

Supplementary Information

Elimination of ultraviolet light-mediated attraction behavior in *Culex* mosquitoes via dsRNA-mediated knockdown of Opsins

Table S1. Summary of experimental conditions for all behavioral assays.

Figure	Assay type	Mosquito species	Light wavelength (nm)	Light intensity ($\mu\text{W}/\text{cm}^2$)	Biological replicates	Total number of mosquitoes tested	Test duration (min)
Fig. 1B	Y-maze assay	<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	150 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	500 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	1000 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	1500 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. 1C	Y-maze assay	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	6	300	5
			345	150 $\mu\text{W}/\text{cm}^2$	6	300	5
			345	500 $\mu\text{W}/\text{cm}^2$	6	300	5
			345	1000 $\mu\text{W}/\text{cm}^2$	4	200	5
			345	1500 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. 1D	Y-maze assay	<i>Ae. aegypti</i>	345	5 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	10 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	500 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	1000 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. 1E	Y-maze assay	Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	150 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	500 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	1000 $\mu\text{W}/\text{cm}^2$	3	150	5
			345	1500 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. 2B	Tube assay	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	3	218	5
		<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	4	200	5
		Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
		<i>Ae. aegypti</i>	345	50 $\mu\text{W}/\text{cm}^2$	3	116	5
		<i>Ae. albopictus</i>	395	50 $\mu\text{W}/\text{cm}^2$	3	150	5

Fig. 2C	Tube assay	<i>Cx. quinquefasciatus</i>	395	50 $\mu\text{W}/\text{cm}^2$	3	150	5	
		Field <i>Cx. quinquefasciatus</i>	395	50 $\mu\text{W}/\text{cm}^2$	3	150	5	
		<i>Ae. aegypti</i>	395	50 $\mu\text{W}/\text{cm}^2$	3	111	5	
Fig. 3C	Automated video-tracking	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	5	400	2	
		<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	6	480	2	
		Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	4	320	2	
Fig. 3D	Automated video-tracking	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	5	400	2	
		<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	6	480	2	
		Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	4	320	2	
Fig. 3E	Automated video-tracking	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	5	400	2	
		<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	6	480	2	
		Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	4	320	2	
Fig. 3F	Automated video-tracking	<i>Ae. albopictus</i>	345	50 $\mu\text{W}/\text{cm}^2$	5	400	2	
		<i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	6	480	2	
		Field <i>Cx. quinquefasciatus</i>	345	50 $\mu\text{W}/\text{cm}^2$	4	320	2	
Fig. 4B	Y-maze assay	<i>Ae. aegypti</i>	dsGFP	345	10 $\mu\text{W}/\text{cm}^2$	4	148	5
			dsAaegOpsin1	345	10 $\mu\text{W}/\text{cm}^2$	3	109	5
			dsAaegOpsin2	345	10 $\mu\text{W}/\text{cm}^2$	3	98	5
			dsAaegOpsin8	345	10 $\mu\text{W}/\text{cm}^2$	4	144	5
			dsAaegOpsin9	345	10 $\mu\text{W}/\text{cm}^2$	3	107	5
Fig. 5B	Y-maze assay	<i>Cx. quinquefasciatus</i>	dsGFP	345	500 $\mu\text{W}/\text{cm}^2$	5	232	5
			dsCqOpsin2	345	500 $\mu\text{W}/\text{cm}^2$	5	180	5
			dsCqOpsin3	345	500 $\mu\text{W}/\text{cm}^2$	5	176	5
			dsCqOpsin5	345	500 $\mu\text{W}/\text{cm}^2$	5	198	5
			dsCqOpsin6	345	500 $\mu\text{W}/\text{cm}^2$	5	189	5
			dsCqOpsin11	345	500 $\mu\text{W}/\text{cm}^2$	5	183	5
Fig. 5C	Y-maze assay	<i>Cx. quinquefasciatus</i>	dsGFP	345	50 $\mu\text{W}/\text{cm}^2$	5	197	5
			dsCqOpsin2	345	50 $\mu\text{W}/\text{cm}^2$	5	203	5
			dsCqOpsin3	345	50 $\mu\text{W}/\text{cm}^2$	5	214	5
			dsCqOpsin5	345	50 $\mu\text{W}/\text{cm}^2$	5	208	5
			dsCqOpsin6	345	50 $\mu\text{W}/\text{cm}^2$	5	198	5
			dsCqOpsin11	345	50 $\mu\text{W}/\text{cm}^2$	5	196	5
			dsGFP	345	500 $\mu\text{W}/\text{cm}^2$	3	112	5
			dsCqOpsin2	345	500 $\mu\text{W}/\text{cm}^2$	3	112	5

