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Transcript profiling of the meiotic drive phenotype in testis of *Aedes aegypti* using suppressive subtractive hybridization

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Abstract

The meiotic drive gene in *Aedes aegypti* is tightly linked with the sex determination locus on chromosome one, and causes highly male-biased sex ratios. We prepared cDNA libraries from testes from the *Ae. aegypti* T37 strain (driving) and RED strain (non-driving), and used suppressive subtraction hybridization techniques to enrich for T37 testes-specific transcripts. Expressed sequence tags (ESTs) were obtained from a total of 2,784 randomly selected clones from the subtracted T37 (subT37) library as well as the primary libraries for each strain (pT37 and pRED). Sequence analysis identified a total of 171 unique genes in the subT37 library and 299 unique genes among the three libraries. The majority of genes enriched in the subT37 library were associated with signal transduction, development, reproduction, metabolic process and cell cycle functions. Further, as observed with meiotic drive systems in *Drosophila* and mouse, a number of these genes were associated with signaling cascades that involve the Ras superfamily of regulatory small GTPases. Differential expression of several of these genes was verified in *Ae. aegypti* pupal testes using qRT-PCR. This study increases our understanding of testes gene expression enriched in adult males from the meiotic drive strain as well as insights into the basic testes transcriptome in *Ae. aegypti*.

Keywords

Culicidae; segregation distortion; expressed sequence tag; testes transcriptome

1. Introduction

Mosquito-borne diseases remain or have re-emerged as significant global health problems with few obvious solutions on the horizon. At present, mosquito population reduction or suppression is the most effective strategy for controlling transmission of these diseases. However, rapid selection for insecticide resistance is common among mosquito populations subjected to concerted control efforts (Hemingway and Ranson, 2000; Nauen, 2007). Alternate disease control strategies based on genetic manipulation of mosquitoes to render them incapable of transmitting pathogens followed by their release into and subsequent replacement of natural vector competent populations are being considered (Hill *et al.*, 2005).

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While considerable progress has been achieved in the development of stable mosquito transgenesis and identification of anti-pathogen effector molecules (Speranca and Capurro, 2007; O'Brochta and Handler, 2008), the greater hurdle of developing efficient methods for driving genes into natural populations remains a significant challenge (Braig, H.R. and Yan, G., 2001; James, 2005; Sinkins and Gould, 2006). Several natural “selfish” genetic elements have been identified that show non-Mendelian inheritance and subsequent potential to spread throughout populations; still, the exact role of such elements in eukaryotic genome evolution remains unclear (Hurst and Werren, 2001) and multiple aspects of their individual utility for genetic control applications remain unresolved (Sinkins and Gould, 2006).

Some populations of the dengue vector mosquito *Aedes aegypti* are known to carry an endogenous meiotic drive system that distorts meiosis in favor of male-determining gametes (Craig et al., 1960; Hickey and Craig, 1966a; Mori et al., 2004; Cha et al., 2006a). Sex determination in *Ae. aegypti* and other mosquitoes in the subfamily Culicinae does not involve chromosome dimorphisms, but instead sex is determined by an autosomal gene located on chromosome one, with the male-determining allele (*M*) being dominant to the female-determining allele (*m*); therefore, males are the heterogametic sex (Gilchrist and Haldane, 1947). In *Ae. aegypti*, a meiotic drive gene (M^D) shows strong linkage disequilibrium with the sex locus (Hickey and Craig, 1966b). The M^D gene product likely interacts with a sensitive responder locus in linkage disequilibrium with the *m* allele (m^s) resulting in breakage of the m^s allele-carrying chromosome during meiosis (Newton et al., 1976). Susceptibility to distortion varies among m^s alleles and insensitive alleles (m^i) (Suguna et al., 1977; Wood and Newton, 1991; Cha et al., 2006a). Cage trials have suggested that release of *Ae. aegypti* males carrying a strong M^D gene into drive sensitive populations has potential as an effective tool for population replacement (Cha et al., 2006b). Although rapid selection for tolerance (i.e., drive suppressor) genes has been reported for the *Ae. aegypti* drive system in some studies (Wood and Ouda, 1987), no evidence of similar selection for tolerance was observed in long-term cage trials (Cha et al., 2006b). Theoretical computer simulations confirmed a potential for use in population replacement, but also indicated that factors that might influence the stability of the M^D drive system could limit its practical utility (Huang et al., 2007).

