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COLOR, BACTERIA, AND MOSQUITO EGGS AS OVIPOSITIONAL  
ATTRACTANTS FOR *Aedes aegypti* AND *Aedes albopictus*  
(DIPTERA: CULICIDAE)

Steven G. Pavlovich<sup>1</sup> and C. Lee Rockett<sup>2</sup>

ABSTRACT

Selected bacterial washes, color, and mosquito eggs were comparatively examined as ovipositional attractants for *Aedes aegypti* and *Aedes albopictus*. Any evidence of additive activity was noted. All colored washes alone were preferred over selected bacterial washes. The combinations of color and bacteria in a single wash were better attractants for oviposition than colored washes alone. The bacterial content of the breeding water was a more important factor than egg presence in oviposition site selection.

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*Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) are important disease vectors. Christophers (1960) indicated that *Ae. aegypti*, the yellow fever mosquito, was probably brought to the United States during colonization through shipping trade with the Old World. *Aedes albopictus*, the Asian tiger mosquito, is a major vector of dengue in many parts of the world. *Aedes albopictus*, a relative newcomer to the United States, was initially found in Texas in 1985 (Sprenger and Wuithiranyagool 1986) and is now well established in numerous states.

Knowledge pertaining to mosquito oviposition behavior is useful in controlling mosquitoes and the diseases they vector. Bates (1949) noted that factors such as water movement, temperature, chemical composition, and background color of water can all influence mosquito oviposition behavior. Sites high in organic matter have been reported to be effective attractants to ovipositing females (Ritchie and Johnson 1991). Bacteria and their metabolites are thought to be essential components in the attractiveness of an ovipositional medium (Wilton 1968). Workers such as Maw (1970), Ikeshoji et al. (1975), and Benzon and Apperson (1988) have noted the role of specific bacterial species and their metabolites as ovipositional attractants. Steelman and Colmer (1970) compared two coliform bacteria (*Escherichia coli* (Migula) and *Enterobacter aerogenes* Hormaeche and Edwards) commonly found in polluted waters and reported that *E. coli* was the better ovipositional attractant. Rockett (1987) compared numerous bacterial species (with their metabolites) in mosquito breeding waters as potential attractants for *Culex pipiens* L. He found that *Pseudomonas maltophilia* Hugh was selected more frequently than any of the other tested bacteria or distilled water controls. In a similar

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work utilizing *Ae. aegypti*, Hasselschwert and Rockett (1988) noted that this species appeared to display discriminatory behavior in selecting oviposition sites with different bacterial species. The presence of *Pseudomonas aeruginosa* (Schroeter) and *Bacillus cereus* Frankland and Frankland were reported to be effective ovipositional attractants. Several other bacterial species and distilled water alone were significantly less effective in promoting oviposition.

Colored ovipositional substrates or containers have been used by numerous authors to examine the role of color as an ovipositional attractant. Bates (1940) examined the use of background color as an ovipositional attractant in *Anopheles atroparvus* van Thiel by placing different colored papers (black, yellow, and white) in the bottom of ovipositional pans. He found that the sites with black paper were most frequently selected by ovipositing mosquitoes. Gubler (1971) placed clear glass egg traps over circular discs of black, brown, or white paper and determined that the black background was preferred by two *Aedes* species: *Ae. albopictus* and *Aedes polynesiensis* Marks. Frank (1985) compared white, green, blue, and black, artificial bromeliad flower ovipositional sites and reported that *Ae. aegypti* was most attracted to the black artificial flowers. However, in the same study, Frank found that two *Wyeomyia* species reacted very differently than *Ae. aegypti* and tended to oviposit in the white and green artificial bromeliads; the black bromeliads received less than one percent of the total eggs deposited. Muir et al. (1992) stated that "much of the literature on the behavioral responses of *Ae. aegypti* to visual stimuli is contradictory."

In addition to bacteria and color, the immature stages of mosquitoes have also been noted as being effective ovipositional attractants. Bentley et al. (1976) noted that conspecific larvae are effective ovipositional attractants. Ahmadi and McClelland (1983) found that gravid *Ochlerotatus* (formerly *Aedes* genus) *sierrensis* (Ludlow) preferred larval water over distilled water and was most attracted to tree-hole water, rich in organics. Reisen and Meyer (1990) and Andreadis (1977) noted that *Culex tarsalis* (Coquillett) and *Culex salinarius* (Coquillett) respectively, had an affinity for ovipositing in conspecific pupal waters. Starratt and Osgood (1972) reported that the glyceryl esters of fatty acids were responsible for egg attractiveness in *Cx. tarsalis*. In working with *Ae. aegypti*, Hasselschwert and Rockett (1988) indicated that egg homogenate washes (ground mosquito eggs and distilled water) were not preferred when compared to the wash of the favorable bacterium, *B. cereus*.