Fig. 5D	Y-maze assay	Field Cx. <i>quinquefasciatus</i>	dsCqOpsin3	345	500 $\mu\text{W}/\text{cm}^2$	3	113	5
			dsCqOpsin5	345	500 $\mu\text{W}/\text{cm}^2$	3	128	5
			dsCqOpsin6	345	500 $\mu\text{W}/\text{cm}^2$	3	120	5
			dsCqOpsin11	345	500 $\mu\text{W}/\text{cm}^2$	3	128	5
Fig. 5E	Tube assay	Cx. <i>quinquefasciatus</i>	dsGFP	345	500 $\mu\text{W}/\text{cm}^2$	4	180	5
			dsCqOpsin2	345	500 $\mu\text{W}/\text{cm}^2$	4	179	5
			dsCqOpsin3	345	500 $\mu\text{W}/\text{cm}^2$	4	187	5
			dsCqOpsin5	345	500 $\mu\text{W}/\text{cm}^2$	4	166	5
			dsCqOpsin6	345	500 $\mu\text{W}/\text{cm}^2$	4	180	5
			dsCqOpsin11	345	500 $\mu\text{W}/\text{cm}^2$	4	157	5
Fig. S1A	Y-maze assay	<i>Ae. albopictus</i>		345	150 $\mu\text{W}/\text{cm}^2$	3	218	5
		Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	3	150	5
		Field Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. S1B	Tube assay	<i>Ae. albopictus</i>		345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
		Cx. <i>quinquefasciatus</i>		345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
		Field Cx. <i>quinquefasciatus</i>		345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
		<i>Ae. aegypti</i>		345	50 $\mu\text{W}/\text{cm}^2$	3	150	5
Fig. S2A	Automated video-tracking	<i>Ae. albopictus</i>		345	150 $\mu\text{W}/\text{cm}^2$	5	400	2
		Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	8	640	2
		Field Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	6	480	2
Fig. S2B	Automated video-tracking	<i>Ae. albopictus</i>		345	150 $\mu\text{W}/\text{cm}^2$	5	400	2
		Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	8	640	2
		Field Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	6	480	2
Fig. S2C	Automated video-tracking	<i>Ae. albopictus</i>		345	150 $\mu\text{W}/\text{cm}^2$	5	400	2
		Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	8	640	2
		Field Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	6	480	2
Fig. S2D	Automated video-tracking	<i>Ae. albopictus</i>		345	150 $\mu\text{W}/\text{cm}^2$	5	400	2
		Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	8	640	2
		Field Cx. <i>quinquefasciatus</i>		345	150 $\mu\text{W}/\text{cm}^2$	6	480	2

Table S2. qPCR primers of opsin genes used in expression profile.

Primers for SYBR Green RT-qPCR	Forward Primer	Reverse Primer
<i>CqOpsin1</i>	CCTTATTCGGATGTTGCTCG	GGAGTT- GGTCAGCGGTTTAC
<i>CqOpsin2</i>	TGCCTTCGGAGATAAGACC	TGACTGATGGCGTAAACCC
<i>CqOpsin3</i>	GAACCACGAGAAGGCACTT	CACAAACAG- GAAACAGACGG
<i>CqOpsin4</i>	TGGAAGTGGAAGGGAAAG	AGCAGTTGACGCTGTTGA
<i>CqOpsin5</i>	ACAACCTCTTCCAACGACTCC	TCCAGTGTGTGCTGTCTTG
<i>CqOpsin6</i>	GAACAGATGCGTGAACAGG	TGAACCACAGGGAAATGG
<i>CqOpsin7</i>	GTTATCGGCAACGGATGT	ATCAGGAAGTCGGA- GAAGG
<i>CqOpsin8</i>	AAGCCGATGACCAACAAC	CGAAGAATGGAGGGA- TAGTC
<i>CqOpsin9</i>	CGTCTCCATCTGGTCAATG	GTTGTTGGTCATCGGCTT
<i>CqOpsin10</i>	CCTCTGGTTTATGGCTTGG	ACTTGGGATGGCTGATTCC
<i>CqOpsin11</i>	TTTCGCTTATCGGGAACG	ATTGGCATCTCGCACATC
<i>CqOpsin12</i>	CTGCTCATCTGGTGTACTCGT	TTCAGGGTTTGC GTTTCC
<i>CqOpsin13</i>	GCTTGCGAGGTCTACGCTAT	TCAGCCGAGATACCGTT- GAC
<i>AaegOpsin1</i>	CGGCTTTGTCTTCGAGTCTG	ACAAATGCCGCCATTC- TATTTT
<i>AaegOpsin2</i>	AGTAGTTCGTTGCTGTGGG	GGCAGCCATTGTCTTCTCCT
<i>AaegOpsin3</i>	CCGATTACCCCACTGGCTAC	GAACTTCTGG- TACAGGGCGG
<i>AaegOpsin4</i>	GATCCTGCCGACGATACGC	AAGTGCCTTAAGCAGAT- TCCG
<i>AaegOpsin5</i>	CTGCCGACGATTTCGCAGTC	TGTCCCTTTAAGCAGAT- TCCGTC
<i>AaegOpsin7</i>	ACAATCCGATCGTGACGGG	AGCCATCGACTTAG- CATCGG
<i>AaegOpsin8</i>	CCTTCGTGAACAGGCCAAGA	GGCTATCCTCAC- CTCAACCG
<i>AaegOpsin9</i>	ACGATTTCGGATACTCGCGTC	CATGCTT- GCTCACATGACCG
<i>AaegOpsin10</i>	TTCGCGCTATACGAGGAACG	GGATTGGTCTCGGTCAGGTC
<i>AaegOpsin12</i>	CGCTGATGTGCAAGGATGTG	TGTGAACGGGTTGCCGA- TAA

Table S3. Primers for dsRNA synthesis and detection.