We previously selected for an *Ae. aegypti* strain (T37) that carries a strong meiotic drive gene, with matings between T37 males and $m^s m^s$ females resulting in highly sex ratio biased F_2 progeny with a mean of ~85% males (Mori et al., 2004). Cytological studies of pupal testes from another meiotic drive-carrying strain (T-30) showed a significantly greater incidence of chromosome breakage in the chiasmate arm of the chromosome one bivalent by the end of anaphase I of meiosis (Newton et al., 1976; Wood and Newton, 1991). Further, Giemsa C-banding (Newton et al., 1974) indicated that the preponderance of breaks occurred in the female-determining chromosome, and suggested that the drive mechanism may be associated with crossing-over (Newton et al., 1976). However, no information exists concerning the molecular basis for the M^D drive system. Here we prepared cDNA libraries from testes of T37 males and males from the highly drive sensitive RED strain (Mori et al., 2004). These were subjected to suppression subtractive hybridization (SSH) techniques (Diatchenko et al., 1996) to enrich for cDNAs specific to T37 strain testes. Because the biology of male mosquitoes remains understudied, we also obtained ESTs from each of the unsubtracted primary libraries.

2. Materials and methods

2.1. Mosquito rearing

Two *Ae. aegypti* laboratory strains were used for these studies. The T37 strain which carries a strong M^D gene and insensitive m^i allele, was selected with mosquitoes collected in

Trinidad (Mori *et al.*, 2004). The RED strain is highly sensitive to drive (Hickey and Craig, 1966a; Mori *et al.*, 2004). Matings between T37 strain males and drive sensitive females result in ~85% male offspring. Larvae for each strain were reared on a suspension of bovine liver powder and kept in an environmental chamber at 26°C, 85% relative humidity, and a 16 hour light/8 hour dark cycle with a 1-h crepuscular period at the beginning and end of each cycle. Pupae were transferred to small cups and placed in 20×20×20 cm cages until adult emergence. Adults were maintained on a 2% sucrose solution. Adult females were blood-fed on anesthetized rats 1 week after emergence. Our protocol for maintenance and care of experimental animals was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Notre Dame.

2.2. Construction of testes cDNA libraries

Testes were dissected from ~150 adults at ~24 hours post-emergence for each strain. Tissue extractions were performed by placing individual males on a glass slide, adding saline, and using an insulin needle to tear the abdomen and separate the testes from the abdominal segments. Although care was taken to isolate testes tissue only, some additional tissues (e.g., accessory glands, etc.) were likely included. These were placed in 1.5 ml centrifuge tubes containing Trizol™ reagent (Invitrogen, Carlsbad, CA, USA) on ice. Total RNA was extracted following the manufacturer's protocol and precipitated with isopropanol. The precipitates were resuspended in transcription buffer and reverse-transcribed using the Clontech Smart PCR cDNA synthesis kit (Clontech, Mountain View, CA). Following first-strand cDNA synthesis using the modified oligo(dT) 3' SMART CDS Primer IIA, suppressive subtractive hybridization (SSH) was performed using the PCR-select cDNA subtraction kit (Clontech) according to the manufacturer's protocol. To identify genes that were specifically expressed in testes from T37 males, the cDNA synthesized from T37 strain testes was used as the tester cDNA, while the cDNA generated from RED strain testes was used as the driver cDNA (Fig. S1). Briefly, the tester and driver cDNAs were restriction digested with *RsaI* to obtain blunt-ended cDNA. The tester cDNA was then subdivided into two groups, and two adaptors (adaptor 1 and adaptor 2) were ligated to the 5' end of each strand. Two hybridizations were performed with the tester and driver cDNAs; thereafter, only the unhybridized sequences remained and the hybridized sequences were eliminated. In the first round of hybridization, the concentrations of high and low-abundance sequences were equalized. During the second hybridization, only the remaining equalized and subtracted single-strand tester cDNAs bearing different end adaptors could re-associate and form double-strand tester molecules. The entire population of molecules was then subjected to PCR to amplify the desired differentially expressed sequences. Only molecules of the tester sample, which had two different adaptors, could be amplified exponentially. A second PCR amplification was performed using nested primers to further reduce any background PCR product and to enrich differentially expressed sequences.

Following SSH cDNA library construction, the remaining primary cDNAs for both the T37 and RED strains were subcloned into the TOPO TA® vector (Invitrogen, Carlsbad, CA, USA) after incubation with 2 units of Taq polymerase at 74°C to extend an adenosine tail at both ends, following the manufacturer's protocol.

