COMPARATIVE OVIPOSITION SELECTION PREFERENCE BY *AEDES DORSALIS* AND *AEDES NIGROMACULIS* FOR THREE INORGANIC SALTS IN THE LABORATORY [1]

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Introduction

It has been suggested that differences in larval sites of different species of mosquitoes are the result of indiscriminate laying of eggs and selective pressures exerted in the larval stage by specific combinations of environmental conditions. It is presently accepted by most workers that ovipositing mosquitoes do not lay their eggs indiscriminately and that restriction of larvae of a particular species to a given habitat is largely a result of selection preference by the ovipositing females.

Aedes nigromaculis (Ludlow) is much more restricted in its larval habitats in northern Utah than is *Aedes dorsalis* (Meigen). A strong correlation exists between the presence or absence of *A. nigromaculis* and *A. dorsalis* in a given habitat and the salinity of the soils on which the females of these two species oviposit (Petersen and Rees, 1966). In an attempt to determine just how inorganic salts affect the distribution of these two mosquito species, oviposition selection preference studies were conducted in the laboratory with *A. dorsalis* and *A. nigromaculis* females to determine their preferences for variations in inorganic salts and to determine the effects of inorganic salts upon the maturation and hatching of the eggs.

Materials and Methods. Since *A. dorsalis* and *A. nigromaculis* resist colonization, biting females were collected in the field and transported to the laboratory. Approximately 150 to 160 engorged females were transferred to 18″ x 8″ x 8″ cages and held in an insectary for 3 days.

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At the end of this period, they were given a choice of oviposition sites.

Each oviposition site consisted of paper toweling protruding from a petri dish so as to provide an oviposition surface of approximately 40 square centimeters. Each site was moistened with 40 ml. of a given salt solution. Test cages contained duplicate sets of oviposition sites of five different dilutions of a given salt. In some tests one series of dilutions was made on white toweling and the other on black toweling. The black oviposition sites were obtained by dipping white paper toweling into a solution of two parts black India ink to 250 parts of distilled water and allowing the towels to dry thoroughly.

Tests consisted of exposing sets of oviposition sites to gravid females for 48 hour periods; the oviposition sites were replaced with fresh ones and replications were continued until excessive adult mortality terminated the tests. The oviposition sites were placed into the cages each time in a random manner, and the cages rotated 180° every 24 hours in an attempt to reduce the influence of physical factors as much as possible. Salt solutions were made by volumetric dilution using reagent grade salts and distilled water. All salt concentrations were based on the percent chloride ion rather than the percent total salt for each of the different salts. This was done to keep the equivalents of each ion constant at a given percent dilution for the three salts used, thus permitting direct comparisons of the results obtained for the various salts at a given chloride concentration. All dilutions were made from 3 percent chloride stock solutions (=4.9% NaCl, 6.3% KCl and 4.7% $CaCl_2$). Tests were conducted at a temperature of 74°±4° F. and a relative humidity of 70%±5%.

Since *Aedes* mosquitoes lay their eggs singly and may lay a few eggs on several oviposition sites before finding the one on which they will lay the majority of their eggs, exact counts were made of the eggs laid on each site. This was done by washing the eggs from the oviposition sites onto gridded filter paper and passing the eggs under a magnifying lens. Counts included the total number of eggs laid as well as the number of eggs which underwent the normal darkening process and those that remained white.

To determine the effects of sodium chloride concentrations on the maturation and hatching of eggs of these two species, gravid females were allowed a choice of oviposition sites consisting of 4″ x 4″ x 3″ plastic containers containing 80 c.c. of finely sifted low salinity soils moistened with 40 ml. of a given sodium chloride concentration. The soils were placed into containers to form an elevation gradient from one side to the other.

These tests were conducted in a similar manner to those with paper toweling except that the original oviposition sites were kept in the cages throughout the test. Following the testing period, the soils were observed for the presence of eggs and then covered and incubated at 80° F. for 10 days. They were then flooded and estimated counts made on the hatched larvae.