Primers for dsRNA synthesis	Forward Primer	Reverse Primer
dsRNA GFP	TAATACGACTCAC- TATAGGGCAACGGTGTG- GACTTTGAC	TAATACGACTCAC- TATAGGGCGGCTGGTTCTTCAG ATAGTT
dsRNA <i>CqOpsin2</i>	TAATACGACTCAC- TATAGGGTGTCTTGCTCAA CGAAACCG	TAATACGACTCAC- TATAGGGATCACAAC- GAACAGCGATGC
dsRNA <i>CqOpsin3</i>	TAATACGACTCAC- TATAGGGTGGTTTGGGCG- TACACCATT	TAATACGACTCAC- TATAGGGAACCACGGTAG- TCGCTTCTG
dsRNA <i>CqOpsin5</i>	TAATACGACTCAC- TATAGGGCGG- TATGTCCCAGAGGGTAA	TAATACGACTCAC- TATAGGGTCGTTGGAAGAGTT- GTCCG
dsRNA <i>CqOpsin6</i>	TAATACGACTCAC- TATAGGGACAGTGGTGC- TATGGTCAAG	TAATACGACTCAC- TATAGGGGGTGTGCTTGGA- GAGTTG
dsRNA <i>CqOpsin11</i>	TAATACGACTCAC- TATAGGGCGGTGGAG- CAATCAGCAATG	TAATACGACTCAC- TATAGGGGTCTAGGCAG- GACACAACCT
dsRNA <i>AaegOpsin1</i>	TAATACGACTCAC- TATAGGGAGTTCCAC- CGATGAATCC	TAATACGACTCAC- TATAGGGGGCGTAAAC- GATGATGTAGG
dsRNA <i>AaegOpsin2</i>	TAATACGACTCAC- TATAGGGTATGAT- TCACCCGCACTG	TAATACGACTCACTATAGGG- GAAGATGGCGTAGATGATGA
dsRNA <i>AaegOpsin8</i>	TAATACGACTCAC- TATAGGGTTGTCAG- TAGGCACCGTGA	TAATACGACTCAC- TATAGGGAGTGAAC- CGATGAAAGCG
dsRNA <i>AaegOpsin9</i>	TAATACGACTCAC- TATAGGGGTATCTTGCGAC- GGTGTTT	TAATACGACTCAC- TATAGGGGCGATGACTGCGTT AGTTATG
Primers for SYBR Green RT-qPCR	Forward Primer	Reverse Primer
<i>CqOpsin2</i>	CTGTTTGTCTGCGCTTGGAC	GCACGAAACAATCTTGCAGC
<i>CqOpsin3</i>	TATCGGCCGAAACTCGCTAC	AGGGCGAAAATTGTGAACGC
<i>CqOpsin5</i>	TACCGCTCTAGACGTTGTGA	ATGTCCACGACGGACATGTTG
<i>CqOpsin6</i>	ACATCTCCACGTGCAG- TTTCTTA	GGTCAAGCGCGTGATTGTTG
<i>CqOpsin11</i>	GCCATCAGCCATCCGAAGT A	CTGCCGGAGGATCCGATATG

<i>AaegOpsin1</i>	CGGCTTTGTCTTCGAGTCTG	ACAAATGCCGCCATTCTATTTC
<i>AaegOpsin2</i>	AGTAGTTCGTTTCGCTGTGGG	GGCAGCCATTGTCTTCTCCT
<i>AaegOpsin8</i>	CCTTCGTGAACAGGCCAAG	GGCTATCCTCACCTCAACCG
	A	
<i>AaegOpsin9</i>	ACGATTCGGATACTCGCGTC	CATGCTTGCTCACATGACCG