The major purpose of this work was to further identify and compare, bacteria, color, and egg washes as ovipositional attractants for two congeneric mosquito species, *Ae. aegypti* and *Ae. albopictus*. An additional objective was to look for any evidence of additive activity when attractants were combined. Isoe and Millar (1995) noted that considerable confusion has arisen with regards to the terms "attraction" and "stimulation" in the context of clues promoting mosquito oviposition. Recognizing a distinction between the two terms, volatile chemical cues serve as attractants and act over a determined distance; contact chemicals stimulate oviposition following initial attraction. Excluding a few screened experiments in this work, no attempt was made to separate "attractants" from "stimulants". In this paper, the inclusive term "attractant" is used.

## MATERIALS AND METHODS

Colonies of *Ae. aegypti* and *Ae. albopictus* were maintained in the laboratory. Eggs were hatched in aged tap water that had been deoxygenated by

bubbling nitrogen gas through the water for 15 min. Larvae were subsequently raised on a mixture of brewers yeast and ground dog food in 15 x 15 x 30 cm plastic containers which were half full with water. Adult mosquitoes were kept in 40 x 40 x 80 cm screen cages at 27C, 80% relative humidity, and a photoperiod of 16L:8D. Adults were maintained, between blood meals, on cotton balls soaked with 10% sucrose. Prior to blood feeding, approximately 100 female mosquitoes per cage were starved for 24 h in order to maximize blood feeding on a restrained, hairless, laboratory rat. Feeding was allowed to continue for approximately 3 h. Larval containers and sucrose solutions were removed from the cages within 24 h after blood feeding in order to eliminate any error that could result from extra ovipositional sites.

Oviposition substrates (washes) were prepared by using 400 ml wide-mouthed glass beakers. A piece of muslin cloth (4 x 12 cm) was draped over the beaker lip and secured with a paper clip. Approximately 200 ml of distilled water was added to each beaker which subsequently moistened the cloth. Bacterial colonies were added to the washes by swabbing previously inoculated petri plates with a sterile cotton swab. A spectrophotometer (Bausch and Lomb Spectronic 20) was then used to standardize the concentration of each replicate bacterial wash. Due to differences in bacterial growth, only individual experiments were standardized to the same transmittance levels. For color comparison experiments, 0.1 ml odorless food dye (black, blue, green, orange, red, or yellow) was added to the wash. In experiments utilizing egg homogenates, approximately 250 mosquito eggs (same species) were crushed with a mortar and pestle and then added to the wash.

Approximately 48 h after blood feeding, egg homogenate, bacterial, and/or food dye washes were placed in the mosquito cages. White backgrounds were used for all experiments. Either two or six oviposition choices were provided in each cage. For experiments using only two oviposition sites, a single wash was placed on either end of the cage. With six ovipositional choices, the containers were spaced in a row from one end of the cage to the other. Positioning of oviposition sites was randomized between replicates. Mosquitoes were not normally restricted from contacting the ovipositional media. Only in the experiments testing for a tactile response effect were screens placed 1 cm over the wash media. In most experiments using color, photoperiod was adjusted to 24L:0D. However, in experiments attempting to show that the preference of individual dye colors was due to visual cues and not olfactory stimuli, the photoperiod was adjusted to 0L:24D.

After 24 h, the washes were removed from the cages and the number of eggs appearing on each muslin cloth were counted with a dissecting microscope. The egg number associated with each wash was used to statistically determine site attractiveness for *Ae. aegypti* and *Ae. albopictus*. In all experiments containing single comparisons, statistical analysis was done by using a Log-Likelihood Ratio (Zar 1984). Multiple comparisons of color were analyzed by using a one-way ANOVA (Zar 1984). Probabilities  $\leq 0.05$  were noted as being significant.

Individual experiments involving both *Ae. aegypti* and *Ae. albopictus* were conducted. Basic procedures for all experiments were similar; any variations were noted. As previous studies such as Frank (1985), Gubler (1971), and Dhileapan (1997) have noted, it was anticipated that both study species would be more attracted to dark colors such as black and red than to lighter colors. However, to better understand and "control" the role that color plays in selection of an oviposition site for the specific populations used in this study, separate sets of three different experiments for both *Ae. aegypti* and *Ae. albopictus* were conducted utilizing food dye washes as ovipositional attractants. A fourth experiment, involving only *Ae. aegypti*, examined a possi-

ble synergistic effect by combining food dye and bacterial washes. Three replicates were done for each experiment.

