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Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens*

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Abstract

The mosquito *Culex quinquefasciatus* poses a significant threat to human and veterinary health as a primary vector of West Nile virus (WNV), the filarial worm *Wuchereria bancrofti*, and an avian malaria parasite. Comparative phylogenomics revealed an expanded canonical *C. quinquefasciatus* immune gene repertoire compared with those of *Aedes aegypti* and *Anopheles gambiae*. Transcriptomic analysis of *C. quinquefasciatus* genes responsive to WNV, *W. bancrofti* and non-native bacteria facilitated an unprecedented meta-analysis of 25 vector-pathogen interactions involving arboviruses, filarial worms, bacteria and malaria parasites, revealing common and distinct responses to these pathogen types in three mosquito genera. Our findings provide support for the hypothesis that mosquito-borne pathogens have evolved to evade innate immune responses in three vector mosquito species of major medical importance.

The Southern house mosquito, *Culex quinquefasciatus*, is a geographically widespread, often abundant mosquito that is an epidemiologically significant vector for an exceptionally diverse array of pathogens including multiple arboviruses, filarial worms and protozoa. *C. quinquefasciatus* transmits West Nile virus (WNV), St. Louis encephalitis virus and other arboviruses, and acts as the primary vector of the causative agent of lymphatic filariasis, *Wuchereria bancrofti*, and *Plasmodium relictum*, an avian malaria parasite. Despite the public health significance of *C. quinquefasciatus*, knowledge of the insect's response capacities to this diverse array of pathogens is limited.

Availability of the *C. quinquefasciatus* genome sequence (1) enabled comparative phylogenomic analyses with *Aedes aegypti* (2), *Anopheles gambiae* (3) and *Drosophila melanogaster*(4) that identified 500 *C. quinquefasciatus* immunity genes from 39 (sub)families or processes (Table S1). Conservation of *C. quinquefasciatus* gene family members follows the species phylogeny, showing greatest similarities with *A. aegypti*. Expansions of C-type lectins (*CTL*s), fibrinogen-related proteins (*FREP*s) and serine protease inhibitors (*SRPN*s) account for much of the 20–30% increase in *C. quinquefasciatus* immunity gene number compared to *A. aegypti* (417 genes) and *A. gambiae* (380 genes) (Figs. S1–S4), This apparent diversification in immune surveillance and immune signal amplification processes seems consistent with selection driven by polluted, microbially complex habitats in which *C. quinquefasciatus* oviposits and develops (5).

Whole genome microarray analysis revealed dynamic changes in infection response gene (IRG) transcription in WNV-infected mosquitoes (Fig. S5). Significant changes are observed for 22 transcripts in the midgut and 309 in the carcass (i.e., the remainder of the body) at 7 days post-infection (dpi), with the greater number of IRGs in the latter apparently reflecting the diversity of infected cell and tissue types in the carcass. At 14 dpi, more IRGs are modulated in midgut (539) and carcass (490) when WNV infection has spread in midgut cells and has disseminated to the salivary glands (6). Few canonical immunity genes are represented among *C. quinquefasciatus* WNV IRGs (Fig. S5). Five CTL genes within a *C. quinquefasciatus*-specific gene expansion (Fig. S3) are up-regulated. Several genes related to the Toll, Imd and JAK-STAT pathways, including *Spaetzle*, *REL1*, *IAP2* and *STAT* orthologs, are activated in *C. quinquefasciatus* and in *A. aegypti* (7, 8) by WNV and Dengue virus (DENV) infection, respectively, further supporting a key role of these defense systems in controlling viral pathogens.

Although the *C. quinquefasciatus* genome encodes orthologs for all components of the anti-viral defense RNA interference (RNAi) pathway (9), none of them is transcriptionally modulated significantly during WNV infection. Similarly, RNAi components are not transcriptionally modulated during arbovirus infection in *A. aegypti* (10), even though RNAi function is key to limiting these infections in mosquitoes (10–12). Apoptosis is evident and *C. quinquefasciatus IAP1* is repressed in WNV-infected salivary glands (6, 13). However, no significant changes in transcript abundance for caspases, caspase activators, *IAP* genes (other than *IAP2*), or autophagy-related genes are evident in WNV-infected *C. quinquefasciatus*, even though modulation of apoptosis (14) or autophagy (15) pathway function affects viral infection in flies. The non-responsiveness of these genes appears to reflect the persistent and generally non-cytolytic nature of arbovirus infections in a susceptible vector; overt activation of these responses would counteract virus persistence and transmission.

Comparative analysis of ESTs (Tables S3–4) from *W. bancrofti*-infected *C. quinquefasciatus* revealed many novel IRGs, presumably because infection with a large metazoan parasite inflicts traumatic injury. Infection with non-native bacteria elicits acute cellular and humoral immune responses in *C. quinquefasciatus* and other vector mosquitoes (16–18). Approximately 60% of *W. bancrofti* or bacteria IRGs are of diverse or unknown function (Fig. S6), and only small proportions (4% *W. bancrofti* and 6% bacteria) are immunity genes. Comparison of *C. quinquefasciatus* virus, filarial worm, and bacteria IRGs reveals unexpected and extensive overlap (548 genes) between *W. bancrofti* and bacteria IRGs (Figs. 1A and S6). Overall, 38 *C. quinquefasciatus* IRGs are common among all three infections (Table S5).

