

# Profiles of Amino Acids and Acylcarnitines Related with Deltamethrin Resistance in *Aedes Albopictus*

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## Research Article

**Keywords:** Amino acids and acylcarnitines profiles, Deltamethrin resistance, *Aedes albopictus*

**Posted Date:** December 28th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-135058/v1>

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## Abstract

**Background:** Fast emerging and increasing insecticide resistance in Asian tiger mosquito, *Aedes albopictus* brings alarm to the dengue epidemic over the world. The genetic and transcriptional sequencing has greatly advanced our understanding of the molecular basis for this crucial adaptation, but less is known about the regulation of insecticide resistance physiology at the metabolomic level.

**Methods:** We captured the relative abundance of 17 amino acids and 34 AcylCNs in the 3<sup>rd</sup> to 4<sup>th</sup> instar larvae of three geographically close strains of *Ae. albopictus*, including a laboratory insecticide susceptible strain (Foshan) and two field deltamethrin-resistant strains (Yuexiu/Baiyun), as well as a laboratory induced deltamethrin-resistant (R18) and its parental insecticide susceptible strains (R0) to characterize their amino acids and acylcarnitines (AcylCNs) profiles by LC-MS/MS spectrometry.

**Results:** Heatmap and PCA analysis of amino acids and AcylCNs profile indicated a clear separation of Foshan from Yuexiu/Baiyun populations, as well as a partly overlapping between Yuexiu and Baiyun populations. All strains were abundant in five amino acids (Pro, Val, Arg, Glu, His) and two AcylCNs (C0, C2). Five amino acids (Tyr, Cit, Pro, Thr, Gly) were significantly higher, while four amino acids (His, Val, Glu, Ala) were obviously lower in Yuexiu strain than Baiyun population. R18 displayed a differential amino acid and AcylCNs profile from its parental R0 strain.

**Conclusion:** This study displayed a distinction of amino acids and AcylCNs profiles between insecticide-resistant and geographically or genetically-close insecticide-susceptible strain of *Ae. albopictus*, suggesting a possible application of amino acids and AcylCNs profiles for rapid diagnosis of deltamethrin resistance in *Ae. albopictus* in the future.

## Background

Asian tiger mosquito, *Aedes albopictus* (*Ae. albopictus*), has spread to 70 countries all over the world out of its native home in Southeast Asia [1], with strong capacity in the transmission of various arboviruses like Dengue and Zika viruses [2]. At present, vector population control strategies, especially usage of insecticides targeting the larval and adult stages, are the most popular way to limit *Ae. albopictus* mosquitoes and virus transmission [3, 4]. Pyrethroids are the most frequently utilized adulticides to control the spread of *Ae. albopictus* to prevent dengue virus transmission as for their low mammalian toxicity and rapid knockdown effect [4–6]. However, extensive and prolonged use of pyrethroids like deltamethrin has imposed selection pressure on *Ae. albopictus* and eventually caused resistance [4, 7]. Fast emerging and increasing resistance to insecticides like deltamethrin in *Ae. albopictus* brings alarm to the dengue epidemic over the world.

Pyrethroids resistance in *Ae. albopictus* occurs mainly through the F1534S and F1534L mutation in the VGSC gene [7, 8], also known as knockdown resistance (kdr) resistance, as well as up-regulation of specific detoxification enzymes like glutathione S-transferase (GST) (GSTE1, AGAP009195-PA) and cytochrome P450 monooxygenases (P450s) CYP6A8, CYP9Z4, CYP6D4 [9]. Pyrethroids resistance was also regarded to be associated with pathways involved in metabolic process and pathways [10, 11], protein digestion and absorption [11]. Metabolic pathways and biosynthesis of secondary metabolites have been identified to be the top-two differential pathway categories between deltamethrin-resistant and susceptible individuals of *Ae. albopictus* [9]. Metabolomics could reflect systematic human metabolic variations in accordance with phenotypic changes, such as resistance to insulin [12] and trastuzumab [13]. Characteristic of amino acids and AcylCNs acylcarnitine profile has become a powerful and popular tool for diagnostic of clinical metabolic alterations [14–18]. However, little is known of the characteristic of amino acids and AcylCNs profiles in mosquitoes. Altered profile of amino acids and AcylCNs has been reported in *Culex quinquefasciatus* L after exposure to chlorpyrifos, temephos and permethrin [19]. In this study, amino acids and AcylCNs profiles of deltamethrin-resistant and susceptible strains of *Ae. albopictus* were compared to observe their alterations in accordance with deltamethrin resistance.