Table S4. Details of the test methods used for each sample analysis

Figure	Test method	Sample	<i>t</i>	<i>df</i>	<i>P</i>
Fig. 1B	One sample <i>t</i> test	50 $\mu\text{W}/\text{cm}^2$	10.36	5	< 0.001
		150 $\mu\text{W}/\text{cm}^2$	12.14	5	< 0.001
		500 $\mu\text{W}/\text{cm}^2$	17.23	5	< 0.001
		1000 $\mu\text{W}/\text{cm}^2$	15.78	5	< 0.001
		1500 $\mu\text{W}/\text{cm}^2$	1.323	5	0.2431
Fig. 1C	One sample <i>t</i> test	50 $\mu\text{W}/\text{cm}^2$	10.65	11	< 0.001
		150 $\mu\text{W}/\text{cm}^2$	7.326	11	< 0.001
		500 $\mu\text{W}/\text{cm}^2$	9.629	11	< 0.001
		1000 $\mu\text{W}/\text{cm}^2$	4.046	7	0.0049
		1500 $\mu\text{W}/\text{cm}^2$	1.518	5	0.1896
Fig. 1D	One sample <i>t</i> test	5 $\mu\text{W}/\text{cm}^2$	8.464	5	< 0.001
	One sample <i>t</i> test	10 $\mu\text{W}/\text{cm}^2$	10.20	5	< 0.001
	Wilcoxon signed-rank test	50 $\mu\text{W}/\text{cm}^2$	$T = 21, N = 6$		0.2810
	One sample <i>t</i> test	150 $\mu\text{W}/\text{cm}^2$	0.8902	5	0.4141
	One sample <i>t</i> test	500 $\mu\text{W}/\text{cm}^2$	0.9807	5	0.3718
	One sample <i>t</i> test	1000 $\mu\text{W}/\text{cm}^2$	0.1514	5	0.8856
Fig. 1E	One sample <i>t</i> test	50 $\mu\text{W}/\text{cm}^2$	18.27	5	< 0.001
		150 $\mu\text{W}/\text{cm}^2$	14.70	5	< 0.001
		500 $\mu\text{W}/\text{cm}^2$	11.67	5	< 0.001
		1000 $\mu\text{W}/\text{cm}^2$	7.018	5	< 0.001
		1500 $\mu\text{W}/\text{cm}^2$	0.5929	5	0.579
Fig. 2B	One sample <i>t</i> test	<i>Ae. albopictus</i>	9.985	5	< 0.001
		<i>Cx. quinquefasciatus</i>	14.32	7	< 0.001
		Field <i>Cx. quinquefasciatus</i>	12.25	5	< 0.001
		<i>Ae. aegypti</i>	1.895	5	0.1166
Fig. 2C	One sample <i>t</i> test	<i>Ae. albopictus</i>	14.23	5	< 0.001
	One sample <i>t</i> test	<i>Cx. quinquefasciatus</i>	5.098	5	0.0038
	One sample <i>t</i> test	Field <i>Cx. quinquefasciatus</i>	3.677	5	0.0143
	Wilcoxon signed-rank test	<i>Ae. aegypti</i>	$T = 9, N = 6$		0.4375
Fig. 3C	One sample <i>t</i> test	<i>Ae. albopictus</i>	8.625	5	< 0.001
		<i>Cx. quinquefasciatus</i>	7.207	5	< 0.001
		Field <i>Cx. quinquefasciatus</i>	6.608	5	< 0.001
Fig. 3D	Unpaired <i>t</i> test	UV vs Dark (<i>Ae. albopictus</i>)	7.377	18	< 0.001
		UV vs Dark (<i>Cx. quinquefasciatus</i>)	5.615	22	< 0.001

		UV vs Dark (Field Cx. <i>quinquefasciatus</i>)	9.247	14	< 0.001
Fig. 3E	Unpaired <i>t</i> test	UV vs Dark (<i>Ae. albopictus</i>)	6.691	18	< 0.001
		UV vs Dark (<i>Cx. quinquefasciatus</i>)	5.884	22	< 0.001
		UV vs Dark (Field Cx. <i>quinquefasciatus</i>)	9.416	14	< 0.001
Fig. 3F	Unpaired <i>t</i> test	UV vs Dark (<i>Ae. albopictus</i>)	4.053	18	< 0.001
		UV vs Dark (<i>Cx. quinquefasciatus</i>)	3.444	22	0.0023
		UV vs Dark (Field Cx. <i>quinquefasciatus</i>)	0.6237	14	0.5371
Fig. 4B	One sample <i>t</i> test	<i>dsAaegOpsin1</i>	11.25	5	< 0.001
	One sample <i>t</i> test	<i>dsAaegOpsin2</i>	13.34	5	< 0.001
	One sample <i>t</i> test	<i>dsAaegOpsin8</i>	7.423	5	< 0.001
	One sample <i>t</i> test	<i>dsAaegOpsin9</i>	6.633	5	< 0.001
	Wilcoxon signed-rank test	<i>dsGFP</i>	$T = 36, N = 8$		0.0078
Fig. 5B	One sample <i>t</i> test	<i>dsGFP</i>	10.89	9	< 0.001
		<i>CqOpsin2</i>	11.52	9	< 0.001
		<i>CqOpsin3</i>	3.377	9	0.0082
		<i>CqOpsin5</i>	0.9304	9	0.3764
		<i>CqOpsin6</i>	1.426	9	0.1876
		<i>CqOpsin11</i>	9.955	9	< 0.001
Fig. 5C	One sample <i>t</i> test	<i>dsGFP</i>	10.32	9	< 0.001
	Wilcoxon signed-rank test	<i>CqOpsin2</i>	$T = 53, N = 10$		0.0039
	One sample <i>t</i> test	<i>CqOpsin3</i>	0.07227	9	0.944
	One sample <i>t</i> test	<i>CqOpsin5</i>	0.4328	9	0.6753
	One sample <i>t</i> test	<i>CqOpsin6</i>	1.392	9	0.1975
	One sample <i>t</i> test	<i>CqOpsin11</i>	7.479	9	< 0.001
Fig. 5D	One sample <i>t</i> test	<i>dsGFP</i>	5.003	5	0.0041
		<i>CqOpsin2</i>	5.464	5	0.0028
		<i>CqOpsin3</i>	1.101	5	0.3212
		<i>CqOpsin5</i>	0.5257	5	0.6216
		<i>CqOpsin6</i>	0.4230	5	0.6899
		<i>CqOpsin11</i>	14.27	5	< 0.001
Fig. 5E	One sample <i>t</i> test	<i>dsGFP</i>	8.874	7	< 0.001
		<i>CqOpsin2</i>	6.691	7	< 0.001
		<i>CqOpsin3</i>	1.596	7	0.1546
		<i>CqOpsin5</i>	1.275	7	0.2429