**Color comparison experiments. Expt. 1.** Black, blue, green, orange, red, and yellow food dye washes (24L:0D) were directly compared to determine which were the better ovipositional attractants.

**Expt. 2.** A direct comparison between black, blue, green, orange, red, and yellow food dye washes was done for both species in complete darkness (0L:24D). This experiment was conducted to determine if odors associated with the dyes were attractive in the absence of color reflection. The door of the environmental chamber was sealed from the outside with duct tape to insure the total absence of light. Three replicates were done for each mosquito species.

**Expt. 3.** Black, blue, green, orange, red, and yellow food dye washes were each compared separately to washes of *B. cereus*. This comparison was done in order to determine whether an "effective" bacterial attractant or colored media is the favored ovipositional attractant for the two species of mosquitoes. Light transmittance for all the *B. cereus* washes was set at 65% transmittance (24L:0D).

**Expt. 4.** Washes of *B. cereus*, *E. coli*, and *P. aeruginosa*, each containing red food dye (the best attractant of the true colors tested), were separately compared to red food dye alone. Light transmittance for *B. cereus*, *E. coli*, and *P. aeruginosa* was 65, 75, and 65%, respectively.

**Bacterial wash experiments.** A series of eight experiments examining bacterial washes as ovipositional attractants for *Ae. albopictus* were conducted in order to directly compare the results to a previous work done with the congeneric *Ae. aegypti* (Hasselschwert and Rockett 1988). The major emphasis was to compare similarities and differences in the discriminating effects exerted by the specific bacterial washes for the two mosquito species. Specific reasons for one bacterial species and their metabolites being a better stimulant for oviposition than another species were not examined.

**Expt. 1.** Four replicates of *E. coli* and *B. cereus* washes were compared. Transmittance values varied between replicates but were standardized within each replicate at 65, 65, 75, and 75%, respectively.

**Expt. 2.** Three replicate comparisons of *B. cereus* and *P. aeruginosa* washes were performed with a constant light transmittance of 65%.

**Expt. 3.** Three replicate comparisons of *P. aeruginosa* and *E. coli* were done with a constant light transmittance of 65%.

**Expt. 4.** The congeneric bacteria *B. cereus* and *Bacillus subtilis* Ehrenberg were compared. Transmittance values for the three replicates were 65, 75, and 75%.

**Expt. 5.** Washes (three replicates at 65% transmittance) of two congeners, *B. cereus* and *Bacillus thuringiensis* Berliner were compared.

**Expt. 6.** Washes of *Staphylococcus epidermidis* (Winslow & Winslow) and *E. aerogenes* (three replicates at 75% transmittance) were examined.

**Expt. 7.** A comparison between washes of *Bacillus cereus* (all 65% transmittance) and washes of *Ae. aegypti* egg homogenate was conducted with three replicates.

**Expt. 8.** Three replicate comparisons of *B. cereus* and *E. aerogenes* were done; all with a light transmittance of 75%.

**Mosquito egg homogenate wash experiments.** Experiments (3 replicates each) were conducted to compare the ovipositional attractiveness of mosquito egg homogenates by the two congeneric species.

**Expt. 1.** For both species, a direct comparison of *Ae. aegypti* and *Ae. albopictus* egg homogenate washes was made to determine if the two mosquito species would select for ovipositional purposes, its own eggs from the other.

Approximately 250 eggs of each species were crushed and added to each of the two washes which were subsequently placed in individual cages of *Ae. aegypti* and *Ae. albopictus*.

**Expt. 2.** For *Ae. albopictus* only, a *B. cereus* wash and a wash of *Ae. albopictus* egg homogenate were placed in a single cage. This experiment was done to determine whether a attractive bacterial wash or egg wash was preferred. All *B. cereus* washes were at 65% transmittance. This experiment was not conducted for *Ae. aegypti* because it had already been performed by Hselschwert and Rockett (1988).

**Expt. 3.** The same procedure was used as described in (1) above except that each wash, in addition to eggs, had *B. cereus* added to them. This was done to test for any "masking" effects for the specific egg homogenates. The washes of *B. cereus* were standardized at 65% transmittance.

**Screened wash experiments.** To separate suspected olfactory and/or visual responses from possible tactile responses in ovipositional site selection, two experiments (*Ae. aegypti* only) were done utilizing screened washes (24L:0D). It should be emphasized that only the screened experiments would absolutely separate olfactory attractants from tactile stimuli. We predominately use the word "attractant" for the non-screened experiments involving bacterial washes realizing that both stimuli may play a role in site selection. A metal window screen was placed 1 cm above the ovipositional media and secured to the beaker with hooks. The mosquitoes were prevented from bypassing the screening by a metal barrier around its base.

