

Morphological variations of *Aedes aegypti* mosquito populations in northern Tulare County, California

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Abstract

The yellow fever mosquito, *Aedes aegypti* (L.), is identified by its contrasting black and white coloration and the distinct lyre shaped marking on its scutum. Morphological variation in the patterns and colorations of tergal scales have been found in *Ae. aegypti* across tropical and subtropical regions. Since 2019, Delta Vector Control District has been finding several adult females with an almost entirely pale, yellow-scaled terga in northern Tulare County. BG-Sentinel traps were used to collect pale types of *Ae. aegypti* specimens for further analysis and photographing morphological differences. A pale type of *Ae. aegypti* was sent to Dr. Leopoldo Rueda, a taxonomist at the Walter Reed Biosystematics Unit, for species confirmation while both pale and wild types of *Ae. aegypti* were sent to San Mateo County Mosquito and Vector Control District for genetic analysis. Sequencing results of the cytochrome c oxidase subunit I gene for the pale type of *Ae. aegypti* showed >99% matching identity to other sequences of *Ae. aegypti* available in GenBank. In addition, Dr. Rueda confirmed the dried specimen as an *Ae. aegypti*. Further genetic and physiological analyses of the pale type of *Ae. aegypti* are warranted to understand its biology and potential ramifications for pesticide resistance and mosquito-borne disease transmission.

Introduction

Morphological variations within mosquito species exist globally. *Aedes aegypti queenslandensis*, the pale type *Ae. aegypti* (L.), have been found in countries within the eastern hemisphere, such as Singapore, Thailand, and Australia (Mattingly et al. 1967, Thongrungrat et al. 2012). In 2019, Delta Vector Control District (DVCD) first identified a potential adult female, pale type of *Ae. aegypti* from a BG-Sentinel (BGS) trap. In 2020, the District collected another pale type of *Ae. aegypti* adult female that prompted further investigation. Three genetic clusters of *Ae. aegypti* have currently been identified in California, but other genetic clusters may have been recently introduced and not yet identified (Lee et al. 2019). The goal of this investigation is to confirm the species identification of the specimen and to identify key morphological differences between the pale and wild types of *Ae. aegypti*.

Materials & Methods

Collection of pale and wild types of *Ae. aegypti* were obtained from the District's routine and supplemental BGS traps. For morphological comparisons, both types of adult *Ae. aegypti* were placed in 0.5x2" polycarbonate vials and stored in a -20°C freezer to maintain the integrity of the specimens. For genetic comparisons of the cytochrome c oxidase subunit I (COI) gene, a common mitochondrial gene that discriminates between vertebrate and invertebrate species, both pale and wild types of *Ae. aegypti* were sent to San Mateo County Mosquito and Vector Control District (SMCMVCD). Sequencing of the COI gene was compared to other *Ae. aegypti* gene sequences available in GenBank by using the Basic Local Alignment Search Tool (BLAST).

One adult pale type *Ae. aegypti* female was sent to Dr. Leopoldo Rueda, a taxonomist from the Walter Reed Biosystematics Unit, for species confirmation. Additionally, incubation paper with suspected mosquito eggs of the pale type of *Ae. aegypti* were sent to Dr. Anthony Cornel for potential rearing and isolation of the adult mosquitoes.

Morphological differences between pale and wild types of *Ae. aegypti* were photographed by rehydrating frozen specimens on top of paper towels soaked in warm water while enclosed in a plastic container for at least 30 minutes. Photographs of the specimens were taken using a Nikon D3100 DSLR camera with a simul-focal trinocular stereo microscope and a NPDL-2(2X) microscope lens adapter.

Job Title	TR_645404-501_DUNK1_CO1R_CO1_reverse_F12.ab1	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
RID	UV15P6YJ013 Search expires on 11-14 01:53 am Download All	Aedes aegypti isolate RDC7 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MN298998.1
Program	BLASTN Citation	Aedes aegypti voucher ECU22361 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890453.1
Database	nt See details	Aedes aegypti voucher ECU22356 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890448.1
Query ID	lcl Query_47769	Aedes aegypti voucher ECU22353 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890445.1
Description	TR_645404-501_DUNK1_CO1R_CO1_reverse_F12.ab1	Aedes aegypti voucher ECU22352 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890444.1
Molecule type	dna	Aedes aegypti voucher ECU22349 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890442.1
Query Length	904	Aedes aegypti voucher ECU22348 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890441.1
		Aedes aegypti voucher ECU22347 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890440.1
		Aedes aegypti voucher ECU20756 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890432.1
		Aedes aegypti voucher ECU20778 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	852	852	51%	0.0	99.57%	MK890429.1

Figure 1. Initial BLAST results for the pale type of *Ae. aegypti* showed >99% matching identity to other COI gene sequences of *Ae. aegypti* available in the GenBank.