2.3. DNA sequence assembly and analysis

Plasmid DNA was isolated using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA, USA). Sequencing was performed using the ABI Prism Big Dye Terminator Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3700 Genetic Analyzer (Applied Biosystems). Clones from the subtracted T37 testes (subT37) cDNA library were sequenced from both ends using M13 forward and reverse primers, while clones from the primary T37 and RED strain testes cDNA libraries (pT37 and pRED) were sequenced from

one end only with the M13 forward primer. Sequences were trimmed to remove vector sequence, poly(A) tails, and poor quality sequences. Trimmed sequences for each library were assembled using SeqMan (<http://www.dnastar.com/>) and the contig assemblies and singleton ESTs were subjected to BLASTn analysis against the *Ae. aegypti* annotated gene set (version AaegL1.1) in VectorBase, (<http://aaegypti.vectorbase.org>). The cutoff for declaring identity matches was arbitrarily set at a threshold e-value less than e^{-70} . Sequences with no significant matches in the *Ae. aegypti* VectorBase transcript database were investigated using identity comparisons with other organisms or ESTs in the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>). Unique sequences were categorized for biological process and molecular function using Gene Ontology (GO) terms and grouped into functional classes based on BLAST2GO results (<http://bioinfo.cipf.es/b2gfar/showspecies?species=7159>).

2.4. Gene Expression Analysis by quantitative Real Time-PCR

Quantitative Real Time-PCR (qRT-PCR) analysis of selected genes was performed in 96-well reaction plates (MicroAmp Optical 96-well Reaction Plate; Applied Biosystem) on an ABI PRISM 7500 Fast System (Applied Biosystems) with the SDS software version 2.2 (Applied Biosystems). RNA was prepared as described previously for cDNA synthesis. Testes samples for RNA extractions were dissected from the early pupal stage in the T37 and RED strains, as well as from F₁ males from a cross between a T37 strain male and RED strain female. RNA samples were treated with DNase I and subjected to first strand cDNA synthesis. For cDNA synthesis, cDNA was synthesized from ~3 ug total RNA with cDNA 600 U SuperScript II RT® (Invitrogen) at 42°C for 90 min in a 60 ul total volume containing 5× Buffer, 100 nM dithiothreitol (DTT), 5 nM of each of the four deoxynucleotide triphosphates (dNTP, Promega), 20 U/ul of RNaseOUT, and then heat inactivated at 70°C for 15 min. Unincorporated primers present in heat inactivated reverse transcription reactions were digested with RNase H (invitrogen) at 37°C for 20 min.

All qRT-PCR reactions were performed in triplicate according to the manufacturer's instructions using SYBR® Green Master Mix (Applied Biosystems) with 300 nM primers in a 25 ml final reaction volume. Reactions for individual genes contained optimized cDNA template quantities based on standard curves obtained from serial dilutions of a cDNA mixture. Primer Express 3.0 software (Applied Biosystems) was used to design primers as shown in Table 1. Expression values for individual genes were normalized against the housekeeping gene *RpS17* (Morlais *et al.*, 2003). Thermocycling was conducted with an initial 2 min incubation at 50°C and 10 min incubation at 95°C, followed by 40 cycles (95°C, 15 s; 60°C, 60 s) with a single fluorescent reading taken at the end of each cycle. Student's t-test was used to compare relative expression levels observed with the RED strain to that observed with the T37 strain and F₁ males, respectively.

3. Results

3.1. Sequencing, contig assembly and annotation

A total of 2,784 randomly selected *Ae. aegypti* testes cDNA clones from the suppressive-subtracted T37 strain (subT37) library, the T37 strain primary (pT37) library, and RED strain primary (pRED) library were sequenced (Table 2). Most of the cDNA inserts ranged from 600 to 1100 base pairs (bp) and the average cDNA insert size was ~700 bp. After trimming vector sequences and low quality sequences, contigs were assembled and annotated using Blast2GO (Conesa *et al.*, 2005) and BLASTn searches against the *Ae. aegypti* annotated gene set (AaegL1.1) at VectorBase. BLAST results, chromosome locations and complete GO term analyses for individual contigs for each library are provided in Table S1. EST BLAST hits to mitochondrial and ribosomal genes or transposable

elements were discarded from further analysis. All EST sequences have been deposited at GenBank (accession numbers: FK707594-FK710317). The subT37 library ESTs represented 171 unique sequences (Table 2). Of these, 111 sequences were annotated and included 23 conserved hypothetical and 13 hypothetical genes. For the pT37 library, 41 of 81 unique sequences were annotated and included nine conserved hypothetical and 10 hypothetical genes. For the pRED library, 23 of 57 unique sequences were annotated and included nine conserved hypothetical genes.