**Expt. 1.** A three replicate comparison of screened *E. coli* and *B. cereus* was performed. Light transmittance of all bacterial washes were 75%.

**Expt. 2.** Three replicate comparisons of screened *B. cereus* and red food dye were performed. All *B. cereus* washes had a light transmittance of 65%.

## RESULTS

**Color comparisons. Expt. 1 (Table 1).** In comparing black, blue, green, orange, red, and yellow food dyes in the presence of light, a significant difference in egg deposition ( $p < 0.014$  for *Ae. aegypti* and  $p < 0.001$  for *Ae. albopictus*) resulted. Egg counts were unevenly distributed among the colors tested. Total egg counts for *Ae. aegypti* were black (33.3%), red (22.7%), orange (19.6%), blue (10.7%), green (9.7%), and yellow (4.0%). Total egg counts for *Ae. albopictus* were black (52.1%), red (19.0%), orange (17.7%), yellow (5.3%), blue (3.9%), and green (1.9%).

**Expt. 2 (Table 1).** The comparisons between black, blue, green, orange, red, and yellow food dyes in the absence of light resulted in a non-significant difference ( $p < 0.786$ ) in degree of egg deposition for both species of mosquitoes.

**Expt. 3 (Table 2).** In the separate comparisons between *B. cereus* and food dyes (black, blue, green, orange, red, yellow), each food dye wash was significantly ( $p < 0.001$ ) chosen over the *B. cereus* wash by both species of mosquitoes.

**Expt. 4 (Table 3).** The ovipositional experiment comparing washes of *B. cereus*, *E. coli*, and *P. aeruginosa* with red food dye to washes containing only red food dye resulted in a significant difference ( $p < 0.001$ ) for all replicates with *Ae. aegypti*. The red dye with bacteria was consistently preferred over food dye alone.

**Bacterial wash experiments. Expt. 1 (Table 4).** In comparing *B. cereus* and *E. coli* washes, a significant difference ( $p < 0.001$ ) in relative attractiveness of each trial resulted. Contrary to work done on *Ae. aegypti*

Table 1. Color comparison Experiments 1 and 2. A comparison of different colors as ovipositional attractants for *Aedes aegypti* and *Aedes albopictus*.

Expt. 1: Food Dye Comparisons for Egg Counts (Illumination) <sup>1</sup>						
	Black		Blue		Green	
	Egg Counts	Total	Egg Counts	Total	Egg Counts	Total
<i>Ae. aegypti</i>	653,301,491	= 1445	132,215,118	= 465	170,28,221	= 419
<i>Ae. albopictus</i>	675,824,682	= 2181	56,25,83	= 164	7,52,20	= 79
	Orange		Red		Yellow	
	Egg Counts	Total	Egg Counts	Total	Egg Counts	Total
<i>Ae. aegypti</i>	424,132,292	= 848	511,263,211	= 985	5,102,68	= 175
<i>Ae. albopictus</i>	232,140,371	= 743	201,171,425	= 797	128,71,25	= 224
Expt. 2: Food Dye Comparisons for Egg Counts (No Illumination) <sup>2</sup>						
	Black		Blue		Green	
	Egg Counts	Total	Egg Counts	Total	Egg Counts	Total
<i>Ae. aegypti</i>	132,45,141	= 318	114,62,77	= 253	119,23,88	= 230
<i>Ae. albopictus</i>	95,68,258	= 421	49,101,64	= 214	70,123,197	= 390
	Orange		Red		Yellow	
	Egg Counts	Total	Egg Counts	Total	Egg Counts	Total
<i>Ae. aegypti</i>	98,11,105	= 214	79,49,97	= 225	165,40,121	= 326
<i>Ae. albopictus</i>	96,91,432	= 619	41,175,316	= 532	63,167,225	= 455

<sup>1</sup>Comparisons were shown to be statistically significant based on a one way Analysis of Variance ( $p < .01$ ).

<sup>2</sup>Comparisons were not significantly different from one another ( $p < .786$ ) based on a one way Analysis of Variance.

(Hasselschwert and Rockett 1988), *E. coli* washes were the more commonly selected ovipositional medium for *Ae. albopictus*. Individual egg count replicates for *E. coli* were 219, 107, 184, and 202; *B. cereus* replicates were 114, 4, 20, and 34.

**Expt. 2 (Table 4).** The comparisons between *P. aeruginosa* and *B. cereus* resulted in a significant difference ( $p < 0.001$ ) for each replicate. *P. aeruginosa* was a significantly better ovipositional attractant than *B. cereus*. *P. aeruginosa* egg counts were 443, 152, and 66; *B. cereus* counts were 265, 61, and 32.

