Supplementary Materials

De novo assembly of cricket transcriptome

Transcriptome short reads were assembled *de novo* by ABySS then Trans-ABySS [1], Velvet-Oases [2] and Trinity [3].

For the ABySS-Trans-ABySS and Velvet-Oases assembly strategies, we assembled datasets from each individual using similar assembly parameters (k-mer value = 43 to 91 with step of 4). The Velvet-Oases assembly employed Velvet (version 1.2.07) using a set of k-mer values [4], followed by Oases (version 0.2.08) with the default parameters [2]. In ABySS-TransABySS assembly, individual k-mer assemblies were carried out by ABySS version 1.3.4 with the scaffolding option off and contig end erosion off [1]. Trans-ABySS (version 1.3.2) was used after ABySS to merge the individual k-mer assemblies with default parameters [5]. After the initial assemblies, contigs of individual samples were merged with both strands information using the accurate mode of CD-HIT-EST version 4.5.4 with the sequence identity threshold at 100% and a word size of 8 [6]. Since the combined set will contain small variations, such as allelic variations, small insertions or deletions, GICL (release date 2010-07-22) was then used to further reduce the redundancy level[7]. Contigs overlapped with at least 50 bp with a minimum identity of 95% were collapsed into single contigs, and the maximum length of unmatched overhangs was set to 100 bp.

Whereas for the Trinity assembly, we used a merged dataset from eight individuals. Trinity release 2012-06-08 was employed with the ALLPATHSLG error correction, and the paired fragment length was set to 200 bp. In the redundancy removal step, only CD-HIT-EST was used remove shorter transcript was entirely covered by longer one with 100% identity.

Performances comparisons among Trans-ABySS, Oases and Trinity

There were no standard criteria to evaluate the quality of transcriptome assemblies [8]. Researchers usually assess the quality of an assembly mostly by looking at the contiguity and accuracy of the assembly [9]. Here, we measured results in terms of transcript completeness, accuracy, and sample specificity, to compare the performance of three publicly available assemblers, Trans-ABySS, Oases and Trinity.

Trans-AbySS and Oases, the two multiple k-mer assembly tools using an assembly merging stratage, outperformed Trinity, the single k-mer assembler performing a single assembly with combined reads. In particular, Oases performed the best among the three assemblers, in assembly accuracy, contiguity and sample specificity. After the initial assembly, the Trinity assembly has the largest N50 and the least number of transcripts being assembled. The procedure of redundancy removal applied to the Trans-ABySS and Oases assemblies has greatly improved the quality of the transcriptome. In the final set of transcripts, the average contig length and N50 of the Oases assembly was significantly higher than those of Trans-ABySS and Trinity assemblies. Shown by the pairwise comparison, the Oases assembly overlapped more of their counterpart (Suppl Table 1). Oases's highest proportion of transcripts being overlapped by other two assemblers also supports it as the best assembler in this study by transcript contiguity. Contiguity indices such as N50 can give an indication about how fragmented the recovered transcripts are.

RNA-Seq analyses often deal with multiple samples. The greatest concern for assembling samples individually is the increase of redundant transcripts, while assembling all samples at the initial step may result in the loss of sample-specific transcripts. We compared the sample specificity of different merging strategies in transcriptome assemblies in this study. For the Trans-ABySS and Oases assemblies merged from individual assemblies, in the initial stage they had more contigs that were much larger than those of Trinity. The merging of assemblies from individual samples using CD-Hit and GICL had greatly reduced the total number and size of transcripts and increase N50s (Suppl Table 2). The Trinity assembly based on merging all reads across all samples did not increase the proportion of mappable reads (Suppl Table 1). Although Trinity in principle provides additional information about isoform/paralog/allele structure of the transcriptome [3], the low mapping percentage in sample specificity has shown that many of the isoforms assembled by Trinity may not truly reflect the real data.