We identified a total of 299 unique genes among the three *Ae. aegypti* testes cDNA libraries, with little overlap between libraries (Fig. 1). The only common gene among the three libraries was an acetylcholinesterase (*Ace1*) gene (AAEL000511), which has been associated with insecticide resistance in several mosquitoes (Weill *et al.*, 2003), although no resistance-specific mutations in the gene have been identified for *Ae. aegypti* (Mori *et al.*, 2007). The subT37 and pRED libraries shared two genes that included actin-related protein 2/3 complex subunit 1A (AAEL007546) and testis-specific serine/threonine kinase 22c (AAEL014017). The subT37 and pT37 libraries shared 5 genes that included ATPase subunit, putative (AAEL012740), conserved hypothetical protein (AAEL003164), ethanolamine-phosphate cytidyltransferase (AAEL005651), hypothetical protein (AAEL005332) and *skp1* (AAEL009160). The pT37 and pRED libraries shared only a single gene, conserved hypothetical gene (AAEL009076). Limited overlap between the subT37 library and either parent library is likely expected because of the SSH selection process. Limited overlap between the parental libraries likely reflects the relatively low-level sequencing efforts directed toward them.

3.2. Functional annotation

Functional analysis of unique sequences from the subT37 library using Gene Ontology (GO) predictions identified several categories under biological process and molecular function (Table 3). GO categories were determined for 50.9% of sequences under biological process, with cellular process (20.5%) and developmental process (11.1%) representing the largest portion of sequences. GO categories were determined for 50.0% of sequences under molecular function, with catalytic activity (24.6%) and binding (9.9%) representing the largest portion of sequences.

We combined results for functional analysis of the pT37 and pRED libraries (Table 4) because these primary testes cDNAs generally represent expression profiles of common genes expressed in *Ae. aegypti* testes. GO categories were determined for 26.7% of sequences under biological process, and as observed with the subT37 library, cellular process (8.4%) and developmental process (6.9%) represented the largest portion of sequences. GO categories were determined for 28.2% of sequences under molecular function, and catalytic activity (12.2%) and enzyme regulator (12.2%) representing the largest portion of sequences. Of note, most annotated genes expressed in testes in the two strains were of unknown function.

3.3. Genes associated with meiotic drive strain

A categorical function listing of annotated genes associated with the meiotic drive phenotype following the suppressive subtractive hybridization technique (subT37 library) is presented in Table S2. The largest numbers of genes were associated with signal transduction (20 genes), development (20 genes), reproduction (11 genes), metabolic process (11 genes) and cell cycle (10 genes). Gene descriptions for the top 12 most frequent ESTs in the subT37 library are shown in Table 5. The most abundant transcripts were associated with cell cycle, reproduction and development functions, although signal transduction, transcription/translation, death and metabolic process were also represented.

The most abundant transcript was *skp1* (AAEL009160), a cell cycle component that has been implicated with chromatin remodeling during prophase I of meiosis (Nayak *et al.*, 2002; Yang *et al.*, 2006). Because most genes in *Ae. aegypti* have not been assigned to chromosome positions (Nene *et al.*, 2007), the majority of transcripts were of unknown genome location; however, among those transcripts for which information exists all three chromosomes were represented (Table S1).

3.4. Genes associated with primary testes libraries

The combined categorical function listing of annotated genes associated with testes of the *Ae. aegypti* pT37 and pRED libraries is presented in Table S3. The largest numbers of genes were associated with development (11 genes), reproduction (7 genes), metabolic process (6 genes), and signal transduction (5 genes). The most abundant transcript was a conserved hypothetical protein (AAEL009076) under transport function, followed by condensin (AAEL011049) under cell cycle function. Condensin plays an important role in chromosome assembly and segregation during both mitosis and meiosis (Koshland and Strunnikov, 1996; Hirano, 2000).