RESULTS AND DISCUSSION. Tests with *A. nigromaculis* using sodium chloride as the selecting factor were repeated three times. Although there was some degree of variation in the percentage of eggs laid on the various oviposition sites for each test, a definite avoidance by ovipositing females to concentrations above 0.5 percent Cl was evident in all three tests (Table 1). In two of the three tests, a preference was exhibited for distilled water to any salt concentrations available, and in one a slight preference was evident for 0.5 percent Cl over the other choices available.

In the two tests using *A. dorsalis* and sodium chloride as the selecting factor, results were similar in that no values for any of the concentrations varied more than 3 percent between the two tests (Table 1). This species demonstrated a preference to 0.5 percent chloride in sodium chloride to any other concentration available in the tests. Preferences by *A. dorsalis* did not drop off rapidly after any particular concentration as did those for *A. nigromaculis*.

In tests with potassium chloride as the selecting factor for ovipositing females, only one test was run using *A. nigromaculis*. No preference was exhibited by this species for the lower concentrations of potassium chloride as was the case with sodium chloride.

Two tests using potassium chloride with *A. dorsalis* were conducted. In both tests the results in percentage of eggs laid showed a stronger preference for distilled water over other solutions available (Table 1). Beyond this, little correlation was shown as to site preference and increasing salt concentrations.

Due to the time factor and the availability of mosquitoes, only one test was run with *A. dorsalis* and *A. nigromaculis* using calcium chloride as the selecting factor. The percentages of eggs laid by *A. nigromaculis* at each concentration compared closely with those values obtained for this species using sodium chloride. Distilled water was clearly preferred over any salt concentrations available to them. The values for *A. dorsalis* indicated no strong preference for any particular concentration, but a decrease in preference was evident as salt concentrations increased (Table 1).

Both *A. dorsalis* and *A. nigromaculis* showed similar preferences to dark over light oviposition sites. Of 28,174 eggs laid in three of the tests using dark and light oviposition sites, *A. dorsalis* laid 18,316 eggs (65 percent) on dark surfaces. *A. nigromaculis* laid 10,895 of 16,334 eggs (67 percent) on dark surfaces.

The most striking results obtained from these oviposition studies were the marked effects that the higher salt concentrations exerted on the freshly laid eggs of *A. nigromaculis* and *A. dorsalis*. Under normal conditions, eggs of both species are a pearly white color when laid and undergo a change to a black color in 30 minutes to one hour after oviposition. This change appears to be a tanning of the proteins in the egg chorion, mainly in the endochorion (Clements, 1963). On higher salt concentrations of sodium chloride and potassium chloride, many of the eggs failed to undergo this physiological change. The eggs of *A. dorsalis* appear to be considerably more resistant to the prevention of color change than the eggs of *A. nigromaculis* (Table 2). When exposed to concentration ranges of sodium chloride, potassium chloride and calcium chloride, a larger percentage of the *A. nigromaculis* eggs failed to turn dark than did those of *A. dorsalis*. In no case was embryonic development observed in the white eggs. The effect of the calcium chloride was less evident for both species than for the other two salts, even though the chloride content was equal at any given concentration.

In an attempt to determine the highest sodium chloride concentrations at which eggs of these mosquitoes would mature and hatch, females of both species were

TABLE 1.—Percentages of *Aedes nigromaculis* and *A. dorsalis* eggs laid on five dilutions of three inorganic salts.

Species	Salt	No. of Tests	Percent of total number of eggs laid at various salt concentrations					Total Number of eggs laid
			0.0% Cl	0.5% Cl	1.0% Cl	1.5% Cl	2.0% Cl	
Aedes								
nigromaculis	NaCl	3	40.9	31.8	10.3	8.0	9.0	8667
dorsalis	NaCl	2	25.5	31.7	19.7	14.1	8.9	26605
nigromaculis	KCl	1	18.4	12.9	22.6	21.8	24.2	4523
dorsalis	KCl	2	47.4	21.3	5.9	8.1	17.2	7045
nigromaculis	$CaCl_2$	1	42.9	20.8	12.3	14.6	9.4	5398
dorsalis	$CaCl_2$	1	25.1	23.4	21.7	15.3	14.4	8934

Table 2.—Percentage of *Aedes dorsalis* and *A. nigromaculis* eggs remaining white when laid on various concentrations of three salts.