**Expt. 3 (Table 4).** Comparisons of *P. aeruginosa* and *E. coli* washes yielded a high degree of selection differences ( $p < 0.001$ ) for each replicate with *P. aeruginosa* being the favored attractant. Egg counts for *P. aeruginosa* were 148, 89, and 517. Counts for *E. coli* were 37, 44, and 401.

**Expt. 4 (Table 4).** In each congeneric bacterial comparison of *B. cereus* and *B. subtilis*, a significant difference ( $p < 0.001$ ) was noted. *B. cereus* was the favored ovipositional wash. Egg counts for *B. cereus* were 365, 220, and 873. Counts for *B. subtilis* were 208, 99, and 617.

**Expt. 5 (Table 4).** Washes of *B. cereus* were selected ( $p < 0.001$ ) over

Table 2. Color comparison Experiment 3. Comparison of different colors (columns on left) versus *Bacillus cereus* washes (columns on right) as ovipositional attractants for *Aedes aegypti* and *Aedes albopictus*.<sup>1</sup>

Black compared to <i>B. cereus</i> wash				
	Egg Counts	Total	Egg Counts	Total
<i>Ae. aegypti</i>	952,1401,594	= 2947	99,289,41	= 429
<i>Ae. albopictus</i>	238,236,627	= 1101	124,41,126	= 291
Blue compared to <i>B. cereus</i> wash				
<i>Ae. aegypti</i>	921,1256,2409	= 4586	272,218,625	= 1115
<i>Ae. albopictus</i>	911,540,911	= 2362	493,207,390	= 1090
Green compared to <i>B. cereus</i> wash				
<i>Ae. aegypti</i>	302,1141,197	= 1640	183,463,24	= 670
<i>Ae. albopictus</i>	88,225,467	= 780	11,62,216	= 289
Orange compared to <i>B. cereus</i> wash				
<i>Ae. aegypti</i>	627,932,1471	= 3030	321,409,683	= 1413
<i>Ae. albopictus</i>	196,467,691	= 1354	42,124,250	= 416
Red compared to <i>B. cereus</i> wash				
<i>Ae. aegypti</i>	419,488,741	= 1648	91,95,198	= 384
<i>Ae. albopictus</i>	833,72,791	= 1696	128,5,243	= 376
Yellow compared to <i>B. cereus</i> wash				
<i>Ae. aegypti</i>	401,709,465	= 1575	97,189,147	= 433
<i>Ae. albopictus</i>	104,437,355	= 896	24,221,189	= 434

<sup>1</sup>Each comparison was shown to be statistically different using a log-likelihood ratio ( $p \leq .001$ ).

washes of the toxic bacterium, *B. thuringiensis*. Egg counts for *B. cereus* were 144, 138, and 360. Counts for *B. thuringiensis* were 97, 47, and 61.

**Expt. 6 (Table 4).** Each comparison of *S. epidermidis* and *E. aerogenes* resulted in a significant difference ( $p < 0.001$ ) in degree of egg deposition. *S. epidermidis* had egg counts of 218, 468, and 386 while *E. aerogenes* was 111, 38, and 183.

**Expt. 7 (Table 4).** In comparing washes of *Ae. aegypti* egg homogenate and *B. cereus*, a significant difference ( $p < 0.001$ ) in relative preference was observed. *B. cereus* was a significantly better ovipositional attractant than the egg homogenate wash. Egg counts for *B. cereus* were 162, 293, and 361; counts for *Ae. aegypti* egg homogenate were 47, 132, and 239.

**Expt. 8 (Table 4).** Each trial comparison of *B. cereus* and *E. aerogenes* resulted in a significant difference ( $p < 0.001$ ) with *B. cereus* being the most commonly selected. Counts were 397, 361, and 221 for *B. cereus* and 328, 182, and 94 for *E. aerogenes*.



Table 3. Color comparison Experiment 4. Comparison of red color alone versus a red-colored bacterial wash of *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* as ovipositional attractants for *Aedes aegypti*.<sup>1</sup>

Red Dye Alone		Red Dye with Bacteria		
Egg Counts	Total	Egg Counts	Total	
258,403,173	= 834	593,11069,321	= 1983	( <i>B. cereus</i> )
39,190,116	= 345	264,403,200	= 867	( <i>E. coli</i> )
81,206,47	= 334	623,778,352	= 1753	( <i>P. aeruginosa</i> )

<sup>1</sup>Each comparison was shown to be statistically significant using a log-likelihood ratio ( $p \leq .001$ ).