3.5. SubT37 library validation by qRT-PCR

In order to validate our EST results for the subT37 library-specific genes as up-regulated in early stages of spermatogenesis, we performed qRT-PCR on genes identified as subT37 library specific and located on chromosome one in *Ae. aegypti*, using total RNA extracted from testes tissues dissected from T37 and RED strain males as well as F₁ males from a cross between a T37 strain male and a RED strain female (Fig. 2). Six genes were chosen on the basis of their putative annotations linking them with biological processes previously considered to be important to the meiotic drive system, including genes within the ras super family and associated with chromatin remodeling. Our results indicate that observed expression patterns were very similar for pupal testes from the T37 strain and the F₁ hybrid males for each of the six genes. For three genes (AAEL001537, AAEL012904, AAEL009160), expression levels were reduced in testes from RED strain males relative to the T37 strain and the F₁ hybrid males. For the remaining three genes (AAEL010506, AAEL009884, AAEL009887), expression levels were higher in testes from RED strain males relative to the T37 strain and the F₁ hybrid males.

4. Discussion

We employed suppressive subtractive hybridization techniques to enrich for and characterize meiotic drive-associated transcripts expressed in testis of an *Ae. aegypti* strain (T37 strain) selected for the meiotic drive phenotype. As this study is among the first to examine gene expression in *Ae. aegypti* testis, we also characterized ESTs from the primary meiotic drive strain and non-meiotic drive strain testis cDNA libraries (pT37 and pRED, respectively). Of note, a large number of the 299 genes we identified among the three cDNA libraries were of unknown function, and the frequency of these was very high (>70%) among the pT37 and pRED libraries. This was likely expected as multiple studies with *Drosophila melanogaster* have shown that not only are ~25% of genes in the testis transcriptome testis-specific or testis-enriched, but the largest predicted functional category is of unknown function (White-Cooper, 2010). Although the *Ae. aegypti* genome assembly remains fairly fragmented and only ~1/3 of the genome supercontig assemblies have been placed to general chromosome regions (Nene *et al.*, 2007), we were able to place a number of testis transcripts to chromosome 1 and individual arms for chromosomes 2 and 3 (see Tables S1–S3). It is well known in *D. melanogaster* that the X chromosome contains a paucity of male-specific genes (including testis-enriched genes) compared to the autosomes, likely due to sexual antagonism (White-Cooper, 2010). *Aedes aegypti* (and other culicine

mosquitoes) are interesting here because as previously indicated (see Introduction) sex determination is determined by an autosomal locus located on chromosome 1 and thus, selection due to sexual antagonism should not apply (i.e., no haploid/diploid-male/female effects on gene expression). Still, however, only seven of 50 genes (14%) enriched in the subT37 library and placed to chromosome were located on chromosome 1.

Phenotypically, the meiotic drive system in *Ae. aegypti* (Newton *et al.*, 1976) is somewhat similar to the segregation distorter system (SD) of *D. melanogaster*, although the SD gene is autosomal and located on chromosome 2 (Sandler *et al.*, 1959). SD is one of the best characterized meiotic drive systems, and causes chromatin condensation of the homologous chromosome bearing a sensitive responder to fail during spermatogenesis. It has been shown that a duplicated but truncated *RanGAP* gene is the effector gene for SD (Merrill *et al.*, 1999). *RanGAP*, a regulatory gene of Ran, a small GTPase in the Ras superfamily, maintains the nucleocytoplasmic RanGTP concentration gradient, catalyzing RanGTP to RanGDP in the cytoplasm. The defective *RanGAP* protein works enzymatically, but is misallocated to the nucleus likely because it is missing one nuclear export signal sequence (NES) (Kusano *et al.*, 2001). This misallocation prevents normal transport of RanGTP to the cytoplasm and leads to chromatin condensation failure during spermatid maturation via a yet unknown mechanism. We previously demonstrated that the *RanGAP* gene in *Ae. aegypti* showed high sequence identity with the *Drosophila* *RanGAP* gene, but was likely unrelated to the meiotic drive phenotype because it mapped to chromosome 2 (Cha *et al.*, 2006c) whereas the drive locus (M^D) is located on chromosome 1 (Hickey and Craig, 1966a).