Salt	Species	Percentage of eggs remaining white at various salt concentrations				
		0.0% Cl	0.5% Cl	1.0% Cl	1.5% Cl	2.0% Cl
	Aedes					
NaCl	*dorsalis*	0	0	2.6	22.4	53.1
	nigromaculis	2.1	3.4	87.9	69.1	92.6
KCl	*dorsalis*	3.9	0	13.5	43.9	6.7
	nigromaculis	9.3	0	20	87.8	96.8
$CaCl_2$	*dorsalis*	0	0	0	0	0
	nigromaculis	0	0	0	3.7	23.3

allowed to oviposit on soil moistened with a range of sodium chloride concentrations. The following three tests were conducted with each species: one consisted of a range of five dilutions from 0.0 percent Cl (distilled water) to 6.0 percent Cl in sodium chloride; one with five dilutions that ranged from 0.0 percent to 2.0 percent Cl; and one with four dilutions that ranged from 1.0 percent Cl to 2.5 percent Cl. The results are summarized in table 3.

In Test 1 using *A. nigromaculis*, eggs were observed on all five salt dilutions even though no hatching was observed above 1.0 percent Cl in sodium chloride. *A. dorsalis*, when given this choice of salt ranges, laid eggs on the four lower dilutions and apparently completely avoided the 6.0 percent Cl concentration. Egg hatching was not observed above 2.0 percent Cl during this test. In Test 2, gravid females of both species were exposed to a lower range of concentrations. Eggs were observed for both species on all oviposition sites except the 2.0 percent Cl concentration in the test with *A. dorsalis*. Again, hatching of *A. nigromaculis* eggs was not observed above 1.0 percent Cl and only very limited at this concentration. *A. dorsalis* eggs hatched on 1.5 percent Cl concentration, the highest upon which the eggs of this species were laid during the test.

In an attempt to force both species to lay eggs on highly saline oviposition sites the lower dilutions were removed for test 3. Eggs were observed on all concentrations for both species. Even though many

Table 3.—Effects of various sodium chloride concentrations on egg laying and hatching in *Aedes dorsalis* and *Aedes nigromaculis* in the laboratory.

Test No.	Species	Estimated number of hatched larvae at various salt concentrations				
		0.0% Cl	1.0% Cl	2.0% Cl	3.0% Cl	6.0% Cl
	Aedes					
1	*nigromaculis*	2600	175	0	0	0
1	*dorsalis*	3000	1000	100	0	0
		0.0% Cl	0.5% Cl	1.0% Cl	1.5% Cl	2.0% Cl
2	*nigromaculis*	2300	1200	1	0	0
2	*dorsalis*	3000	600	250	10	0
		1.0% Cl	1.5% Cl	2.0% Cl	2.5% Cl	
3	*nigromaculis*	0	0	0	0	..
3	*dorsalis*	150	0	0	30	..

A. nigromaculis eggs were present on the soils during this test, none were observed to hatch upon flooding, and nearly all of the eggs exhibited the effects of the sodium chloride present on the soil. Eggs of *A. dorsalis* were observed to hatch on the highest concentration available (2.5 percent Cl) during this test.

It is evident from this study that inorganic salts play an important role in oviposition selection preferences as well as exerting a selective effect upon egg development and hatching in these two species. The results clearly indicate that both species can and do select for and against specific inorganic salt concentrations on oviposition sites. They do this apparently by "tasting" the salts present in a given site with chemoreceptors on their legs (Wallis, 1954). It is difficult to conclude from this study just how inorganic salts exert their effect on ovipositing mosquitoes. It does not appear, however, to be a simple matter of osmotic pressure differences since both species exhibited very different reactions to potassium chloride than they did to sodium chloride and both salts exert similar osmotic pressures at equivalent chloride concentrations. Further, both species exhibited similar preferences to both sodium chloride and calcium chloride, and calcium chloride exerts an osmotic pressure of about one-half that of sodium chloride. There may be some selective response by the gravid females to concentration ranges of the particular cation (Na^+, K^+ and Ca^{++}) involved. A great deal of work needs to be done before any conclusions can be reached as to the effects of a particular cation or anion as selecting factors in oviposition sites of mosquitoes.