Table 4. Bacterial wash Experiments 1-8. Comparison of selected bacteria as ovipositional attractants for *Aedes albopictus*.<sup>1</sup>

Expt.	Reps.	Bacterial Species	Total Egg Count	Bacterial Species	Total Egg Count
1	4	<i>Escherichia coli</i>	712	<i>Bacillus cereus</i>	172
2	3	<i>Pseudomonas aeruginosa</i>	661	<i>Bacillus cereus</i>	358
3	3	<i>Pseudomonas aeruginosa</i>	754	<i>Escherichia coli</i>	482
4	3	<i>Bacillus cereus</i>	1458	<i>Bacillus subtilis</i>	924
5	3	<i>Bacillus cereus</i>	642	<i>Bacillus thuringiensis</i>	205
6	3	<i>Staphylococcus epidermis</i>	1072	<i>Enterobacter aerogenes</i>	332
7	3	<i>Bacillus cereus</i>	816	[ <i>Aedes aegypti</i> egg homogenate]	418
8	3	<i>Bacillus cereus</i>	979	<i>Enterobacter aerogenes</i>	604

<sup>1</sup>Each comparison was shown to be significant using a log-likelihood ratio ( $p \leq .001$ ).

**Comparisons of egg homogenate and bacterial wash combinations for *Ae. aegypti* and *Ae. albopictus* (Table 5). Expt. 1a.** In the comparison between *Ae. aegypti* egg homogenate and *Ae. albopictus* egg homogenate, a significant difference ( $p < 0.001$ ) was noted for *Ae. aegypti* mosquitoes. *Aedes aegypti* egg homogenate was preferred over *Ae. albopictus* (egg counts of 602, 435, and 482 versus *Ae. albopictus* counts of 142, 251, and 169).

**Expt. 1b (Table 5).** When *Ae. aegypti* egg washes were set against *Ae. albopictus* egg washes using *Ae. albopictus* mosquitoes, *Ae. albopictus* eggs were preferred ( $p < 0.001$ ) in all trials using *Ae. albopictus* mosquitoes. *Ae. albopictus* egg counts were 356, 377, and 411. *Ae. aegypti* counts were 89, 195, and 164.

**Expt. 2 (Table 5).** In comparing each replicate of *B. cereus* and *Ae. albopictus* egg homogenate washes for *Ae. albopictus* mosquitoes, a significant difference ( $p < 0.001$ ) was noted. *B. cereus* was favored when compared to *Ae. albopictus* egg washes. Individual replicate counts for *B. cereus* were 555, 387, and 170; counts for *Ae. albopictus* egg washes were 149, 101, and 34.

**Expt. 3a (Table 5).** *Aedes aegypti* egg homogenate with *B. cereus* was a significantly ( $p < 0.001$ ) better ovipositional attractant than *Ae. albopictus* egg homogenate with *B. cereus* for *Ae. aegypti* mosquitoes. Individual counts for *Ae. aegypti* egg homogenate with *B. cereus* were 572, 723, and 418; counts for *Ae. albopictus* egg homogenate with *B. cereus* were 147, 290, and 71.

Table 5. Experiments 1-3: Comparison of egg homogenate and bacterial wash combinations as ovipositional attractants for *Aedes aegypti* and *Aedes albopictus*.<sup>1</sup>

Expt. <sup>2</sup>	Adults Tested	Wash Components	Egg Total	Wash Components	Egg Total
1a	<i>Ae. aegypti</i>	<i>Ae. aegypti</i> eggs	1519	<i>Ae. albopictus</i> eggs	562
b	<i>Ae. albopictus</i>	<i>Ae. albopictus</i> eggs	1144	<i>Ae. aegypti</i>	448
2	<i>Ae. albopictus</i>	<i>Ae. albopictus</i> eggs	284	<i>Bacillus cereus</i>	1112
3a	<i>Ae. aegypti</i>	<i>Ae. aegypti</i> with <i>Bacillus cereus</i>	1713	<i>Ae. albopictus</i> with <i>Bacillus cereus</i>	508
b	<i>Ae. albopictus</i>	<i>Ae. albopictus</i> with <i>Bacillus cereus</i>	1625	<i>Ae. aegypti</i> with <i>Bacillus cereus</i>	520

<sup>1</sup>Each experimental comparison was shown to be significant using a log-likelihood ratio ( $p \leq .001$ ).

<sup>2</sup>Three replicates per experiment.

**Expt. 3b (Table 5).** With *Ae. albopictus*, its own egg washes combined with *B. cereus* were significantly better attractants ( $p > 0.001$ ) than the mixture of *Ae. aegypti* eggs with *B. cereus*. Counts for *Ae. albopictus* egg and *B. cereus* were 640, 482, and 503. Counts for *Ae. aegypti* egg with *B. cereus* were 191, 265, and 64.

## DISCUSSION

The results of this work further support the idea that a variety of complex biological, chemical, and physical factors are utilized in oviposition site selection by mosquitoes.