		<i>CqOpsin6</i>	3.202	7	0.015
		<i>CqOpsin11</i>	11.64	7	< 0.001
Fig. S1A	One sample <i>t</i> test	<i>Ae. albopictus</i>	5.944	5	< 0.001
		<i>Cx. quinquefasciatus</i>	8.450	5	< 0.001
		Field <i>Cx. quinquefasciatus</i>	21.27	5	< 0.001
Fig. S1B	One sample <i>t</i> test	<i>Ae. albopictus</i>	8.638	5	< 0.001
		<i>Cx. quinquefasciatus</i>	4.628	5	0.0057
		Field <i>Cx. quinquefasciatus</i>	9.004	5	< 0.001
		<i>Ae. aegypti</i>	40.73	5	< 0.001
Fig. S2A	One sample <i>t</i> test	<i>Ae. albopictus</i>	11.42	9	< 0.001
		<i>Cx. quinquefasciatus</i>	5.206	15	< 0.001
		Field <i>Cx. quinquefasciatus</i>	8.288	11	< 0.001
Fig. S2B	Unpaired <i>t</i> test	UV vs Dark (<i>Ae. albopictus</i>)	11.54	18	< 0.001
		UV vs Dark (<i>Cx. quinquefasciatus</i>)	6.473	30	< 0.001
		UV vs Dark (Field <i>Cx. quinquefasciatus</i>)	5.844	22	< 0.001
Fig. S2C	Unpaired <i>t</i> test	UV vs Dark (<i>Ae. albopictus</i>)	9.816	18	< 0.001
		UV vs Dark (<i>Cx. quinquefasciatus</i>)	6.227	30	< 0.001
		UV vs Dark (Field <i>Cx. quinquefasciatus</i>)	5.402	22	< 0.001
Fig. S2D	Mann Whitney <i>U</i> test	UV vs Dark (<i>Ae. albopictus</i>)	$U = 10, N_1 = N_2 = 10$		0.0015
	Unpaired <i>t</i> test	UV vs Dark (<i>Cx. quinquefasciatus</i>)	4.227	30	
	Mann Whitney <i>U</i> test	UV vs Dark (Field <i>Cx. quinquefasciatus</i>)	$U = 10, N_1 = N_2 = 12$		0.5137
Fig. S3A	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin1</i>	11.31	18	< 0.001
Fig. S3B	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	9.599	18	< 0.001
Fig. S3C	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin8</i>	5.852	18	< 0.001
Fig. S3D	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin9</i>	7.152	18	< 0.001
Fig. S4A	Mann-Whitney <i>U</i> test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	$U = 9, N_1 = N_2 = 10$		0.0011
Fig. S4B	Mann-Whitney <i>U</i> test	<i>dsGFP</i> vs. <i>dsOpsin3</i>	$U = 3, N_1 = N_2 = 10$		< 0.001
Fig. S4C	Mann-Whitney <i>U</i> test	<i>dsGFP</i> vs. <i>dsOpsin5</i>	$U = 10, N_1 = N_2 = 10$		0.0015
Fig. S4D	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin6</i>	2.928	18	0.009
Fig. S4E	Unpaired <i>t</i> test	<i>dsGFP</i> vs. <i>dsOpsin11</i>	4.278	18	< 0.001

"*t*" represents the test statistic value of the one sample *t* test, "*df*" represents the degrees of freedom, "*T*" represents the test statistic of the Wilcoxon signed-ranks test, "*N*" represents the sample size, and "*U*" represents the test statistic value of the Mann-Whitney *U* test.

Table S5. The results of multiple comparisons test for testing among different groups.