Due to lack of genomic resources for the Australian black field cricket, the completeness of the transcriptomes was firstly measure by the BLAST search to an existing Hawaiian trigonidiine cricket gene index. Although the total number of hits from Oases assembly is slightly lower than that of from Trans-ABySS assembly, the number of high quality hits from

Oases is higher. However, the completeness of the Hawaiian trigonidiine cricket gene index is remaining unknown, the number of hits to the Hawaiian trigonidiine cricket gene index cannot be used as an indication of transcriptome completeness, the *Drosopila* transcriptome from Flybase were considered as 'gold standard' reference in our studies. Among the three assemblers, Oases had the highest number of hits and high quality hits to the Drosophila transcriptome, it also had the highest number of high quality unique Drosophila transcript hits.

Transcriptome Annotation

Gene name assignment is crucial for drawing biologically meaningful conclusions from RNA-seq experiments and for comparing results among different studies. Vijay and colleagues [10] suggested that in assigning orthologous genes from distantly related genomes, BLAST-based orthorlogy detection such as BLAST2GO would potentially have higher assignment success than suffix-tree based methods such as NUCmer and PROmer [11]. Stringent filtering on blast scores, alignment length and reciprocal-best-hits are thus crucial to guard against false detection of orthologous genes [12].

To functionally annotate the cricket transcriptome, the final assembled transcripts (≥200 bp) were submitted for homology and annotation searches using Blast2GO software (version 2.4.4; http://www.blast2go.org/webcite). For BLASTX against the NR database, the threshold was set to E-value≤10^{-6}. GO classification was achieved using WEGO software [13]. Enzyme codes were extracted and Kyoto Encyclopedia of Genes and Genomes (KEGG)[14] pathways were retrieved from KEGG web server (http://www.genome.jp/kegg/).

Using BLAST2GO (version 2.4.4), we were able to assign gene annotations to 46,774 of the 80,476 transcripts from the Oases assembly. Gene ontologies (GOs) were also assigned to the assembled transcripts by BLAST2GO. There were a total of 90,357 gene ontology (GO) terms on all GO-levels associated with the 46,774 identified genes. Of these, assignments to level two GO-terms Molecular Function (40,244) made up the highest category, followed by Biological Process (33,225) and Cellular Components (16,888).

Supplementary Results

Age-related gene expression differences

We also found that females reared in the calling treatment and males in the silent treatment overexpressed Osiris proteins compared to females in the silent treatment and males in the calling treatment, respectively. The Osiris gene family is a family of approximately 20 genes first described in *D. melanogaster* that are highly conserved and only found within insects [15]. Although the genes are still of unknown function, they are the molecular basis of the unique Triplo-lethal locus [16] and have a secretion signal peptide and four domains, one of those being a putative transmembrane domain.

Treatment and sex related differences

Although we did not study mating, spermatogenesis, CHCpheromonal communication or learning in this study, these aspects have were the focus of previous studies in this and a sister-species, *T. oceanicus* [17]. As these studies followed a similar protocol, we discuss the genes associated with mating and oogenesis in greater detail here.

Males reared with recorded calls

Four wheel drive is also associated with an increase in spermatogenesis during cytokinesis [18-20] which may explain why *T. oceanicus* males reared in a calling environment demonstrate greater sperm viability [17]. In line with these increases in spermatogenesis, males also overexpressed *spargel*, a gene whose expression is associated with increased energy metabolism associated with mitochondrial regulation [21, 22]. However, males reared in our calling treatment also demonstrated an increased expression of *Nascent polypeptide associated complex protein alpha subunit* [23] and *Chromodomain-helicase-DNA-binding protein 1 [24, 25]*, genes associated with decreased fertility, in part due to decreased success in zygotic mitoses [24]; these gene expression results do not support the results from *T. oceanicus*. In addition, although males from the silent treatment had an increased expression of *lingerer*, a gene associated with increased copulation duration [26], males also had increased expression of *discs large 1* where mutants show decreased mating behaviour.

The results regarding spermatogenesis and mating behavior in our males reared in silence are unfortunately not as clear as our other gene-phenotype associations specifically examined in our experiment. For example, males in reared in silence also overexpressed single genes associated with mating success (*yellow*) [27], and successful spermatogenesis (*Receptor for Activated C Kinase 1*) [28]. Nonetheless, we mention then here as exploring these candidate genes in future research specifically examining sperm competitive ability and mating behavior may prove fruitful.