## Materials And Methods

### Mosquito strains, analysis of insecticide resistance and kdr mutations

Larvae of two field strains of *Ae. albopictus* were collected from public land like parks, schools and residential areas in 2017 in two districts named Yuexiu and Baiyun of Guangzhou city respectively, with dengue incidence of > 5 and 1–5 cases per 100,000 people respectively as previous report [7]. The larvae were housed [7] and have been tested for their larval resistance to four insecticides: (1) organophosphate, temephos; (2) microbial bacterial toxin, Bti; (3) hormonal insect growth regulators, pyriproxyfen (PPF); (4) the chitin biosynthesis inhibitor, hexaflumuron; as well as their adult resistance to five adulticides including deltamethrin, permethrin, malathion, bendiocarb, DDT following WHO guidelines [7]. The larvae housed from both Yuexiu and Baiyun districts were also tested for their resistance to adulticides including deltamethrin, permethrin, DDT, bendiocarb, malathion following WHO guidelines as previous report [7, 20]. For larval bioassays, the resistant status was evaluated by the resistant ratio (RR<sub>50</sub>) (RR<sub>50</sub> < 5, susceptible; 5 < RR<sub>50</sub> < 10, moderately resistant; RR<sub>50</sub> > 10, highly resistant), for adult bioassays, resistant status was defined by mortality rate: resistant if mortality < 90%, probably resistant if mortality was between 90 and 98%, and susceptible if mortality > 98% [7]. The kdr mutation in resistant and susceptible individuals from Yuexiu and Baiyun districts in 2017 has been genotyped at the voltage-gated sodium channel (VGSC) gene after exposure to deltamethrin, permethrin, and DDT to detect mutations within domains II, III and IV [7], samples exposed to bendiocarb have been genotyped at the ace-1 gene to detect mutations within G119 [7]. The Foshan strain of *Ae. albopictus*, an insecticide-susceptible strain which was reared in laboratory without exposure to any insecticides since its collection in 1983 in Foshan (a smaller city adjacent to Guangzhou city), was used as the control strain for metabolomic comparison with these field strains of *Ae. albopictus*.

R18 is a laboratory resistant strain (LC<sub>50</sub> = 0.039, RR<sub>50</sub> = 13) after deltamethrin selection of an insecticide susceptible *Ae. albopictus* populations from Shanghai CDC for 17 generations, which was primarily screened by 0.005 mg/L of deltamethrin (Sigma, USA) from the 1st to the 10th generation, followed by

deltamethrin screening at 50% lethal concentration (LC<sub>50</sub>) from the 11th to the 22nd generation as described previously [21]. The parental strain of *Ae. albopictus* without any exposure to insecticides (R0) was used as an insecticide-susceptible control strain for R18 strains of *Ae. albopictus* in this study, LC<sub>50</sub> (95%) = 0.003 (0.001, 0.006) mg/L. The larval and adult resistance of R18 to deltamethrin was also analyzed following WHO guidelines as previous report [7, 20]. The resistant and susceptible adults against deltamethrin from R18 strains of *Ae. albopictus* were also genotyped for kdr mutation as previous report [7].

LC-MS/MS sample preparation and metabolites extraction

Six replicates of ten numbers of 3rd to 4th instar larvae (approximately 2 mg/larvae) of Foshan, Yuexiu, Baiyun, R0, R18 strains were sampled respectively. Then, they were homogenized (TIANGEN OSE-Y10-plus) in 500 µl of ddH<sub>2</sub>O, followed by centrifugation at 4 °C,13000 rpm for 10 min. The lysate was transferred to a sterile syringe which was attached to a 0.22 µm acrodisc for filtration. Finally, 30 µl of the filtered lysate was added to the filter paper (S&S903) and dried for storage. A paper circle with 3.2 mm diameter was punched from the dry larval lysate filter paper by a Panthera-Puncher TM 9 Puncher (PerkinElmer, Waltham, MA, USA)]. A NeoBase non-derivatized MS/MS kit (Perkin Elmer) was employed for extraction of amino acids and AcylICNs.

Metabolomics analysis

LC-MS/MS (API 2000, ABSciex, Framingham, MA, USA) platform, with stable isotopes labelled amino acid standards (Cambrige Isotope Labs) as an internal solution, was selected for quantification of 17 amino acids including alanine, arginine, aspartate, citrulline, glutamine, glutamate, glycine, histidine, leucine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, valine) and 34 AcylICNs including C0, C2, C3, C3DC, C4, C4-OH, C4DC, C5, C5:1, C5-OH, C5DC, C6, C6:1, C6DC, C8, C8:1, C10, C10:1, C10:2, C12, C12:1, C14, C14-OH, C14:1, C14:2, C16, C16:1, C16-OH, C16:1-OH, C18, C18:1, C18-OH, C18:2, C18:1-OH (34AC). Analysis of 17 amino acids and 34 AcylICNs was interpreted in multiple reaction monitoring (MRM) mode with Analyst 1.6.2 software (ABSciex) and NeoBase database.