Figure	Test methods	Groups	Mean rank diff.	<i>P</i>
Fig. 4B	Kruskal-Wallis test		$H_4 = 7.53$	0.1104
	Dunn's multiple comparisons test	<i>dsGFP</i> vs. <i>dsOpsin1</i>	-3.458	> 0.9999
		<i>dsGFP</i> vs. <i>dsOpsin2</i>	-4.042	> 0.9999
		<i>dsGFP</i> vs. <i>dsOpsin8</i>	8.500	0.8712
		<i>dsGFP</i> vs. <i>dsOpsin9</i>	2.542	> 0.9999
Fig. 5B	One-way ANOVA		$F(5, 54) = 20.39$	< 0.001
	Tukey's multiple comparisons test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	-0.08493	0.7313
		<i>dsGFP</i> vs. <i>dsOpsin3</i>	-0.3485	< 0.001
		<i>dsGFP</i> vs. <i>dsOpsin5</i>	-0.4367	< 0.001
		<i>dsGFP</i> vs. <i>dsOpsin6</i>	-0.4408	< 0.001
		<i>dsGFP</i> vs. <i>dsOpsin11</i>	-0.1088	0.4841
Fig. 5C	Kruskal-Wallis test		$H_5 = 33.44$	< 0.001
	Dunn's multiple comparisons test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	-5.15	> 0.9999
		<i>dsGFP</i> vs. <i>dsOpsin3</i>	-31.15	0.001
		<i>dsGFP</i> vs. <i>dsOpsin5</i>	-29	0.0031
		<i>dsGFP</i> vs. <i>dsOpsin6</i>	-26.45	0.0106
		<i>dsGFP</i> vs. <i>dsOpsin11</i>	-4.25	> 0.9999
Fig. 5D	One-way ANOVA		$F(5, 30) = 6.693$	< 0.001
	Tukey's multiple comparisons test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	-0.04459	0.9976
		<i>dsGFP</i> vs. <i>dsOpsin3</i>	-0.3146	0.0412
		<i>dsGFP</i> vs. <i>dsOpsin5</i>	-0.3538	0.0162
		<i>dsGFP</i> vs. <i>dsOpsin6</i>	-0.3564	0.0152
		<i>dsGFP</i> vs. <i>dsOpsin11</i>	0.01875	> 0.9999
Fig. 5E	One-way ANOVA		$F(5, 42) = 13.04$	< 0.001
	Tukey's multiple comparisons test	<i>dsGFP</i> vs. <i>dsOpsin2</i>	-0.0104	> 0.9999
		<i>dsGFP</i> vs. <i>dsOpsin3</i>	-0.308	< 0.001
		<i>dsGFP</i> vs. <i>dsOpsin5</i>	-0.3209	< 0.001
		<i>dsGFP</i> vs. <i>dsOpsin6</i>	-0.2455	0.0054
		<i>dsGFP</i> vs. <i>dsOpsin11</i>	0.02093	0.9995

"*H*" represents the test statistic value of the Kruskal-Wallis test and "*F*" represents the test statistic value of the One-way ANOVA.

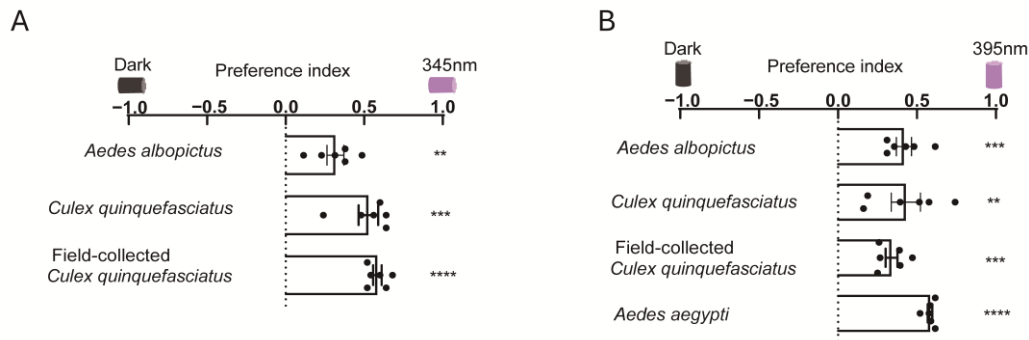


Figure. S1 The photobehaviour of female mosquitoes under different wavelengths of UV light. (A) photobehaviour of laboratory-reared *Ae. albopictus*, *Cx. quinquefasciatus* and field-collected *Cx. quinquefasciatus* under 345 nm UV light at intensities of 150 $\mu\text{W}/\text{cm}^2$. (B) photobehaviour of laboratory-reared *Ae. albopictus*, *Cx. quinquefasciatus*, *Ae. aegypti*, and field-collected *Cx. quinquefasciatus* under 395 nm UV light at intensities of 50 $\mu\text{W}/\text{cm}^2$. (A-B) Values represent mean $\pm$ SEM of ≥ 3 biological replicates (two technical replicates each). The total number of female mosquitoes tested in each group is presented in Table S1. Statistical significance of phototaxis relative to chance was computed using a one-sample *t*-test. Significance: ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

