4a and 4b). Compared with DM, five genes were significantly up-regulated in both the starch and sucrose metabolism and galactose metabolism pathways. Furthermore, within the starch and sucrose metabolism and galactose metabolism pathways, the gene with the greatest fold change (1.7601) was maltase D (Table 5). When A12HM was compared with A3HM, three and two genes were significantly up-regulated in the starch and sucrose metabolism and galactose metabolism pathways, respectively; maltase D showed the greatest fold change at 2.4211 (Table 5). In both metabolism pathways, the differentially expressed gene substrate was D-glucose.

Table 4 | Sample mapping rates

Sample name	Total reads	Total mapped (rate)
DM1	6,958,232	6,513,572 (93.61%)
DM2	8,197,823	7,638,547 (93.18%)
DM3	8,219,090	7,833,589 (95.31%)
A3HM1	7,216,539	6,742,771 (93.43%)
A3HM2	7,313,492	6,879,123 (94.06%)
A3HM3	7,158,418	6,758,732 (94.42%)
A12HM1	8,644,886	8,261,558 (95.57%)
A12HM2	8,166,843	7,661,566 (93.81%)
A12HM3	8,022,262	7,510,493 (93.62%)

DM1, DM2, and DM3: 3 repeats of DM; A3HM1, A3HM2, and A3HM3: 3 repeats of A3HM; and A12HM1, A12HM2, and A12HM3: 3 repeats of A12H.

D-glucose content in males with different reproductive statuses. The D-glucose levels in males 12 h after mating (A12HM) were significantly higher than in males during mating (DM) and 3 h after mating (A3HM) ($F = 17.502$, $df = 2$, $p = 0.001$) (Figure 5). The results were identical to the expression profiles and KEGG analysis results.

Performance (body weight, maltase expression, and mating competition) of males reared with three different media. The body weights of the males reared with D-glucose mixed with yeast extract and sucrose mixed with yeast extract were significantly higher than those of the males reared with yeast extract alone ($F = 9.995$, $df = 2$, $p = 0.003$). However, the body weights of the males reared with D-glucose mixed with yeast extract and sucrose mixed with yeast extract did not differ (Figure 6). Furthermore, maltase D gene expression was significantly higher in the males reared with sucrose mixed with yeast extract and D-glucose mixed with yeast extract than in the males of the yeast extract only group ($F = 40.965$, $df = 2$, $p < 0.001$) (Figure 7). Mating competition experiments showed that the numbers of successfully mated males from the sucrose mixed with yeast extract and D-glucose mixed with yeast extract groups were significantly higher than the number of males in the yeast extract only group ($F = 108.545$, $df = 2$, $p < 0.001$). However, a significant difference was not observed between the males in the sucrose mixed with yeast extract and D-glucose mixed with yeast extract groups (Figure 8). Moreover, the D-glucose concentration in individual males showed a significant peak that was greatest at 18:00 during the day ($F = 3.419$, $df = 5$, $p = 0.026$) (Figure 9).

Discussion

To our knowledge, this is the first time that the male *B. dorsalis* transcriptome has been sequenced and annotated during different reproductive statuses. Compared with the published transcriptomes for *B. dorsalis* eggs, third-instar larvae, pupae, and newly emerged adults[21], the transcriptome herein is smaller, and most genes are involved in signal transduction pathways as well as translation and folding pathways but not metabolic pathways. In recent decades, studies have shown that male behavior in mating systems is important[22–24], and the mechanisms used by males to assess female mate quality vary greatly[24–26]. In animals, male-male competition typically enables high mating success in high-quality males[27–29]. For example, for male flies to successfully mate with a female, the males must obtain sufficient nutrition, reach a specific size and be able to synthesize pheromones[5,8,15,30], processes that are closely related to signal