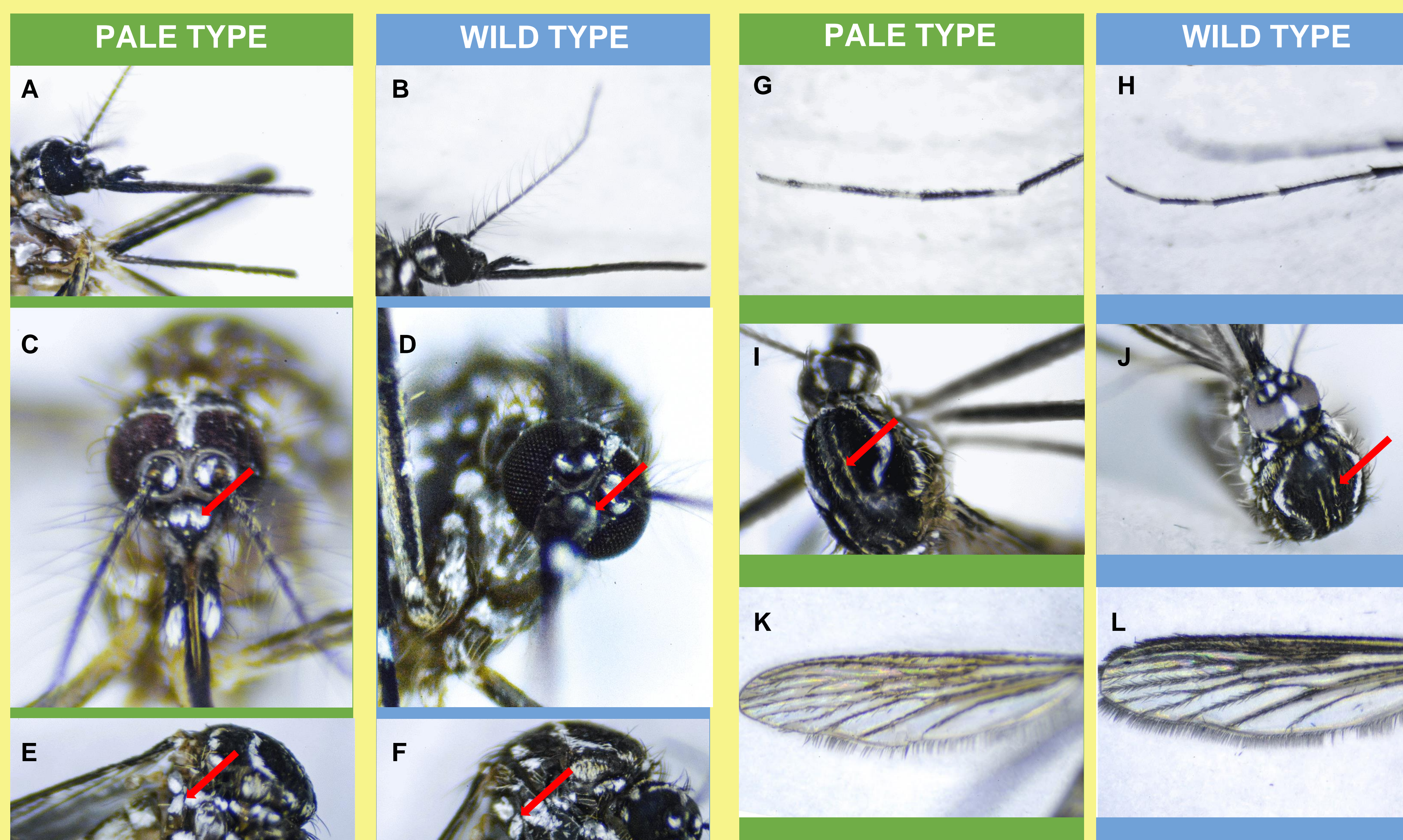


Figure 2. No significant difference between the heads (A-D), mesopleura (E-F), hindlegs (G-H), scuti (I-J), and wing venations (K-L) of the pale and wild types of *Ae. aegypti*.