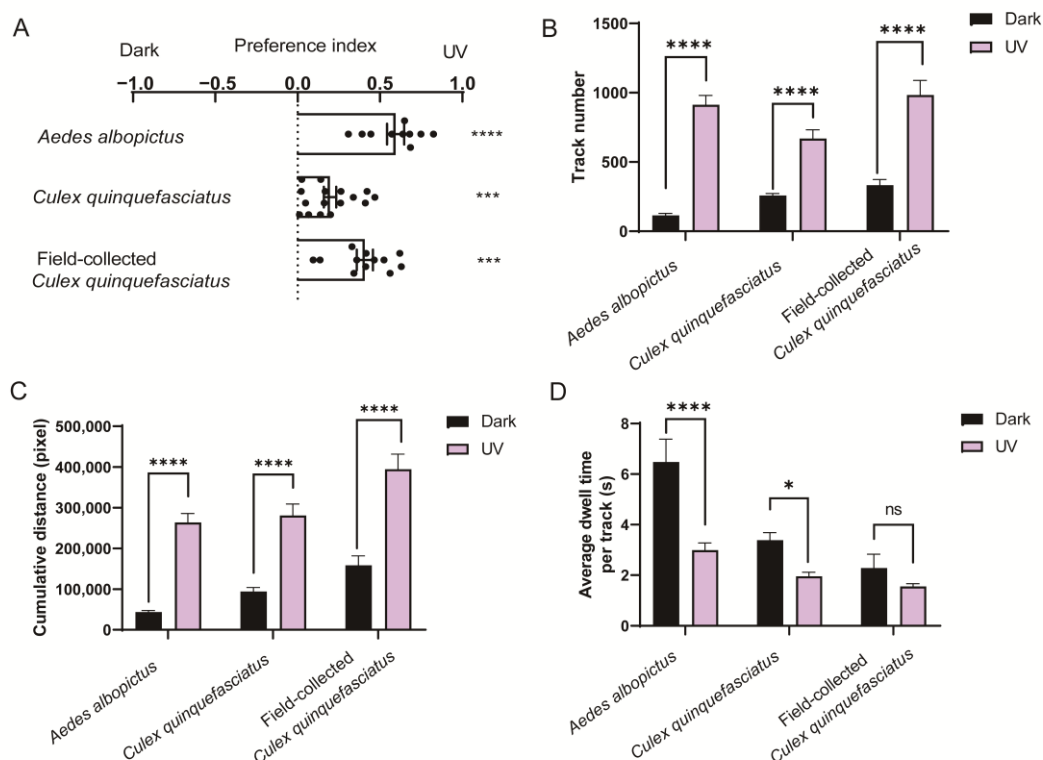


Figure S2. Video recordings and automated analysis were conducted to assess mosquito photobehaviour under 345 nm UV light at an intensity of 150 $\mu\text{W}/\text{cm}^2$. (A-D) Photopreference between darkness and 345nm UV light of 150 $\mu\text{W}/\text{cm}^2$. (A) The preference index was calculated by summing the PI values for each frame and dividing by the total number of frames. (B) The number of mosquito tracks presented at respective window. (C) The cumulative distance mosquitoes moved on respective mesh window of all tracks. (D) The average dwell time that each mosquito track spent on respective mesh window. (A-D) Values represent mean $\pm$ SEM of ≥ 3 biological replicates (two technical replicates each). The total number of female mosquitoes tested in each group is presented in Table S1. (A) Data were analyzed using one sample *t*-test. (B-D) Group comparisons were performed using unpaired *t*-test. or Mann-Whitney test. Significance: * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$, ns: not significant.

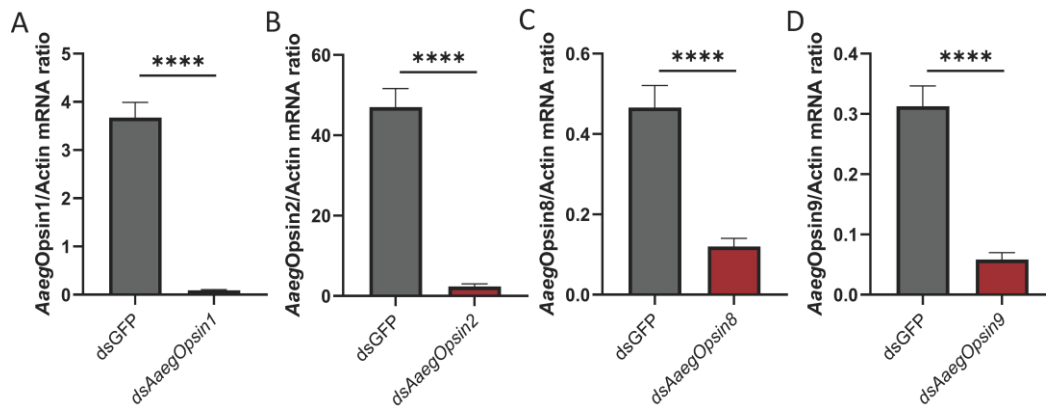


Figure S3. RNAi-mediated knockdown efficiency of *Ae. aegypti* opsin genes. Gene expression levels were quantified by RT-qPCR following intrathoracic injection of species-specific dsRNA. Expression of (A) *AaegOpsin1* after ds*AaegOpsin1* treatment, (B) *AaegOpsin2* after ds*AaegOpsin2* treatment, (C) *AaegOpsin8* after ds*AaegOpsin8* treatment, and (D) *AaegOpsin9* after ds*AaegOpsin9* treatment. Each group contained 30 female mosquitoes. (A-D) Values represent mean $\pm$ SEM of ≥ 3 biological replicates. Expression levels of opsin genes were normalized against *Ae. aegypti* Actin (*AAEL011197*). Group comparisons were performed using unpaired *t*-test. Significance: **** $P < 0.0001$.