Statistical analysis

Finally, the contents of 17 amino acids and 34 AcylICNs were scaled according to the preparation procedure of dry larval lysate filter paper. The concentration of the detected metabolites was normalized and analyzed by orthogonal pattern recognition methods principal component analysis (PCA). PCA with performed using Metaboanalyst (v3.0) (<https://www.metaboanalyst.ca/faces/home.xhtml>) to display the characteristic of amino acids and AcylICNs profiles of *Ae. albopictus* with different levels of deltamethrin resistance. SPSS 17.0 was applied for the spearman correlation analysis between the deltamethrin LC<sub>50</sub> values and the contents of amino acids and AcylICNs in *Ae. albopictus*.LC<sub>50</sub> and KDT<sub>50</sub> were estimated using the log-probit models [7]. For larvae bioassays, the resistant status was measured by the resistant ratio (RR<sub>50</sub>), i.e., the ratio of LC<sub>50</sub> (or IE<sub>50</sub>) for the field population over LC<sub>50</sub> (or IE<sub>50</sub>) for the laboratory-susceptible strain [7]. Larval resistance status was defined as susceptible if RR<sub>50</sub> < 5, moderately resistant if 5 < RR<sub>50</sub> < 10, and highly resistant if RR<sub>50</sub> > 10 [7]. For adult bioassays, resistant status was defined by mortality rate: Resistant if mortality < 90%, probably resistant if mortality was between 90 and 98%, and susceptible if mortality > 98% [7, 9]. The relationship between nonsynonymous mutations and resistance was verified by Fisher's exact test or the χ<sup>2</sup>-test (when all n > 5), and the odds ratio (OR) was calculated for each mutation [7].

Results

Insecticide resistance and kdr mutation of Ae. albopictus larvae from Yuexiu and Baiyun districts in 2017

*Ae. albopictus* larvae from both Yuexiu and Baiyun districts had moderate to high resistance to adulticides like deltamethrin and permethrin, both of them remained susceptible to malathion (Table 1). Moreover, larvae from Yuexiu district had developed much higher level of resistance to deltamethrin than the larvae from Baiyun district, but larvae from Baiyun district was more resistant to both permethrin and DDT (Table 1). In 2017, the larvae and adult populations of *Ae. albopictus* from Baiyun/Yuexiu districts of Guangzhou city, China has developed high resistance to currently used insecticides (deltamethrin, permethrin, DDT and bendiocarb, mortality < 90%) except malathion (mortality > 98%) [7]. The mortality rate against deltamethrin, permethrin, DDT and bendiocarb was 5.6%, 55.2%, 52.1%, 42.4% in the adult populations from Yuexiu district, and 38.4%, 68.8%, 74.7% and 51.2 in the adult population from Baiyun district, respectively [7]. *Ae. albopictus* larvae from both districts were still sensitive to Bti and hexaflumuron (RR<sub>50</sub> < 5), but high resistance to pyriproxyfen (RR<sub>50</sub> > 10) [7].

Table 1  
The larval resistance of *Ae. albopictus* from both Yuexiu and Baiyun districts to five adulticides (deltamethrin, permethrin, DDT, bendiocarb, malathion) in 2017

	deltamethrin		Permethrin		DDT		Bendiocarb		Malathion	
	LC <sub>50</sub> (95%)(mg/L)	RR <sub>50</sub>	LC <sub>50</sub> (95%)(mg/L)	RR <sub>50</sub>	LC <sub>50</sub> (95%)(mg/L)	RR <sub>50</sub>	LC <sub>50</sub> (95%)(mg/L)	RR <sub>50</sub>	LC <sub>50</sub> (95%)(mg/L)	RR <sub>50</sub>
Baiyun	0.027(0.020,0.049)	27	0.628(0.527,0.730)	15	5.281(5.073,5.538)	12.1	1.460(0.995,1.745)	0.77	0.073(0.068,0.080)	
Yuexiu	0.066(0.061,0.094)	66	0.356(0.113,0.533)	8.47	1.912(1.560,2.227)	4.37	5.37(4.826,6.031)	5.54	0.092(0.081,0.109)	
Foshan	0.001(0.001,0.006)	1	0.042(0.031,0.053)	1	0.438(0.389,0.484)	1	0.969(0.908,1.038)	1	0.043(0.039,0.046)	