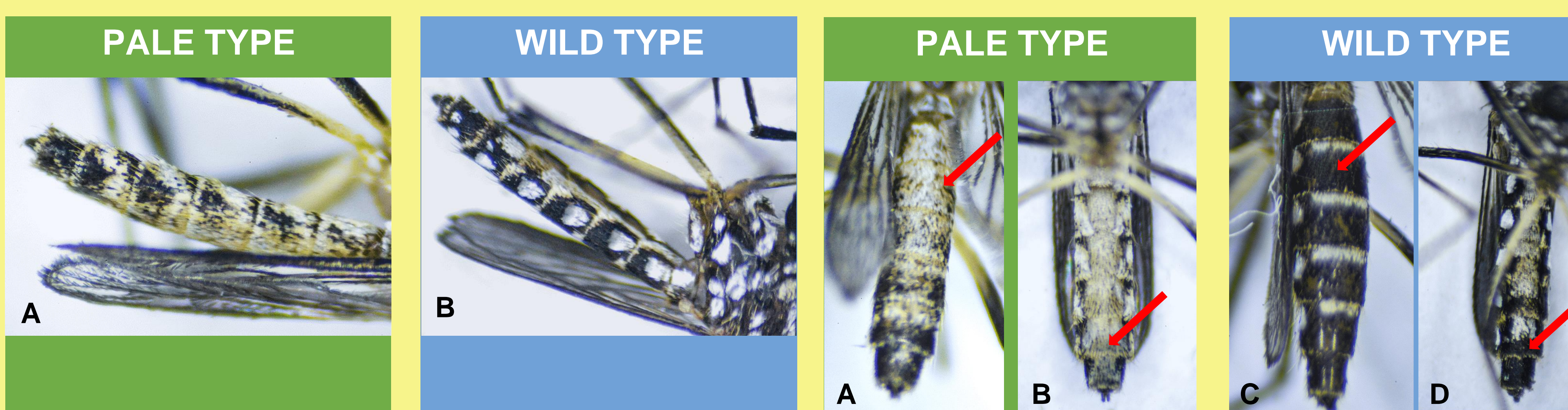


Figure 3. Abundant pale-yellow scales can be seen on the side of the abdomen of the pale type of *Ae. aegypti* (A) but not on the wild type (B).