Another well-characterized meiotic drive system is the mouse t-complex. Segregation distortion is known to be caused by multiple t-complex distorters (*Tcd* loci) that act additively in *trans* on a single responder locus (*Smok1*) to cause abnormal flagellar movement of affected spermatozoa. The *Tcd1* locus has been shown to be the *Tagap1* gene that encodes a Rho GTPase-activating protein (Bauer *et al.*, 2005). As observed with *RanGAP* in *Drosophila* (Kusano *et al.*, 2002), overexpression of wild-type *Tagap1* also results in distortion (Bauer *et al.*, 2005). A second locus, *Tcd2*, has been identified as the *Fgd2* gene that encodes a truncated guanine nucleotide exchange factor (GEF) for Rho small G proteins (Bauer *et al.*, 2007). Of note, evidence from these studies suggests that *Tagap1* and *Fgd2* regulate different pathways in the Rho signaling cascade.

Interestingly, *RanGAP*, *Tagap1* and *Fgd2* are each linked with the Ras superfamily of regulatory small GTPases, which are divided among five major subfamilies Ras, Rho, Rab, Ran and Arf (Takai *et al.*, 2001; Wennerberg *et al.*, 2005). Each subfamily shares the common core G domain, which provides essential GTPase and nucleotide exchange activity. The Ras superfamily is generally responsible for a number of roles in cells including regulation of gene expression, cytoskeletal reorganization, vesicle trafficking, nucleocytoplasmic transport and microtubule organization. Thus, it is likely not surprising that we identified a number of enriched transcripts in the *Ae. aegypti* subT37 library that are associated with signal transduction and cell cycle functions. Of note, these included four Ras-related genes (AAEL013068, protein phosphatase-2a; AAEL001659, misexpression suppressor of Ras; AAEL009887, wd-repeat protein; AAEL007708, wd-repeat protein) and three Ras superfamily-related genes (AAEL009884, Ran-binding protein; AAEL010506, GTP-binding protein alpha subunit; AAEL012904, Rab GDP-dissociation inhibitor).

Spermatogenesis in *Ae. aegypti* occurs primarily during the late 4th instar and pupal stages but continues in adults at decreasing levels over time (see review in Clements, 1992). Testes of young adults (0–18 h) show similar morphology to those in pupae, with only a slight reduction in the total numbers of spermatogonial cysts undergoing development (Wandall, 1986). More recent evidence indicates that sperm development in *Ae. aegypti* continues to

increase up to 10 days post-eclosion (Ponlawat and Harrington, 2007). Our qRT-PCR analyses of a subset of genes identified in the subT37 library, which was constructed from testes isolated at <24 h post adult eclosion, provided good support for our SSR results. These analyses confirmed that at least half of these genes are differentially over-expressed at the pupal stage in both T37 strain males and T37 strain male by RED strain female F₁ hybrid males. Therefore, continued evaluation of genes isolated in the subT37 library should provide new information on the molecular basis for the meiotic drive phenotype.

In conclusion, the results presented here provide the first insights into the molecular and physiological processes associated with the *Ae. aegypti* meiotic drive phenotype as well as testes gene expression in general. When compared with the well-characterized *D. melanogaster* and mouse systems, our results suggest that the *Ae. aegypti* system may involve signaling cascades under regulation by a Ras superfamily GTPase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Distribution of unique genes from *Aedes aegypti* ESTs derived from suppressive
Subtractive hybri

Figure 1.
Distribution of unique genes from each *Aedes aegypti* testis cDNA library.

Figure 2. qRT-PCR expression profiles for pupal testes of select genes from the subT37 library. AAEL001537, bax inhibitor; AAEL009160, *skp1*; AAEL009884, ran-binding protein; AAEL009887, wd-repeat protein; AAEL010506, GTP-binding protein alpha unit; AAEL012904, rab gdp-dissociation inhibitor. RED, RED Strain; T37, T37 strain; F1, T37 strain male by RED strain female F₁ males. *Indicates significantly different from RED strain ($P < 0.05$).

Table 1

Selected candidate gene and primer list for qRT-PCR analysis.