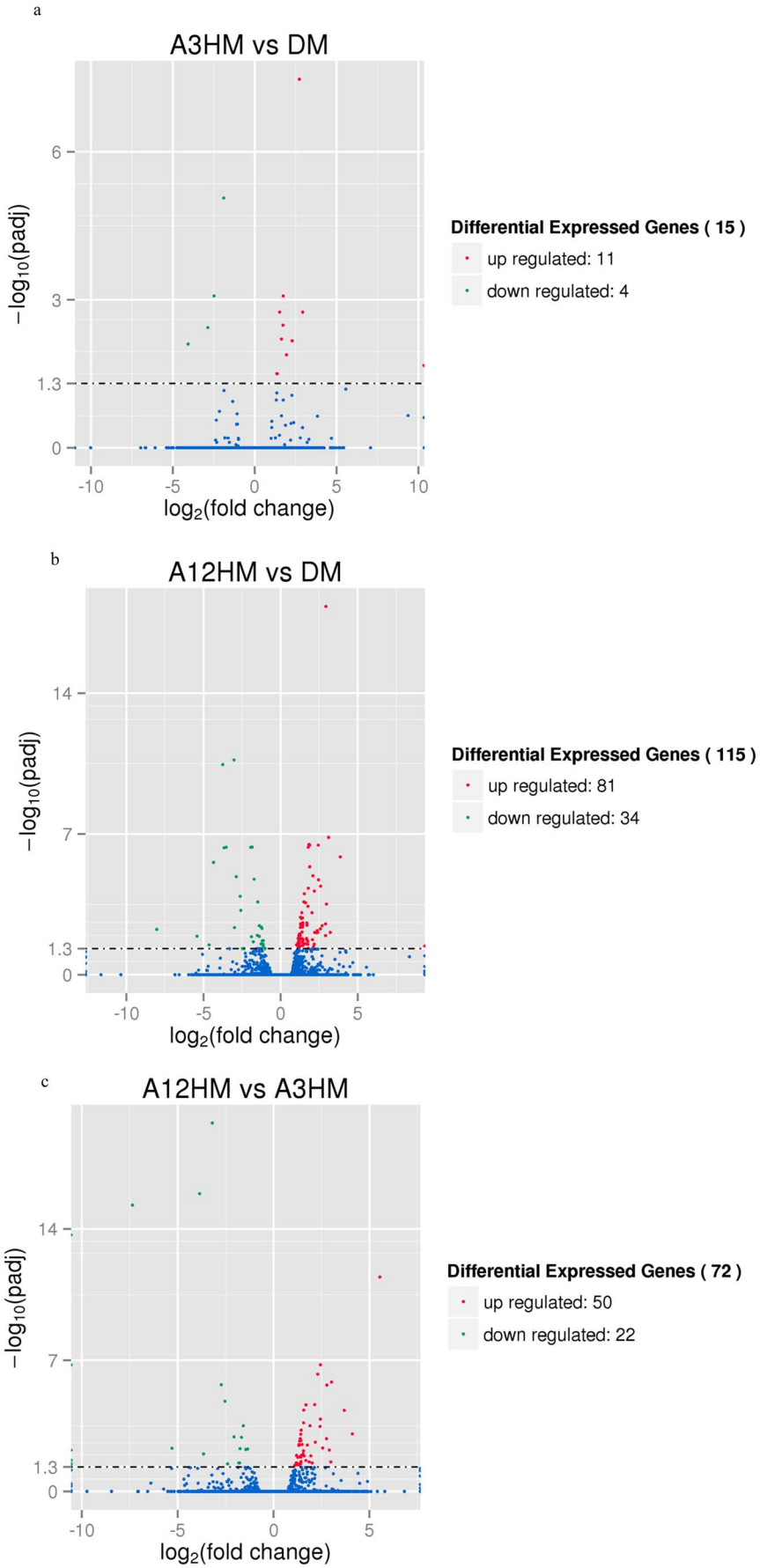

Figure 2 | Analysis of differentially expressed genes among the treatments. Figure 2a: genes that were differentially expressed between A3HM and DM; Figure 2b: genes that were differentially expressed between A12HM and DM; Figure 2c: genes that were differentially expressed between A3HM and A12HM.