Kdr mutation associates with deltamethrin resistance in R18 strain

The larvae and adult populations of *Ae. albopictus* from R18 has developed high resistance to deltamethrin (larval LC<sub>50</sub> = 0.039, RR<sub>50</sub> = 13, adult mortality = 85%). Sequences of domains II (480 bp), III (346 bp) and IV (280 bp) of the VGSC gene were also obtained from the resistant and susceptible mosquitoes after deltamethrin adult bioassays for R18 [7]. Kdr genotyping showed non-synonymous mutations of F1534S could be detected at codon 1534 in domain III of

R18, where wild-type TTC (Phe) was changed to TCC (Ser) at a high frequency of 78.38% (29/37), indicating a significant correlation with deltamethrin resistance (Table 2,  $p < 0.01$ ).

Table 2  
Kdr mutations at position F1534 of the VGSC gene in adults of *Ae. albopictus* from R18 strain and its parental susceptible R0 strain.

Insecticide	N	Genotype					Odds Ratio(95%CI)	
		FF	FS	SS	FL	LL	F1534S	F1534L
Susceptible strain (R0)	40	40	0	0	0	0	1	—
R18	37	9	23	6	0	0	123.00 (14.30,1013.39)**	—

\*\* $p < 0.01$

Differential larval amino acids and AcylCNs profiles between insecticide-resistant *Ae. albopictus* from Baiyun/Yuexiu and susceptible Foshan strain

Amino acids and AcylCNs profile analysis demonstrated that Pro, Val, Arg, Glu and His were the five most abundant amino acids (Fig. 1A) whereas C0, C2 were the richest AcylCNs in the larvae of *Ae. albopictus* (Table S2) & (Figs. 1B and 1C). The metabolites in Baiyun/Yuexiu larvae displaced fluctuation in comparison to Foshan larvae. T test analysis showed the concentration of five amino acids Tyr, Cit, Pro, Thr, Gly (Fig. 2A) and five AcylCNs C3DC, C6-1, C10, C10-1,C10-2 (Fig. 2B) were significantly higher, while the levels of C2 (Fig. 2B) and four amino acids His, Val, Glu, Ala (Fig. 2A) were obviously lower in Yuexiu strain in comparison to Foshan strain. Similarly, Cit and Arg were increased while His was decreased in Baiyun strain (Fig. 2C). Heatmap and PCA analysis of 17 amino acids and 34 AcylCNs contents indicated significant discrepancy of amino acids and AcylCNs profile between Foshan and Baiyun/Yuexiu larvae (Figs. 3A and 3B). However, profile of Yuexiu larvae was partly overlapped with that of Baiyun larvae (Fig. 3B).

Differential larval amino acids and AcylCNs profiles between deltamethrin-resistant (R18) and its parental susceptible (R0) strains

Amino acids and AcylCNs profile analysis demonstrated that Pro, Val, Arg, Glu and His were the five most abundant amino acids whereas C0, C2 were the richest AcylCNs in the deltamethrin-resistant (R18) and -susceptible (R0) larvae of *Ae. albopictus* (Table S2). Between deltamethrin-resistant R18 and susceptible (R0) strains, no significant difference of amino acids and AcylCNs were found, but the heatmap and PCA analysis of 17 amino acids and 34 AcylCNs contents indicated discrepancy of amino acids and AcylCNs profile (Figs. 4A and 4B).

Table 3  
Kdr mutations at position F1534 of the VGSC gene in resistant and susceptible adults of *Ae. albopictus* against deltamethrin, permethrin and DDT from both Yuexiu and Baiyun districts in 2017 [7]

Insecticide	Phenotype	N	Genotype					Odds Ratio(95%CI)	
			FF	FS	SS	FL	LL	F1534S	F1534L
Deltamethrin	S	20	17	3	0	0	0	1	1
	R	41	2	1	17	3	18	4.44(1.12,17.52)*	NA
permethrin	S	19	11	5	0	1	1	1	1
	R	42	1	4	22	5	11	4.55(1.38,15.05)*	27.20(4.60,160.69)**
DDT	S	18	15	2	1	0	0	1	1
	R	44	5	7	15	11	6	5.00(1.27,19.74)	NA**

NA: Not Available. Significance level. \* $p < 0.05$ , \*\* $P < 0.01$ .