Females reared with recorded calls

The calling treatment increased the expression of genes associated with mating and sexual communication. Females increased expression of *Desaturase 1*, which is associated with sexual communication through pheromones [29, 30], and along with increased oogenesis, *spinster* is also associated with increased mate receptivity [31]. Females in the silent treatment only increase a single gene involved in mating decisions, *pale*, a gene associated with increased attractiveness between males and may also increase receptivity of females [32]. These results may help explain why females reared in higher densities of calls show increased receptivity and motivation to find males when searching [33].

Learning and memory

In addition to the above, we also observed several unique genes involved in learning and memory expressed by males and females from the different treatments (Figure 3, Supplemental Excel file). Of these treatment by sex combinations, females reared in the silent treatment increased the expression of three different genes: *CRMP [34]*, *Ankyrin 2 [35]*, and *Argonaute-1* [36] where disruption of the latter two results in cognitive impairment in learning and memory. Males in the silent treatment increased expression of *cAMP-dependent protein kinase 1* associated with memory increases [37] and learning [38, 39] and *Neuroglian* which is positively associated with neurogenesis [40, 41]. In contrast, females in the calling treatment only expressed a single unique gene, *aru*, which is associated with memory formation [42], and males in the calling treatment increased expression of *lethal (2) giant larvae*, which is involved in 26 different biological processes associated with neuronal and nervous system development.

Our results may help explain as to why individuals form other species that are reared under non-social conditions (in our case, in silence) can possess improved learning and memory retention [43]. Future studies will be necessary to determine whether *T. commodus* reared in silence also have improved learning and memory.

Transcription factors

Here we outline the identified transcription factors that were not directly related to our phenotypic study, but yield interesting information for future studies in this and other organisms.

Individuals in the silent treatment

Two other transcription factors seem to play a greater role in males. The first, *spalt-related*, is associated with male genital development [44] and is also associated with antennal development and the sensory perception of sound [45]. Sound perception in *Drosophila*, however, is associated with antennal development, which is not the case in crickets. Thus, whether *spalt-related* has the same role in *T. commodus* is unknown. The second gene, *extra macrochaetae*, is associate with inter-male aggression [46], spermatid development [47] and brain development [48].

Individuals from the calling treatment

Individuals from the calling treatment also showed an overexpression of transcription factors associated with reproduction: *daughterless* coordinates differentiation during follicle formation [49], and *spindle E* is involved in 14 different roles associated with meiosis and oogenesis [50]. These fall in line with our results that females from the calling treatment produced more eggs through their lifetime.

Expressed by females

Females expressed four unique transcription factors associated with neurogenesis when compared to males. *Domino* is a chromatin regulator that, among being associated with *E2F* (a key regulator of cell proliferation and differentiation, [51], it regulates dendrite development resulting in a greater number of longer branches [52, 53]. A second transcription factor,

cubitus interruptus, is a component of hedgehog signaling and is essential for the development of dorsal class I da neurons [51]. *Leonardo* (14-3-3ζ) is a gene that regulates protein folding and stabilizations [54]. In regards to our study, *Leonardo* is of particular interest as it is involved in facilitating olfactory learning and long-term memory in *Drosophila* [55]. These neuronal and sensory system transcription factors are likely to be particularly relevant for females as they are the mate-searching species.

Expressed by males

neutralized and *schnurri*, are associated with neurogenesis [48] and learning [56], respectively. The other two transcription factors are not well documented; *PNUTS*, is associated with development and growth, however little is known about its exact function [57] and *female sterile*, is associated with gametogenesis in females [58].

Learning and Memory

We also found an overexpression of transcription factors associated with neuronal development. Interestingly there was a mixture of genes that positively and negatively regulate neuronal branching. There was an increase in expression of *14-3-3ε*, which, although involved in axon guidance, is not documented to play as central a role in neuronal development as *Leonardo* [59]. Two other transcription factors negatively regulated neurogenesis; *Sin3A* functions in transcriptional repression [51] and *brain tumor* negatively regulates cell proliferation in brain development [60] and neuromuscular junctions [61]. We also found an overexpression of three other transcription factors that are associated with neurogenesis according to FlyBase: *Without children, brahma*, and *daughterless;* however, their exact role in brain development is not well characterized.

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