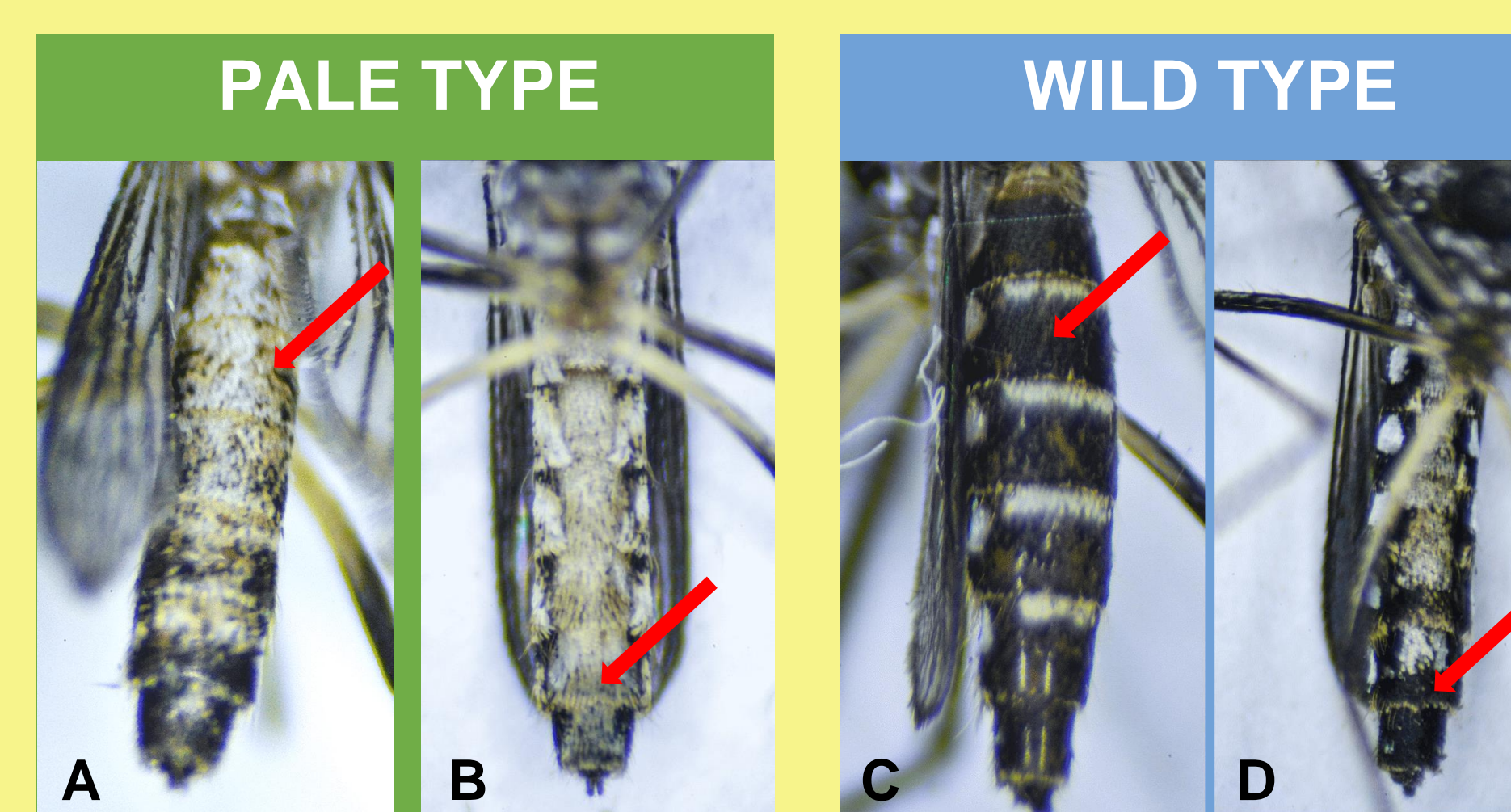


Figure 4. Abundant pale-yellow scales can be seen on the dorsal and ventral sides of the abdomen of the pale type of *Ae. aegypti* (A and B) but not on the wild type (C and D)



Figure 5. A damaged pale type of *Ae. aegypti* (left) compared to the wild type (right). Pale-yellow scales can be seen across the center of the tergal segments.

Results & Discussion

In 2020, the District collected and identified 17,330 adult *Ae. aegypti* of which 23 were pale types of *Ae. aegypti* females. Sequencing of the COI gene of the pale type of *Ae. aegypti* showed >99% matching identity to *Ae. aegypti* sequences available in the GenBank (Fig. 1). Previous studies involving the coexistence of the pale type, *Ae. aegypti queenslandensis*, with wild types of *Ae. aegypti* showed indistinguishable mitochondrial phylogeny with African and global *Ae. aegypti* known to date as well as no significant difference in vector competencies (Rašić et al. 2016). In this case, the presence of the pale types of *Ae. aegypti* in DVCD indicates a new genetic cluster of *Ae. aegypti* that has not yet been identified. Although there are no significant differences in sequences of the COI gene, genetic differences between the pale and wild types of *Ae. aegypti* found within the DVCD boundaries might exist in other regions of the genome.

The pale type of *Ae. aegypti* had unique physical characteristics compared to the wild type of *Ae. aegypti*. The main identifiable characteristics of the specimen that was sent to Dr. Rueda were on the clypeus, mesopleuron, and legs (Fig. 2A-H). No major difference was observed on the scutum and wing venation between both types (Fig. 2I-L). While the main features of *Ae. aegypti* were present, the pale types of *Ae. aegypti* had pale-yellow scales covering the ventral and dorsal sides of the abdomen (Fig. 3-5).

Conclusion

The pale type of *Ae. aegypti* is a variant of the darker, wild type *Ae. aegypti*. Further studies are being conducted by Dr. Anthony Cornel to rear *Ae. aegypti* eggs that we collected for this project, so that the pale types of *Ae. aegypti* can be isolated and analyzed. In-depth genetic and biological studies on the pale types of *Ae. aegypti* found in northern Tulare County are needed to classify the specimens and understand the potential effect on pesticide resistance and mosquito-borne disease transmission.

Acknowledgement

We thank Angie Nakano and San Mateo County Mosquito and Vector Control District staff, for conducting COI tests on our pale and dark types of *Ae. aegypti* samples and providing us with results.

We also thank Dr. Leopoldo Rueda for identifying and confirming the species of the specimen that we sent him and Dr. Anthony Cornel for accepting our samples and conducting future experiments on this matter.

REFERENCES
1. Lee, Y., H. Schmidt, T. C. Collier, W. R. Conner, M. J. Hanemaaijer, M. Slatkin, J. M. Marshall, J. C. Chiu, C. T. Smartt, G. C. Lanzaro, F. S. Mulligan, and A. J. Cornel. 2019. Genome-wide divergence among invasive populations of *Aedes aegypti* in California. *BMC Genomics*. 20: 204
2. Mattingly, P. F. 1967. Taxonomy of *Aedes aegypti* and related species. *Bull. World Health Organ.* 36: 552-554

3. Rašić, G., I. Filipović, A. G. Callahan, D. Stanford, A. Chan, S. G. Lam-Phua, C. H. Tan, and A. A. Hoffmann. 2016. The queenslandensis and the type form of the dengue fever mosquito (*Aedes aegypti* L.) are genomically indistinguishable. *PLoS Negl. Trop. Dis.* 10(11): e0005096
4. Thongrungrat, S., P. Maneekan, L. Wasinpiyamongkol, and S. Prummongkol. 2011. Prospective field study of transovarial dengue-virus transmission by two different forms of *Aedes aegypti* in an urban area of Bangkok, Thailand. *J. Vector Ecol.* 36(1): 147-152