Discussion

For insecticide resistance monitoring, the bioassays like WHO susceptibility test [22] and CDC bottle assay [23], molecular detection of knock-down resistant gene mutations, analysis of metabolic enzymes GSTs, p450s and CCEs activities have been frequently used, but limitations existed, such as time and labor-consuming, difficult for standardization with complicated procedures, requirement of special insectary laboratory to rear mosquitoes and subjective judgement of larval and adult death, etc. Amino acids and AcylCNs profile have been popular for diagnostic of clinical metabolic diseases. In this study, we first tried to explore the characteristic of amino acids and AcylCNs profile among geographically or genetically-close strains of *Ae. albopictus* with differential resistance to insecticides especially deltamethrin, and compared their differences. The insecticide resistance status and the corresponding possible genetic mechanism of the test strains was analyzed thoroughly before their metabolomic analysis. Deltamethrin was the most popular adulticide for vector control in urban areas of Guangzhou city [7]. Accordingly, the most significant difference of insecticide resistance between the larvae and adults from different districts were their differential resistance to deltamethrin, such as significantly higher larval and adult resistance to deltamethrin in Yuexiu strain (larval  $RR_{50} = 66$ , adult mortality of 5.6%) than Baiyun strain (larval  $RR_{50} = 27$ , adult mortality of 38.4%) (Table 1) [7]. In *Ae. albopictus* adults from both districts, the resistance to pyrethroids (deltamethrin, permethrin) and DDT has been proved to be significantly associated with non-synonymous 1534 codon mutations from homozygote wild-type TTC (Phe) (F1534F) to homozygote TCC (Ser) (F1534S) or CTC (Leu) (F1534L) to at domain III (346 bp) of the VGSC gene, with five detected kdr genotype (FF, FS, SS, FL, LL) in the resistant and susceptible individuals of *Ae. albopictus* adults for three insecticides (deltamethrin, permethrin and DDT) from both districts (Table 3) [7] as previous report [8]. Similarly, Kdr genotyping detected F1534S mutation at codon 1534 in domain III of R18 strain

at a frequency of 78.38% (29/37), indicating a significant correlation of F1534S mutation with laboratory-induced deltamethrin resistance in *Ae. albopictus* (Table 2,  $p < 0.01$ ).

The PCA analysis of amino acids and AcylCNs profiles among different strains demonstrated that *Ae. albopictus* larvae with differential deltamethrin resistant level displaced differential amino acids and AcylCNs profiles, including clear distinction of amino acids and AcylCNs profile between insecticide-susceptible larvae (Foshan strain) and larvae collected from geographically-close and insecticide-resistant Baiyun/Yuexiu districts, or obvious difference between a laboratory-induced deltamethrin-resistant strain (R18) and its parental susceptible Shanghai strain (R0), whereas it was relatively similar between insecticide-resistant larvae from relative geographically-close Baiyun and Yuexiu districts. This may suggest a potential role of amino acids and AcylCNs profile in predicting larval insecticide resistance of *Ae. albopictus*.

Moreover, five amino acids (Tyr, Cit, Pro, Thr, Gly) were significantly higher, whereas four amino acids (His, Val, Glu, Ala) were obviously lower in larvae from Yuexiu district than the susceptible Foshan strain. However, only Cit and Arg were increased while His was decreased in larvae from Baiyun district than the susceptible Foshan strain, this phenomenon was probably due to their differential resistance to insecticides especially deltamethrin. Amino acids are metabolites required for protein synthesis, and have roles in metabolism as energy sources and nucleic acid precursors, they could be transformed into carbon-hydrates and fatty acids. The significant difference of amino acids between insecticide-susceptible larvae (Foshan strain) and insecticide-resistant larvae collected from geographically-close Baiyun/Yuexiu districts, was probably associated with the amino acid changes in correspondence with the deltamethrin-resistance correlated genetic changes [7] and altered expression of P450s, cuticle proteins, glutathione S transferase, serine proteases, heat shock protein cytochrome s, esterase [9], as well as P450 gene SNPs such as Arg226Ser in CYP6A8, Pro175Gln in CYP9B2, His877Tyr in CYP9B2, Cys212Ser in CYP1A1 in *Ae. albopictus* [9]. The change of amino acids and proteins was also reported to be involved in insecticide resistance of other mosquitoes, such as arginine (ARG) changes for energy production and insecticide detoxification of *Culex quinquefasciatus* L [19], elevated P450s and esterases (EST) in the insecticide-resistant housefly *M. domestica* [24], more proteins involved in oxidoreductase, transferase, hydrolase, transporter and catalytic activity, cuticle formation in the pyrethroid resistant strain of *Culex pipiens pallens* [25, 26]. Changes of amino acids and insect proteases also occurred under insecticide exposure for protein metabolism, such as for re-synthesis of up-regulated detoxification enzymes, suggesting a role of protein metabolism under insecticide pressure [24, 27].