As expected from earlier literature reports, both species displayed significant differences in the relative attractiveness to different colors. Dhileepan (1997) noted that black and red were the most preferred colors for oviposition of *Culex annulirostris* Skuse and *Culex molestus* Forskal, whereas yellow and green were the least preferred colors. With black and red being the most preferred colors, and yellow and green being among the least preferred, color preference results of this study on *Ae. aegypti* and *Ae. albopictus* confirmed Dhileepan's (1997) results. In studying the vision responses of *Ae. aegypti*, Muir et al. (1992) noted a preference for black and red. They further mentioned that this species is unable to see red and would actually see this color as black.

While previous studies have indicated the use of colored or bacterial washes as ovipositional attractants for mosquitoes, the selection of color versus washes of specific bacterial species has not been extensively compared with each other. The color of ovipositional media may not be considered to be a factor in site selection because of the time of day mosquito species are laying their eggs. Hazard et al. (1967) indicated that optical cues were probably not a factor in their experiments with mosquitoes because most of the oviposition occurred at night. It should be noted that according to Christophers (1960), *Ae. aegypti* has a peak in oviposition during late afternoon. The con-

generic *Ae. albopictus* has been shown to be an aggressive daytime feeder and prefers certain colored substrates for oviposition (Novak 1992).

In our study, the combination of color and bacteria in a single wash appeared to function as a better ovipositional attractant when compared to washes of only colored media. It was expected that the use of an "attractive" bacterial wash would increase oviposition, but it was unknown whether or not a "non-attractive" bacterial wash would have an additive effect on the colored wash. In an earlier work (Hasselschwert and Rockett 1988), *E. coli* was noted as being a poor attractant for *Ae. aegypti* oviposition. Although mosquitoes rarely exhibit an "all or nothing" effect, Hasselschwert and Rockett (1988) found that in some ovipositional comparisons, *E. coli* washes yielded no resultant eggs. In the current experiments with *Ae. aegypti*, all of the bacterial combinations with red food dye, including *E. coli*, had higher egg totals than washes with red food dye alone. Preliminary results would indicate a reinforced effect occurring when attractive colors are mixed with even poor bacterial washes. Surprisingly, all colored washes alone were preferred over plain bacterial washes. Even the lesser favored colors, such as yellow and green, had a higher percentage of egg deposition than any of the bacterial washes alone. From these investigations which were conducted in relatively small cages, it would appear that optical stimuli from apparently odorless substrates (dyes) is more important than stimuli from bacterial washes in promoting oviposition by gravid *Ae. aegypti* and *Ae. albopictus*. In a limited comparison of vision and olfaction with *Ae. aegypti*, O'Gower (1963) noted that black, clean washes were preferred for oviposition when compared with gray, polluted (organic rich) washes. Possibly, this type of behavior is common to numerous mosquitoes other than the selected *Aedes* species. With *Ae. aegypti* and *Ae. albopictus*, the observed behavior pattern of ovipositing more commonly in color than bacterial washes may simply result from increased visual over olfactory perception. Even though our work was done in small cages, it is possible that the bacterial washes are still limited in their volatility and ability to evoke responses in mosquitoes. Bidlingmayer (1994), in studying the visual responses of mosquitoes, noted that visual cues are more important than olfactory cues in some attraction flights. He further mentioned that the role of odor is reduced to short range and probably used to evaluate the suitability of an oviposition site rather than location of stimulus. In these searches, odor detection would follow and not precede visual orientation. It is also possible that the time of mosquito feeding prior to oviposition is important in site selection. Isoe and Millar (1987) observed that starved, but not sugar fed mosquitoes, laid more egg rafts in Bermuda grass infusions than black dyed water. The authors suggested that the starved mosquitoes were more sensitive to chemical cues than sugar fed mosquitoes. Our knowledge of culicid sensory physiology is still very rudimentary and additional work is needed to fully understand this process.

Hasselschwert and Rockett (1988) successfully utilized eight bacterial species in discriminatory studies for *Ae. aegypti*. Oviposition sites high in bacterial content provide needed food. Benson and Apperson (1988) indicated that the survival rates of larvae might increase if adult mosquitoes have the ability to discriminate among habitats, based on bacterial content. Hasselschwert and Rockett (1988) reported that *P. aeruginosa* and *B. cereus* were good ovipositional attractants for *Ae. aegypti* and that bacteria such as *E. coli* were not as effective. In the current study, *B. cereus* and *P. aeruginosa* were again compared to *E. coli* utilizing *Ae. albopictus*. As in the work of Hasselschwert and Rockett (1988), *P. aeruginosa* was a better ovipositional attractant than *E. coli* or *B. cereus* for *Ae. albopictus*. Numerous other similarities existed when comparing bacterial washes as ovipositional attractants for