Gene ID	Description	Forward primer	Reverse primer	Size (bp)
AAEL001537-RA	bax inhibitor	TGGGCTTGATGATCGAACAAG	GAGGGCGGTAATGACGAATG	59
AAEL010506-RA	GTP-binding protein alpha subunit, gna	ACTTCGAAACGGTGACCACAT	GCCCACAGATCTTTGATTGCTT	60
AAEL012904-RA	rab gdp-dissociation inhibitor	GAGCCGGCCGTGAAGAC	GGCCAGCGAGTCCGAGTAC	53
AAEL009884-RA	ran-binding protein	TGTTCAATTTTGGTTGCCAGAA	CGTGTCGATTTTGTCTGGTATTT	65
AAEL009160-RA	skp1	GCCATCCTGCGGAAGGT	GTGCCGGATCGTCTTTGTG	54
AAEL009887-RA	wd-repeat protein	AGAAATCGGCCCGGTGAT	GCCGGCAATGTGTTGAGAT	55
AY927787	RpS17	AGCGGATCGTCGAAGAAGTG	GTCACGAAACCAGCGATCTTAT	70

Table 2Summary of ESTs from *Aedes aegypti* testes cDNA libraries.

Group	Number		
	Sub T37 ^a	pT37 ^b	pRED ^b
Total clones sequenced	1056	864	864
Contigs	484	376	522
Singletons	111	57	29
Unique sequences	171	81	57
Annotated sequences	111	41	23
Conserved hypothetical genes	23	9	9
Hypothetical genes	13	10	0

^a both end sequencing^b one end sequencing

Table 3

Distribution of subT37 unique sequences (US) in Biological process and Molecular function groups.

	% US		% US
Biological Process	50.9	Molecular Function	50.9
Cellular process	20.5	Binding	9.9
Cell cycle	5.3	Protein binding	2.3
Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	4.7	Nucleotide binding	2.3
Signal transduction	10.5	Catalytic activity	24.6
Developmental process	11.1	GTPase activity	2.9
Embryonic development	1.8	Hydrolase activity	4.1
Organ development	1.8	Phosphatase activity	1.8
Reproduction	6.4	Kinase activity	4.1
Metabolic process	5.3	Transferase activity	4.1
Transport	4.1	Electron carrier activity	2.3
Death	1.8	Signal transducer activity	4.1
Cellular component organization	0.6	Structural molecular activity	1.2
Growth	0.6	Transporter activity	3.5
Multicellular organismal process	0.6	Enzyme regulator	1.2
		GTPase regulator activity	1.2
		Transcription regulator activity	4.1
Non-categorized	49.1	Non-categorized	49.1

Table 4

Distribution of pT37 and pRED libraries unique sequences (US) in Biological process and Molecular function groups.

	% US		% US
Biological Process	26.7	Molecular Function	28.2
Cellular process	8.4	Catalytic activity	12.2
Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3.1		5.3
		Hydrolase activity	
Signal transduction	3.8	Kinase activity	1.5
Developmental process	6.9	Transferase activity	2.3
Larval development	2.3	GTPase activity	0.8
Reproduction	4.6	Phosphatase activity	7.6
Metabolic process	0.8	Binding	4.6
Transport	4.6	Protein binding	0.8
Cellular component organization	1.5	Transcription regulator activity	0.8
		Signal transducer activity	2.3
		Transporter activity	1.5
		Electron carrier activity	0.8
		Structural molecular activity	2.3
		Enzyme regulator	3.2
Non-categorized	73.3	Non-categorized	71.8

Table 5

Gene descriptions for the 12 most frequent ESTs enriched in the subT37 cDNA library.

Category	Gene ID	Gene Description	#ESTs	Score	e-value	Supercontig	Chromosome Location
Signal transduction							
	AAEL011380	high mobility group B1, putative	4	216	1.00E-99	1.574	n/a
	AAEL012904	rab gdp-dissociation inhibitor	4	621	0	1.757	n/a
Cell cycle							
	AAEL006642	tubulin alpha chain	4	420	5.00E-114	1.215	3p
	AAEL009160	skp1	31	542	3.00E-153	1.373	n/a
Reproduction							
	AAEL005471	Sec61 protein complex gamma subunit, putative	13	387	1.00E-107	1.158	n/a
Development							
	AAEL000637	hypothetical protein	5	1125	0.00E+00	1.13	3q
	AAEL001240	chaoptin	7	428	0	1.27	2q
	AAEL005885	arginyltransferase, putative	4	1027	0	1.178	2p
	AAEL008166	malate dehydrogenase	10	289	1.00E-77	1.304	3p
Transcription/Translation							
	AAEL005736	conserved hypothetical protein	4	952	0	1.172	2p
Death							
	AAEL009637	cathepsin b	4	483	1.00E-135	1.414	n/a
Metabolic process							
	AAEL005651	ethanolamine-phosphate cytidyltransferase	4	444	3.00E-121	1.168	n/a