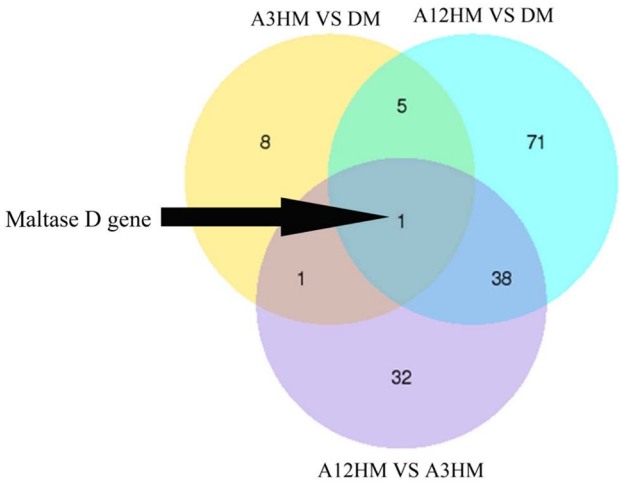

Figure 3 | Venn diagram of the genes that were differentially expressed between the evaluated samples.

transduction pathways. Therefore, studies addressing the molecular mechanisms underlying mating processes will not only enable us to better understand tephritid fruit fly ecology and evolution but may also aid in developing sustainable management strategies that rely on mating disruption[18]. In recent years, sterile males have been used to control the oriental fruit fly[8,31]; therefore, the ability of sterile males to compete with other males in the field is important. We found that males fed more D-glucose or sucrose became stronger and mated more often than males fed yeast extract alone. Thus, we can breed males by rearing them with more D-glucose or sucrose to compete with wild males while using sterile males to control oriental fruit flies.

Many studies have shown that nutrient reserves drive male reproductive behavioral patterns. For example, male Mediterranean fruit flies that are engaged in alternative mating tactics exhibit relatively high sugar levels[32,33], and lekking males are significantly heavier and present significantly higher sugar and protein levels than do resting males[6]. In the Caribbean fruit fly *Anastrepha suspense* (Loew), even sex pheromone production is significantly affected by sugar[34]. Mating behavior is also constrained by the cost of searching and assessment as well as the accuracy of mate assessment mechanisms[35]. Moreover, the time lost searching for an alternative mate may reduce reproductive rates[36], a process that can be energetically expensive. In the oriental fruit fly, the starch and sucrose metabolism and galactose metabolism pathways were significantly enriched; furthermore, the differentially expressed maltase D gene expression and D-glucose levels were higher after mating (Figure 4 and Figure 5). These results indicate that the mating process requires high levels of D-glucose and that males must reserve D-glucose after one mating process prior to another mating event.

Higher body weight and maltase D expression were observed in the males reared with sucrose mixed with yeast extract and D-glucose mixed with yeast extract compared with males reared with yeast extract alone (Figure 6 and Figure 7). Furthermore, during mating competition, more males reared with sucrose mixed with yeast extract and D-glucose mixed with yeast extract mated than males reared with yeast extract alone (Figure 8). These results indicate that D-glucose can dramatically affect male mating competition, which is consistent with previous studies[32,33]. However, the body weights and competition abilities of males reared with sucrose did not differ significantly compared with those of males reared with D-glucose (Figure 6 and Figure 8). Sucrose can be converted into glucose through the starch and sucrose metabolism and galactose metabolism pathways; thus, sucrose may be converted into D-glucose to control male body weight and mating competition. To investigate this possibility, additional experiments are required to discern the mechanisms by which sucrose and D-glucose regulate mating competitiveness.

We have clearly demonstrated that D-glucose, which is the metabolic substrate of maltase D, is critically important to male fly mating competition due to its effect on body weight and competitive fitness during mating (Figure 6 and Figure 8). Significantly higher D-glucose levels were observed in males at 18:00 h, when flies display the most active mating behavior (Figure 9). Sugars are critical substrates for insect metabolism[37], and glucose is the main fuel for insect metabolism but is normally present at low concentrations in the hemolymph due to its reducing and osmotic properties[38]. However, within body fat, glucose can be converted into trehalose, which is abundantly available in the hemolymph[39]. Studies have indicated that trehalose is vitally important to various activities during the insect lifetime[40,41]. However, our male fly experiments did not examine the conversion of D-glucose to trehalose, and whether the trehalose concentration affects male fly mating remains unknown. These issues should be investigated in depth.

Finally, a study on sugar utilization mechanisms and their roles in regulating mating competition in *B. dorsalis* males will be critically important for defining and/or refining artificial diets for this species. These results will mainly be used for the biocontrol of this pest and will lead to the release of stronger sterile males fed with higher D-glucose or sucrose levels. By clearly identifying the molecular mechanism that mediates mating success in male oriental fruit flies, it may be easier to control the damage caused by this pest by releasing sterile males or through molecular methods.