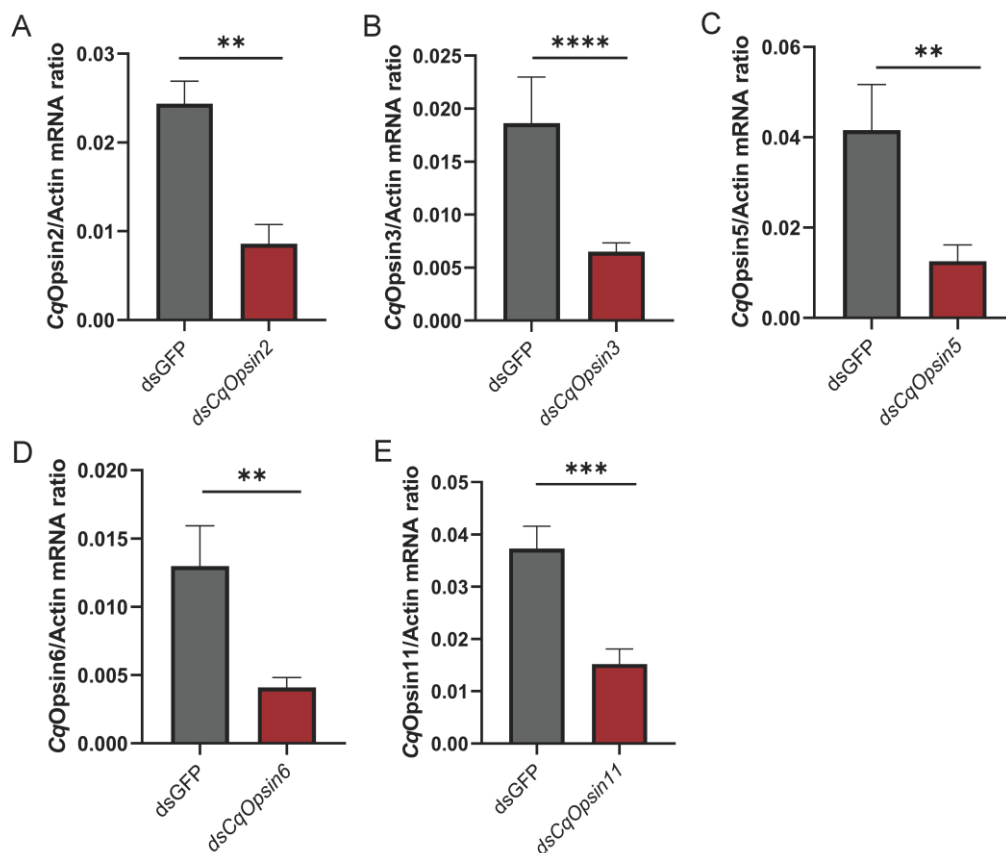


Figure S4. RNAi-mediated knockdown efficiency of *Cx. quinquefasciatus* opsin genes. Gene expression levels were quantified by RT-qPCR following intrathoracic injection of species-specific dsRNA. Expression of (A) *CqOpsin2* after ds*CqOpsin2* treatment, (B) *CqOpsin3* after ds*CqOpsin3* treatment, (C) *CqOpsin5* after ds*CqOpsin5* treatment, (D) *CqOpsin6* after ds*CqOpsin6* treatment, and (E) *CqOpsin11* after ds*CqOpsin11* treatment. Each group contained 30 female mosquitoes. (A-E) Values represent mean $\pm$ SEM of ≥ 3 biological replicates. Expression levels of opsin genes were

normalized against *Cx. quinquefasciatus* Rpl8 (LOC6031076) (A-C) Statistical comparisons between groups were conducted using Mann-Whitney test. (D-E) Statistical comparisons between groups were conducted using unpaired t-test. Significance: ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Video S1. Representative recording of mosquito photobehavior. This video documents the behavioral responses of mosquitoes exposed to 345 nm UV illumination, delivered from a computer-controlled tunable light source directed toward the right mesh windows. The footage spans 2 minutes and is presented at real-time speed without modification or acceleration.

Video S2. Automated analysis of mosquito activity. The video shows an automated program that records and analyzes mosquito activity. We used an automated object-tracking and PI calculating script based on MATLAB, to analyze mosquitoes in recorded video.