Carnitine is derived from amino acids lysine and methionine, AcylCNs are metabolites of organic acids and beta oxidation of fats. In this study, C0 and C2 were the two most abundant AcylCNs in larvae of all five strains. The changes of free carnitine (C0) and acetyl-carnitine (C2) were regarded to be involved in energy production and insecticide detoxification of *Culex quinquefasciatus* L [19]. In this study, five AcylCNs (C3DC, C6-1, C10, C10-1, C10-2) were found to be significantly higher in larvae from Yuexiu strain than the susceptible Foshan strain, while the levels of C2 were obviously lower, suggesting a probable variation of these AcylCNs in association with the development of insecticide resistance in *Ae. albopictus*.

## Conclusions

This study first captured the characteristic of amino acids and AcylCNs profile of *Ae. albopictus* larvae with differential resistance to insecticides especially deltamethrin, indicating the possibility of LC-MS/MS based amino acids and AcylCNs profile as a tool for fast discrimination of deltamethrin-resistant strain from -susceptible strain of *Ae. albopictus*, as well as a potential way for seeking potential biomarkers for early diagnosis of insecticide resistance like deltamethrin in *Ae. albopictus*.

## Abbreviations

AA, amino acids. AC, acylcarnitine. AcylCNs, acylcarnitine. *Ae. albopictus*, *Aedes albopictus*. PCA, principal component analysis.

## Declarations

### Acknowledgements

The authors would like to thank Hongxia Liu from Shanghai CDC for assistance in induction of laboratory deltamethrin-resistant strains of *Ae. albopictus*.

### Authors' contributions

Conceptualization: Lianfen Huang, Ruili Xie, Xiao-Guang Chen, Hua Li. Data curation: Lianfen Huang, Xinhua Su, Chenfang Tang. Formal analysis: Lianfen Huang, Ruili Xie, Lilan Peng, Chenfang Tang, Jun Li, Xinhua Su. Funding acquisition: Xiao-Guang Chen, Hua Li. Investigation: Lianfen Huang, Ruili Xie, Lilan Peng, Xinhua Su. Methodology: Lianfen Huang, Ruili Xie, Lilan Peng, Chenfang Tang, Yonglan Huang, Xinhua Su. Project administration: Lianfen Huang, Ruili Xie, Jun Li. Supervision: Xiao-Guang Chen, Hua Li, Yonglan Huang. Final approval: all authors have read and approved the manuscript.

### Funding

This study was supported by the National Institutes of Health, USA(R01AI083202), Guangzhou Science and technology project, China (202002030491) and the Medical Scientific Research Foundation of Guangdong Province, China (A2019512).

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

#### Availability of supporting data

The insecticide resistance and Kdr mutation analysis data of the adults of *Ae. albopictus* from Yuexiu/Baiyun districts of Guangzhou city in 2017 is available from the paper (Su X, Guo Y, Deng J, Xu J, Zhou G, Zhou T, et al. (2019) Fast emerging insecticide resistance in *Aedes albopictus* in Guangzhou, China: Alarm to the dengue epidemic. *PLoS Negl Trop Dis* 13(9): e0007665.) at the website (<https://doi.org/10.1371/journal.pntd.0007665>).

#### Competing interests

The authors declare no conflicts of interest in this work.