Methods

Insects. Oriental fruit flies were reared in the laboratory at 25 ± 1°C under a 16 : 8 h light:dark photoperiod and 70–80% RH. The positive artificial diets for adults were yeast extract and sucrose mixed at a 1 : 1 ratio[42]. Artificial diets with yeast extract or D-glucose mixed with yeast extract at a 1 : 1 ratio were also used to identify the roles of D-glucose and sucrose in male mating. Males were collected during mating (DM), 3 h after mating (A3HM) and 12 h after mating (A12HM); 1 individual was collected for each sample. Three replicate samples were collected for each treatment. To prepare the reference transcriptome, males both engaged in the mating process and after the mating process were collected. The insects herein were collected 7 days after emergence, when mating behavior is most active.

Preparing the total RNA and cDNA libraries and Illumina sequencing. The total RNA was extracted using the TRIzol reagent (Invitrogen, California, USA) following the manufacturer's instructions. The RNA sample quality was examined through 4 steps: (1) analysis of the RNA sample degradation and contamination via agarose gel electrophoresis; (2) examination of the RNA purity using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific); (3) precise quantification of the RNA concentration using a Qubit® 2.0 fluorometer (Life Technologies); and (4) accurate detection of the RNA integrity using an Agilent 2100 Bioanalyzer (Agilent Technologies). The quantified RNA samples were enriched for mRNA using magnetic beads with an oligonucleotide (dT), and the enriched mRNA was then fragmented into 400–600 bp fragments using fragmentation buffer and used as a template to synthesize both the first-strand cDNA and the second-strand cDNA. The double-stranded cDNA that was generated was purified using AMPure XP beads, the end of the double-stranded cDNA was then repaired, a base A tail was added, and sequencing adapters were connected to the end of the double-stranded cDNA. Finally, the fragments were selected based on size (400–600 bp fragments) using AMPure XP beads. PCR was used for amplification, and the PCR products were purified using AMPure XP beads to generate the cDNA libraries. The prepared libraries were sequenced using the Illumina HiSeq platform. The paired-end method was used for sequencing. The sequencing read length was 200 bp. We sequenced 2.66 G clean bases for each sample.

Reference transcriptome preparation. *Detecting the sequencing error rate distribution.* Using the Illumina HiSeq TM2000 platform, the relationship between the sequencing error rate (e) and the base quality value (Qphred) can be described as follows.