Indiscriminate laying was evident to some extent with both species during the tests. This indiscriminate oviposition was probably due to a great extent to the artificial environment in which the mosquitoes were placed. The authors believe that discrimination is much more marked in nature by ovipositing females of both species.

It is evident from the values obtained from the oviposition selection preferences and the results of the observed effects of these salts on the eggs of these species, that *A. nigromaculis* is less tolerant of high salinity than *A. dorsalis*. Sodium chloride solutions of 1.0 percent chloride (1.65 percent NaCl) under the conditions of this study seem to be about the highest concentration at which embryonic development and hatching will occur in the eggs of *A. nigromaculis*. *A. dorsalis*, on the other hand, shows a definite preference to oviposit on the lower salinity soils, but when forced to lay eggs on the higher salt concentrations, normal development will occur. Development and hatching of *A. dorsalis* eggs will occur on sodium chloride concentrations of 2.5 percent chloride (4.1 percent NaCl) and possibly higher.

The tolerance difference between the eggs of *A. dorsalis* and *A. nigromaculis* to inorganic salts is very evident and undoubtedly plays an important role in habitat differences between the two species. It is surprising that so little work has been conducted on the effects of inorganic salts on the egg stage of the mosquito life cycle. From the information obtained during this study, it is difficult to establish whether death of the egg in unfavorable salt concentrations is a result of toxic effects exerted upon the eggs by specific ions or is a result of lethal dehydration due to osmotic pressure when laid in hypertonic environments. It is certain, however, that whatever the cause, the eggs are affected soon after being laid as evidenced by the failure to turn dark.

Oviposition selection preference by gravid females alone cannot account for the marked habitat distribution differences between these two species, for it can be assumed that females on occasion will lay indiscriminately. This would result in the occasional presence of *A. nigromaculis* in such areas as the marsh habitats of northern Utah in which only *A. dorsalis* is known to develop. Inorganic salt concentrations of the water from the larval habitats of these two species do not seem to be a limiting factor in most cases (Petersen and Rees, 1966). It would seem, therefore, that the effects of inorganic salts upon the eggs must exert a definite effect on the distribution of these mosquitoes.

SUMMARY. The effects of three inorganic salts on oviposition selection preference and egg hatching of *A. dorsalis* and *A. nigromaculis* were studied. Both species exhibited selective preferences to increasing concentrations of sodium chloride and calcium chloride, with *A. nigromaculis* more selective for the lower concentrations of both salts. Selective preferences were not evident to any extent when using potassium chloride as a selecting factor. Many of the eggs of both species when laid on the higher salt concentrations failed to undergo the normal darkening process. The eggs of *A. dorsalis* were more resistant to this effect than were those of *A. nigromaculis*. When allowed to oviposit on soils moistened with a range of sodium chloride concentrations, the eggs of *A. dorsalis* were observed to hatch on concentrations as high as 4.1 percent NaCl while the eggs of *A. nigromaculis* were not observed to hatch on concentrations above 1.65 percent NaCl. Both oviposition selective preference and environmental effects upon the newly laid eggs appear to be important factors in limiting the larval habitats of these two mosquito species.

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PRELIMINARY REPORT ON SOLUBLE ANTIGEN FLUORESCENT ANTIBODY TECHNIQUE FOR IDENTIFICATION OF HOST SOURCE OF MOSQUITO BLOOD MEALS [1]

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The ability to identify the host source of blood meals in captured engorged mosquitoes is a valuable aid in studies involving mosquito-borne diseases transmitted to man and other vertebrates. The precipitin test is normally used for obtaining this information but has remained largely unsatisfactory due to the time and effort expended, the difficulty often encountered with weak reactions and false positives, and because of the requirement for training and experience in interpretation of results. Due to these factors there is a desire among many workers for a simpler, more accurate method for determining mosquito blood meals.

Recent advances in fluorescent antibody techniques (Toussaint and Anderson, 1965) permitting use of soluble antigens, significantly extended the capabilities and versatility of immunofluorescence procedures. The basic methodology developed for this procedure, and used in a direct rather than indirect immunofluorescence technique, appeared to offer promise as a

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