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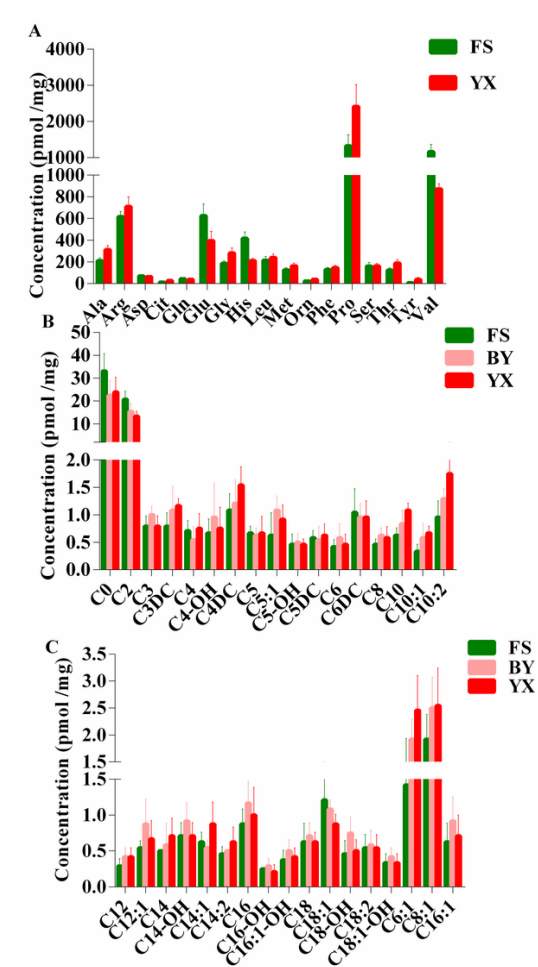
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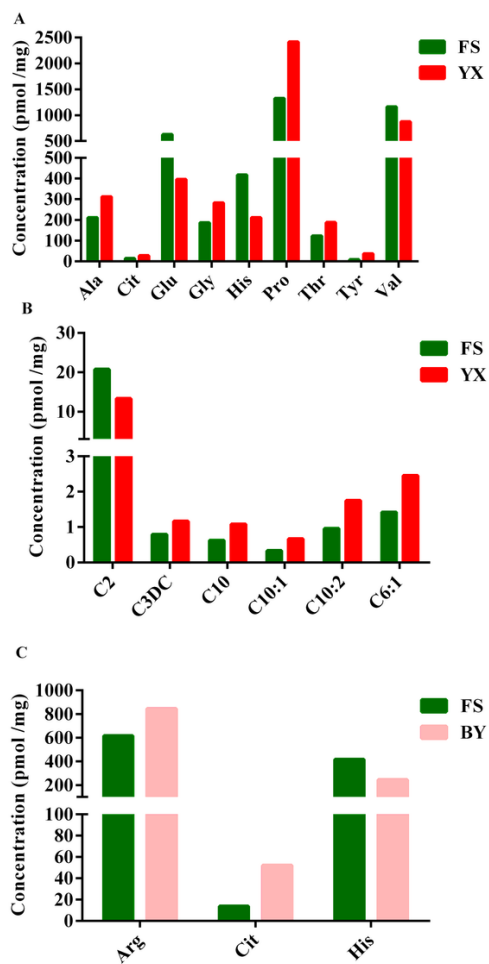
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## Figures



**Figure 1**

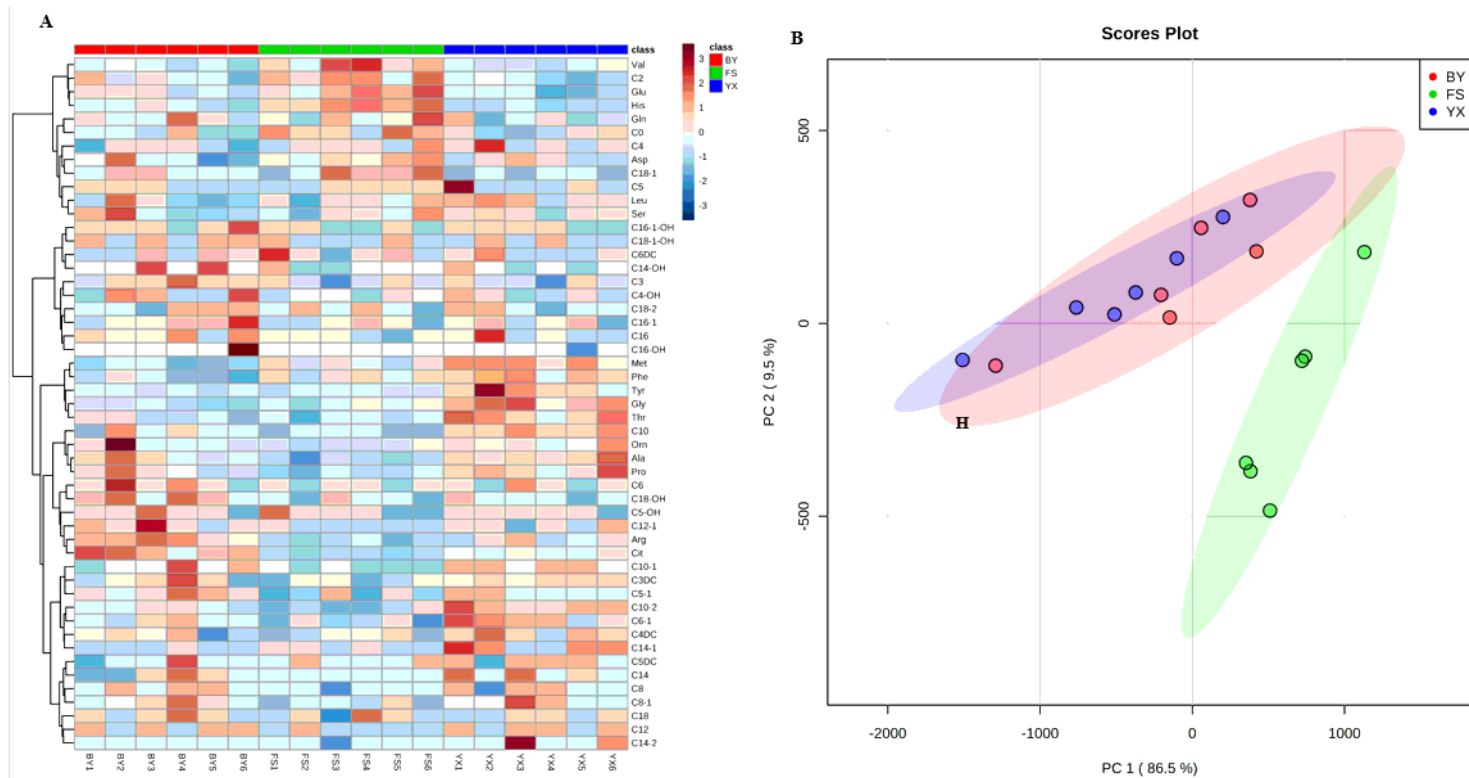
The concentrations of amino acids and acylcarnitines in Foshan (FS), Baiyun (BY) and Yuexiu (YX) strains