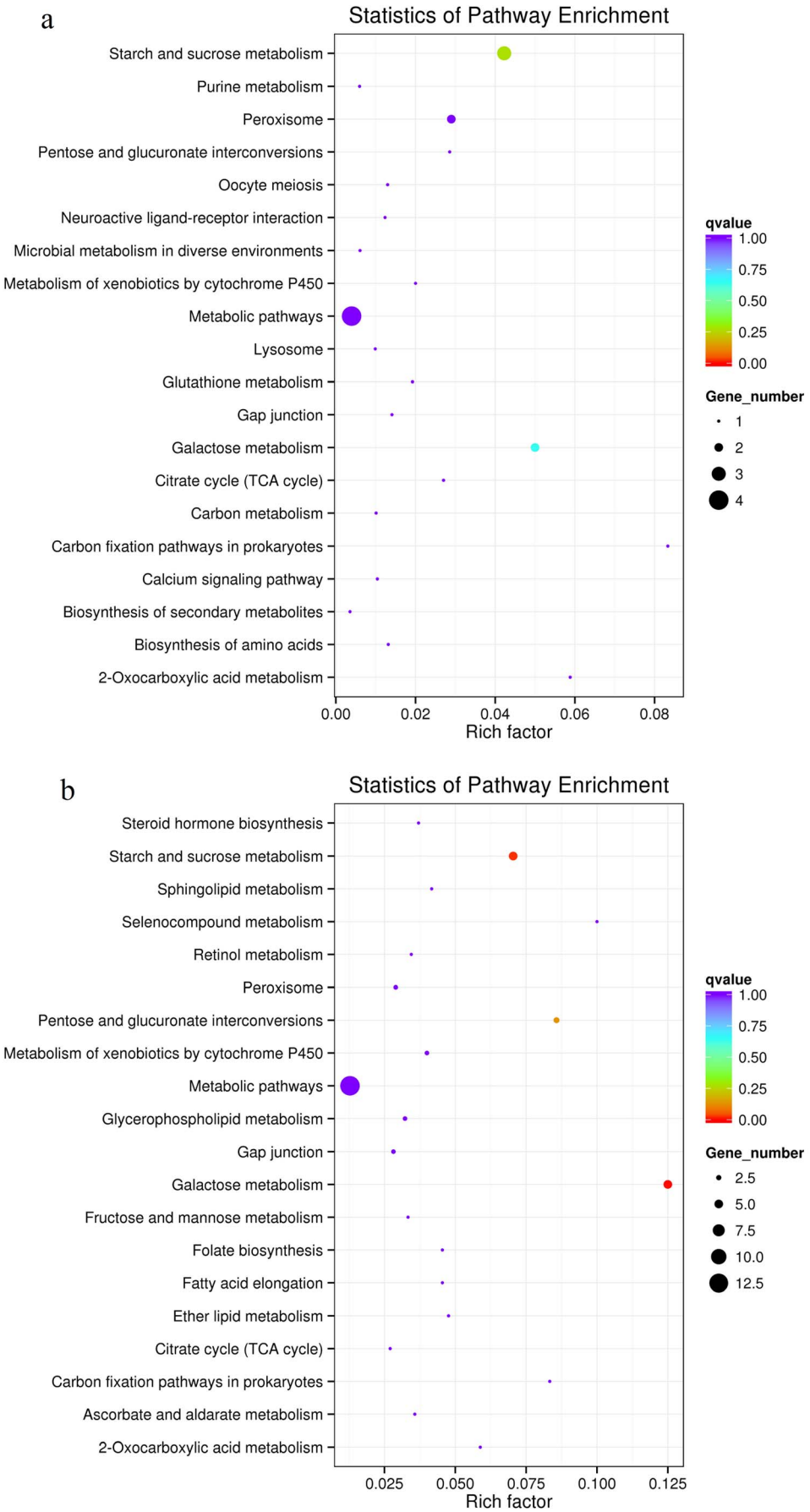

Figure 4 | KEGG significant enrichment analysis for differentially expressed genes. Figure 4a: KEGG significant enrichment analysis for the genes that were differentially expressed between A12HM and A3HM; Figure 4b: KEGG significant enrichment analysis for the genes that were differentially expressed between A12HM and DM.

Table 5 | Differentially expressed genes involved in significantly enriched pathways

Combination	Pathway	Gene ID	Fold change	padj	Function description
A12HM vs. DM	Galactose metabolism	comp42195_c0	1.2073	0.0093	maltase H
		comp42350_c0	1.1787	0.0116	UTP-glucose-1-phosphate uridylyltransferase
		comp41920_c0	1.7601	0.0303	maltase D
		comp42405_c0	1.1465	0.0239	beta-glucosidase
		comp40303_c0	1.0816	0.0296	alpha-glucosidase
	Starch and sucrose metabolism	comp41512_c0	1.1007	0.0338	UDP-glucuronosyltransferase 2B13
		comp42195_c0	1.2073	0.0093	maltase H
		comp42350_c0	1.1787	0.0116	UTP-glucose-1-phosphate uridylyltransferase
		comp41920_c0	1.7601	0.0303	maltase D
		comp40303_c0	1.0816	0.0296	alpha-glucosidase
A12HM vs. A3HM	Galactose metabolism	comp42195_c0	1.5703	4.59E-05	maltase H
		comp41920_c0	2.4211	0.000345	maltase D
	Starch and sucrose metabolism	comp42195_c0	1.5703	4.59E-05	maltase H
		comp39387_c0	1.3355	0.003135	trehalase
		comp41920_c0	2.4211	0.000345	maltase D

$$Qphred = -10\lg(e)$$

Using Illumina Casava version 1.8 (http://support.illumina.com/downloads/casava_182.html), the relationship between the base calling and Phred score is described (Table 6).

Detecting A/T/G/C content distribution. Because the sequences were randomly fragmented, the G and C or A and T base contents should theoretically be equal. Thus, the G and C distribution levels were detected for each sequencing cycle to determine whether the A and T or G and C levels differed.