The identification of *C. quinquefasciatus* IRGs provided an unprecedented opportunity to undertake a meta-analysis of 25 vector-pathogen interactions in *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae* infected with arboviruses, parasites and bacteria (Fig. 1B, Table S6) within the context of orthologous groups (OGs) that define evolutionarily related genes. A set of 69 arbovirus IRG OGs (representing 93 *C. quinquefasciatus* and 89 *A. aegypti* genes) was implicated in *C. quinquefasciatus*-WNV, *A. aegypti*-DENV and *A. aegypti*-Sindbis virus (SINV) responses (Fig. S7, Table S7). A cytochrome P450 DENV IRG from *Drosophila* (*Cyp6a19*, FBgn0033979) and mammalian cells (19) is similar to genes that respond significantly in *C. quinquefasciatus*-*WNV* (CPIJ004411) infection, and in *A. aegypti*-SINV and *A. aegypti*-DENV (AAEL009117) infections, highlighting the potential importance of this molecule as a universal arbovirus IRG. Filarial worm IRGs comprised 41 OGs modulated during *C. quinquefasciatus*-*W. bancrofti* infection and infection of *A. aegypti* with *Brugia malayi* (Fig. S8, Table S8). The IRGs represented most frequently include serine proteases and cuticle proteins. Changes in the latter may be associated with tissue repair necessitated by parasite invasion, migration and development (20). Increased representation of heat shock protein and cytochrome P450 IRGs appears to reflect stress during the infection response. The most extensive overlap (113 OGs) in bacterial IRGs was observed between Culicine mosquitoes, *C. quinquefasciatus* and *A. aegypti* (Table S9). Only 34 OGs and 26 OGs represent IRGs (Fig. S9) in bacteria-infected *C. quinquefasciatus* and *A. gambiae* (Table S10), and *A. aegypti* and *A. gambiae*, respectively. Among 16 OGs containing bacteria IRGs from all three species, serine proteases, cecropins, myosin light chain, and components of the 26S proteasome are highly represented (Table S11). A meta-analysis of bacteria, filarial worm, virus, and malaria parasite infection datasets from *C. quinquefasciatus*, *A. aegypti* and *A. gambiae* reveals 95 orthologous IRGs that span mosquito species and pathogen types (Figs. 1B and S10, Table S12).

Orthology data (21) were employed to distinguish universal (see Fig. 1) multi- or single-copy OGs from mosquito-specific OGs, revealing that the majority of IRGs have orthologs

across Arthropoda (Fig. 1C, Table S13). Universal multi-copy OGs are overrepresented among IRGs for viruses, filaria and bacteria; and universal single-copy OGs are underrepresented among arbovirus and filarial worm IRGs (and among IRGs common to all pathogens in *C. quinquefasciatus*, *A. aegypti* and *A. gambiae*) compared to the complete set of mosquito OGs (Fig 1C). Immunity genes (IMM, Fig. 1D), including CTLs, CLIPs and SRPNs, are generally more prevalent among responsive multi-copy OGs than among responsive single-copy OGs. In fact, no canonical immunity genes are found among arbovirus- or filarial worm-responsive universal single-copy OGs.

The cosmopolitan distribution of *C. quinquefasciatus* across continents and ecozones generally south of 39° N latitude implies that this species has the plasticity to adapt to diverse habitats, and this plasticity may be enhanced by an expanded immunity gene repertoire. Overrepresentation of universal multi-copy OGs among pathogen IRGs implies that members of expanded gene families have been recruited into pathogen-responsive defense pathways. Arboviral and filarial worm infections constitute susceptible, long-term vector-pathogen interactions in which the pathogen undergoes amplification or develops intracellularly, while acute infections with non-native bacteria trigger systemic immunity and are cleared rapidly (22, 23). Our meta-analysis reveals that arboviral and filarial worm pathogens transmitted by vector mosquitoes modulate very few canonical immunity genes, and fail to affect expression of RNAi and most programmed cell death pathway genes in these vectors. Our results therefore provide strong support for the hypothesis that pathogens that successfully develop in, and are transmitted by, vector mosquitoes have evolved to avoid most immune responses in the three mosquito genera responsible for the vast majority of human morbidity and mortality attributable to insect-transmitted pathogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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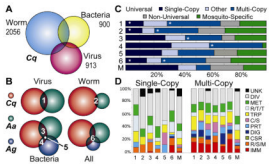

Figure 1.

Infection response genes (IRGs) in the mosquitoes *Culex quinquefasciatus* (*Cq*), *Aedes aegypti* (*Aa*) and *Anopheles gambiae* (*Ag*). **(A)** Shared and unique infection response genes in *C. quinquefasciatus* infected with a filarial worm, bacteria, or virus. **(B)** Proportions of shared and unique IRGs post-infection with viruses (1), filaria (2) or bacteria (3) in *C. quinquefasciatus* and *A. aegypti*, in *C. quinquefasciatus* and *A. gambiae* (4), and in all three species (5); and common IRGs in *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae* (6). **(C)** Orthology relationships for IRG sets (Rows 1–6). IRGs with orthologs in at least 20 arthropod species were classified as Universal, as compared to Non-Universal or Mosquito-Specific. Gene copy-number counts distinguish mostly single- and multi-copy orthologous groups. IRG sets 1–6 were compared to 10,083 mosquito OGs (Row M) to identify significantly greater or smaller (asterisks) proportions (Fisher's Exact Tests: p<1e-5). **(D)** Consensus functional categories of universal single-copy (left) and multi-copy (right) orthologous groups of IRG sets Rows 1–6, and all mosquito groups (Row M). Functional groups are described in SOM, and (24). Each set of IRGs is described in Supplemental Tables S7–12.