**Figure 2**

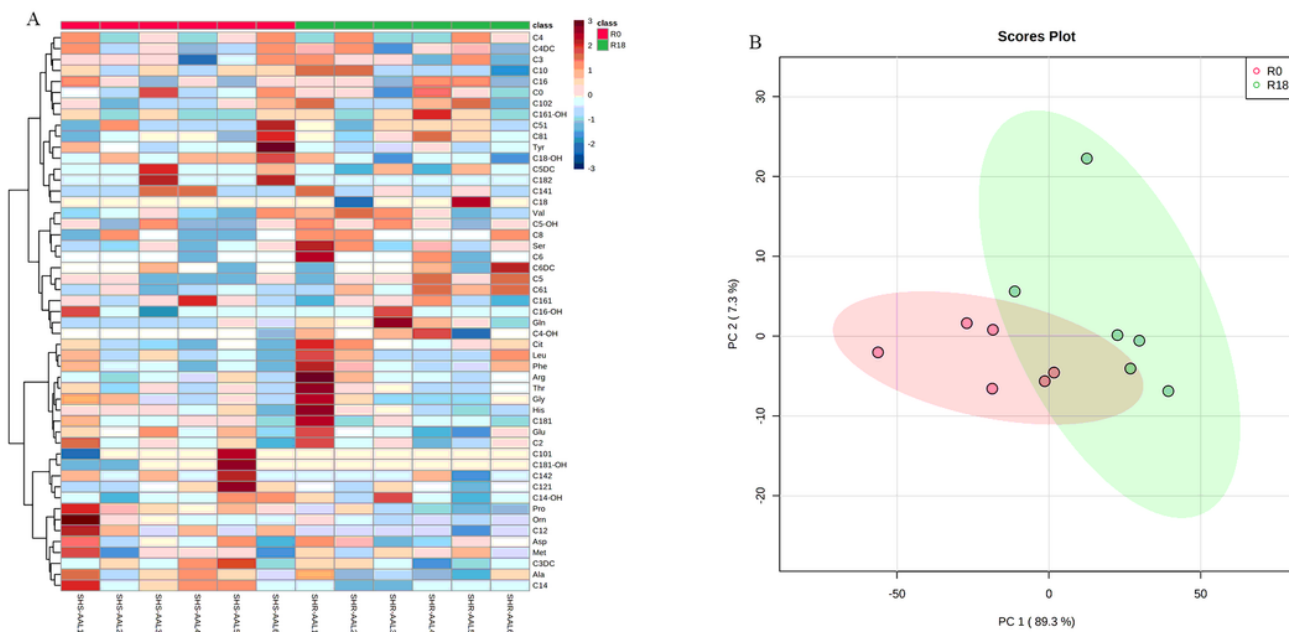
Differential amino acids and AcylCNss between DS-FS and DR-BY/YX larvae. (A) & (B) significant differential amino acids and AcylCNs between susceptible Foshan (FS) and resistant Yuexiu (YX) larvae; (C) significant differential amino acids between susceptible DS-FS and Foshan (FS) and resistant Baiyun (BY) larvae.





**Figure 3**

Heatmap (A) and PCA analysis (B) showed Differential amino acids and AcylCNS profiles between Foshan (FS) and Baiyun (BY)/Yuexiu (YX) larvae.



**Figure 4**

Heatmap (A) and PCA analysis (B) showed differential amino acids and AcylCNS profiles between larvae from deltamethrin-resistant R18 and its parental susceptible strains (R0)

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
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