*Ae. albopictus* and *Ae. aegypti*. For both mosquito species, *B. cereus* was preferred over *B. subtilis*, *B. thuringiensis*, *E. aerogenes*, and *Ae. aegypti* egg homogenate. Also, both mosquito species preferred *S. epidermidis* over *E. aerogenes*. In comparing *Ae. aegypti* (Hasselschwert and Rockett 1988) with *Ae. albopictus* in the current study, one notable difference existed. In the *Ae. albopictus* multi-replicate comparisons between *B. cereus* and *E. coli*; *E. coli* was the better ovipositional attractant. *Escherichia coli* is a common bacterial species in water and the authors are at a loss to explain why washes with *E. coli* were not a good ovipositional attractant for *Ae. aegypti*, but were for *Ae. albopictus*. Additional work is needed in comparing these two bacterial washes with the two *Aedes* mosquitoes. Works such as Rockett (1987) and Steelman and Colmer (1970) did find *E. coli* to be an effective attractant for *Cx. pipiens*. Other studies have indicated that congeneric mosquitoes may exhibit similar oviposition behaviors. Gubler (1971) reported that *Ae. albopictus* and *Ae. polynesiensis* Marks had only slight differences in ovipositional habits. While Frank (1985) noted striking differences between the oviposition behavior of some *Wyeomyia*, *Aedes*, and *Culex* species, he found that the congeners *Wy. vanduzeei* Dyer & Knab and *Wy. mitchellii* (Theobald) had very similar ovipositional habits. Since *Ae. aegypti* and *Ae. albopictus* have similar feeding patterns, breeding sites, and even vector some of the same diseases such as dengue and filariasis, it does not seem surprising that these two mosquito species display numerous similarities in oviposition behavior.

In these experiments, *Ae. aegypti* and *Ae. albopictus* were both found to have the ability to distinguish their own eggs from the other. Even when bacteria were added to the egg wash, both mosquito species could still distinguish and be drawn to their own eggs for ovipositional purposes. Ahmadi and McClelland (1983) concluded that mosquito-produced attractants were favorable for adult ovipositing *Aedes* females. Possibly, pheromone production by immature mosquitoes provides physiological cues indicating that the site is suitable for subsequent egg development. Davis and Bowen (1994) reported that "to date" only *Culex quinquefasciatus* (Say) is known to produce a pheromone from its egg raft that attracts conspecific gravid females. They further stated that most of the attractive compounds isolated from oviposition sites appear to be bacterial or fungal metabolites. Millar et al. (1994) reported that attempts to demonstrate that conspecific egg rafts influenced *Cx. quinquefasciatus* oviposition failed to elicit responses from gravid females. While additional research on the role of eggs as ovipositional attractants for mosquitoes is needed, other ovipositional attractants may be better in comparison. Rockett and Hasselschwert (1988) found that *Ae. aegypti* preferred to oviposit in washes containing *B. cereus* when compared to their own egg washes. In this current study, *Ae. albopictus* also chose selected bacterial washes over their own egg washes. Although both *Ae. aegypti* and *Ae. albopictus* display discriminatory behavior in selecting their own egg washes, it would appear that at least for some bacterial species, the bacterial content of the breeding water is a more important factor than egg presence in oviposition site selection.

The screened media experiments indicated that *Ae. aegypti* mosquitoes do not have to rely on tactile or contact senses in selecting an oviposition site. It should be emphasized that only the screened experiment would absolutely separate olfactory from possible contact stimuli. The results of the "screened" media experiments produced similar results to previous unscreened experiments done in this work and by Hasselschwert and Rockett (1988). Hazard et al. (1967) noted similar results for *Cx. quinquefasciatus* using screened olfactometers as described by Gouck and Schreck (1965). They found that hay infusions were still preferred over both distilled water and bacterial washes

containing *E. aerogenes*. In using *Ae. aegypti*, O'Gower (1963) indicated that screened experiments with manure infusions obtained the same results as non-screened work. It is probable that tactile stimuli plays a part in the final selection of an oviposition site for many mosquito species, but initial attraction by visual and olfactory stimuli are sufficient to promote oviposition if contact is not possible.

Additional investigations concerning the role of color, bacteria, and other ovipositional cues are warranted. Continued laboratory investigations are required to address the specific metabolites produced by attractive bacterial species. Field trials examining color, bacteria, and egg attractants need to be conducted to better understand the ovipositional behavior of these mosquitoes in their natural habitat. The utilization of ovipositional attractants as "lures" has a role in future mosquito pest management techniques.

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