Screening the sequenced data. Raw sequencing data contain adapters and low-quality data. To generate accurate analysis results, the data should be screened to produce clean reads that can be used in subsequent analyses. The screening process[43] can be described as follows: (1) discard the data with adapters; (2) discard those data for which the percentage of N bases (N: the bases that cannot be sequenced) is greater than 10%; and (3) discard low-quality data (for which the percentage of Qphred <5 bases is greater than 50%).

Transcript assembly. Trinity software[44] was used for transcript assembly (version: v2012-10-05; min_kmer_cov = 2; the default settings were used for the remaining parameters). The assembly process was previously described[45]. The sequences assembled by Trinity were used as reference sequences for the subsequent analysis. For each gene, the longest assembled sequence (more than one assembled sequence (transcript) for each gene) was regarded as a unigene for functional annotation and the length of the unigene was calculated.

Annotation. BLAST searches against the non-redundant (NR) and nucleotide sequence (NT) databases NCBI, SWISS-PROT, KEGG and KOG were performed with an e-value cut-off at 1e-5. Gene Ontology terms were assigned using Blast2GO v2.5[46] by searching the NR database.

Sequencing the treated samples and gene expression analysis. *Sequencing and cDNA libraries were prepared for the treated samples at different reproductive statuses.* For each sample with a different reproductive status, cDNA libraries (DM, A3HM and A12HM) were prepared using the reference transcriptome preparation method.

Gene expression levels. Clean reads (extracted by the screening sequenced data process used in the preparation of reference transcriptome) for each sample were mapped onto the reference transcriptome. In the mapping process, the software RSEM was used following the manufacturer's instructions[47]. The mapping results from RSEM were calculated to generate the read count for each gene and converted to RPKM (reads per kilobase per million mapped reads) using the estimation method in Mortazavi et al. (2008)[48]. To verify the expression profile of each sample, the RPKM density distribution was generated.

Overall RNA-Seq data quality assessment. To determine whether the sequence data volume was sufficient, a saturation curve was generated for each sample, and to examine the reliability and rationality of samples, correlations between repeated samples were examined.

Analyzing the differentially expressed genes. To detect the RPKM distribution under different experimental conditions, the gene RPKM density distributions were com-

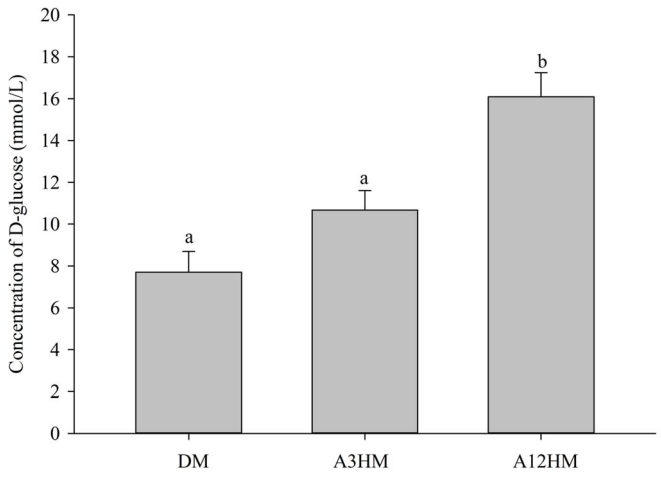

Figure 5 | D-glucose content in males with different reproductive statuses. DM: males during mating; A3HM: males 3 h after mating; A12HM: males 12 h after mating.

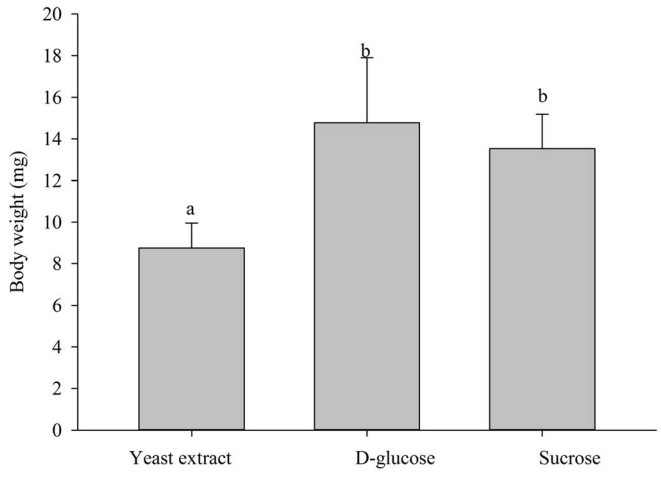

Figure 6 | Comparison of the body weights (mean ± SE) for male flies reared with yeast extract, D-glucose and sucrose. Yeast extract: males reared with only yeast extract; D-glucose: males reared with D-glucose mixed with yeast extract at a 1 : 1 ratio; sucrose: males reared with sucrose mixed with yeast extract at a 1 : 1 ratio. Bars labeled with the same letter do not differ significantly from each other ($p > 0.05$, Turkey's test).

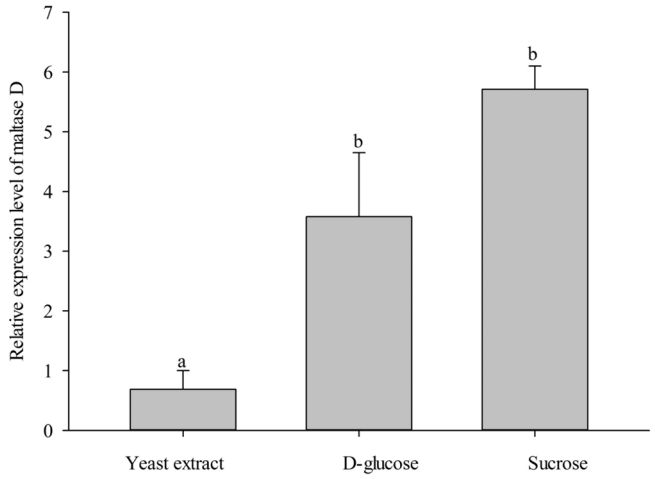

Figure 7 | Relative expression levels of maltase D (mean ± SE) in males reared with yeast extract, D-glucose and sucrose. Yeast extract: males reared with only yeast extract; D-glucose: males reared with D-glucose mixed with yeast extract at a 1 : 1 ratio; sucrose: males reared with sucrose mixed with yeast extract at a 1 : 1 ratio. Bars labeled with the same letter do not differ significantly from each other ($p > 0.05$, Turkey's test).

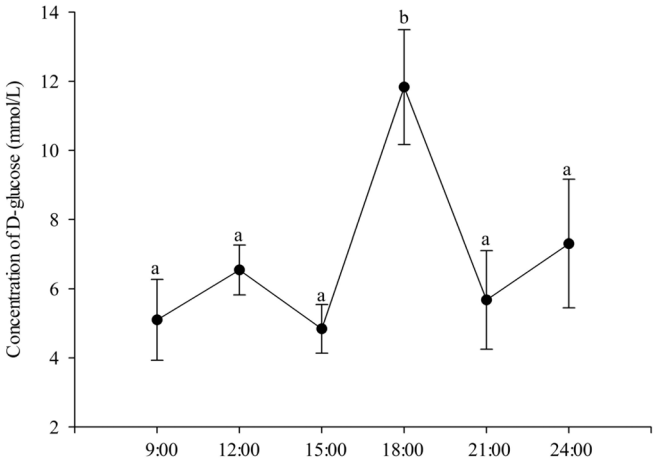

Figure 9 | Dynamics of D-glucose concentrations (mean ± SE) as detected in male oriental fruit flies. Bars labeled with the same letter do not differ significantly from each other ($p > 0.05$, Turkey's test).

pared. With a screening threshold at padj <0.05, DESeq[48] was introduced to analyze the read count data and to identify differentially expressed genes under different experimental conditions.

KEGG analysis of the significant enrichment of differentially expressed genes. To identify the main biochemical pathways and the signal transduction pathways in which differentially expressed genes are involved, a significant pathway enrichment analysis was performed using the KEGG database. Furthermore, the downstream products of the differentially expressed genes in various pathways were investigated to identify the substrate that affects male fly mating competitiveness.

D-glucose content measurements in males with different reproductive statuses. D-glucose levels in males with different reproductive statuses (DM, A3HM and A12HM) were measured individually using a D-glucose determination kit produced by the Nanjing Jiancheng Bioengineering Institute following the manufacturer's instructions; 5 repeats were performed for each treatment.

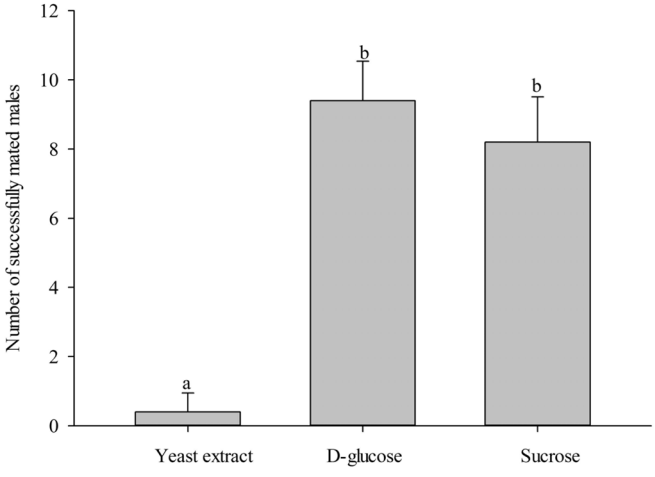

Figure 8 | Number (mean ± SE) of mated males from the groups reared with yeast extract, D-glucose and sucrose. Yeast extract: males reared with only yeast extract; D-glucose: males reared with D-glucose mixed with yeast extract at a 1 : 1 ratio; sucrose: males reared with sucrose mixed with yeast extract at a 1 : 1 ratio. Bars labeled with the same letter do not differ significantly from each other ($p > 0.05$, Turkey's test).

Maltase D expression in males reared with different diets. The TIANscript RT Kit (Tiangen, China) was used to reverse-transcribe complementary DNA using 1 μg of total RNA from males reared with yeast extract alone, sucrose mixed with yeast extract and D-glucose mixed with yeast extract. The $2^{-\Delta\Delta Ct}$ method[49] was used to measure the relative expression levels of maltase D, which were normalized by α-tube[21]. Each sample was analyzed using three independent replicates in a total reaction volume of 25 μL containing 0.5 μL of each primer (diluted to 10 μM), 12.5 μL of SYBR® Premix Ex Taq™, 9.5 μL of ddH2O and 2 μL of template. The maltase D gene primers used for amplification were as follows: forward: TCGGCGTCAATTTTGCCATC; reverse: GGCCACTTCGGTTATGACA. PCR amplification was performed using an Mx3000P spectrofluorometric thermal cycler (Stratagene), beginning with a 2 min incubation at 95°C followed by 40 cycles of 95°C for 20 s, 55°C for 30 s and 72°C for 30 s. A melting curve analysis was performed to confirm the amplification specificity.

Body weight and mating competition of males reared with different diets. After emergence, the males were selected and reared with yeast extract only, D-glucose mixed with yeast extract or sucrose mixed with yeast extract. After 7 days, the fresh body weight of the males was measured individually with 20 repeats for each treatment; mating competition experiments were performed as described in Shelly & McInnis (2001)[50], with 20 females reared with sucrose mixed with yeast extract and 60 males from the groups reared with yeast extract, D-glucose mixed with yeast extract and sucrose mixed with yeast extract (20 males from each group), which were placed in one cage (30 cm*30 cm*30 cm). The abdomens of the males reared with D-glucose and sucrose were painted green and red, respectively. In the cage, the males and females that successfully mated were recorded and removed. The number of successfully mated males was observed and recorded from 17:00–20:00; 5 repeats were performed. The male mating competitiveness was compared by assessing the numbers of mated males.

D-glucose concentration measurements in males from 9:00-24:00. Sexually mature males (unmated and reared with sucrose mixed with yeast extract) were sampled every 3 h from 9:00–24:00, and the D-glucose levels were measured individually using a D-glucose determination kit produced by the Nanjing Jiancheng Bioengineering Institute and following the manufacturer's instructions; 3 or more repeats were performed for each treatment.

Statistical analysis. Differences in the relative expression of maltase D; fresh body weight; number of mating males in groups of males reared with yeast extract alone, D-glucose mixed with yeast extract and sucrose mixed with yeast extract; and D-glucose levels in male hemolymph were compared via a one-way analysis

Table 6 | Relationship between base calling and Phred score

Phred score	Base calling errors	Accurate base calling rate	Q-score
10	1/10	90%	Q10
20	1/100	99%	Q20
30	1/1,000	99.9%	Q30
40	1/10,000	99.99%	Q40

of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons.

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Author contributions

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