AN ANALYSIS OF PREFERENCE IN THE COLOR OF OVIPOSITION SITES EXHIBITED BY FEMALE *TOXORHYNCHITES R. RUTILUS* IN THE LABORATORY

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ABSTRACT. The majority of female *Toxorhynchites r. rutilus* oviposited only in black containers, but about 21% of the females laid some eggs in a white container even in the presence of a black one and 26% used a white container only if a black one was not available. The choice of oviposition site was based on the color of the container and was unaltered either by the degree of contrast between the container and its surroundings or by preconditioning the young females to only one color of container. Despite an apparent genetic basis for selection of white oviposition site, the strength of inheritance appeared weak. Many generations of selective breeding would be necessary to greatly alter oviposition site choice behavior in this species.

INTRODUCTION

Computer simulations indicated that *Toxorhynchites* sp. could control certain species of mosquitoes if the adult females are efficient in finding the breeding sites of the prey (Focks et al. 1978). Focks et al. (1980) observed that females of a natural population of *Toxorhynchites r. rutilus* (Coquillett) oviposited into 100% of their experimental breeding sites (automobile tires containing *Aedes aegypti* (L.) larvae). In another experiment (Focks et al. 1979) *Tx. r. rutilus* females released into a residential area laid eggs in 80% of ovitraps attached to tree trunks.

The effectiveness of *Tx. r. rutilus* was adversely affected by a situation dissimilar to its normal ecological habitat. When this sylvan predator was released in a city where *Ae. aegypti* was breeding in artificial containers on the ground, only 20% to 30% of the breeding sites subsequently contained *Toxorhynchites* eggs (Focks et al. 1983). This level of predation was too low to control the prey. These results indicated that this species may be of limited value except against treehole mosquitoes. However, in this release some eggs were found in the ground level containers. Even though the observed overlap in oviposition site selection of the predator and the prey was insufficient to control the prey, the desired behavior in the predator might be increased by selection.

This study examined the plasticity and basis of oviposition behavior in *Tx. r. rutilus* with regard to color. Color was chosen for examination because *Toxorhynchites* females preferentially oviposit in black containers. The following aspects of oviposition behavior were studied: 1) the difference between individual females in their preference for color of the oviposition site; 2) the effect on choice of oviposition site of the color contrast between the site and its surrounding environment; 3) the choice of oviposition site color as affected by what is commonly available to the young female. The results should indicate if there is a genetic basis of the behavior for oviposition site selection.

MATERIALS AND METHODS

The *Toxorhynchites r. rutilus* used in the parental generation of this experiment were a random sample of one generation from a colony at the Insects Affecting Man and Animals Research Laboratory, Gainesville, FL. The mosquitoes were separated to sex as pupae and allowed to emerge in two large cages supplied with cotton pads saturated with a 1:1 honey:water mixture as a food source. Four days after emergence, each female was forcemated and held in an individual test cage made from a 1-gallon paper can. The top was replaced with a piece of plastic wrap and access to the cage was through a hole in one side that had a sleeve of Tube Gauze® tied off as a closure, stapled around it. Honey solution on a cotton pad was the food. Each cage had a humidifier made of 3 tubes of dental packing (1 cm diam× 10 cm length) wrapped with cloth in a 20 dram jar filled with water-soaked cotton balls covered with the same type cloth. Oviposition jars were 30 dram jars that were painted on the outside with either a flat black or a flat white enamel paint and half-filled with well water.

The parental females were used in an experiment designed to simultaneously test the randomness of oviposition in the white vessel, the importance of color rather than contrast in the selection process, and the effect of precon-

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ditioning to one color on site choice when jars of both colors were later provided. Ninety females were individually placed into cages; 30 with interiors and humidifiers black, 30 with colors gray, and 30 with colors white. In 10 of the cages of each color the female was offered only black oviposition jars, in 10 only white jars, and in 10 controls the females were given both black and white jars. After 10 days, jars of both colors were placed in all cages.

During the test, the cages were held at room temperature inside a plexiglass cabinet to reduce intercage environmental differences. Every 2 days, the numbers of eggs observed in the black jar, the white jar, or elsewhere in the cage were recorded. The eggs were removed from the cage and placed into a pan of water labelled with the identification number of the female and the water in the oviposition jars changed. At this time water was also added to the humidifier and the food pad was changed if necessary. To negate any position effects, the black and white jars were randomly placed into the cage with respect to the food and the cage opening and each cage was placed in the cabinet in a position determined by a table of random numbers.

To test the inheritance of the trait for choosing the white container, the progeny of certain females were reared to adults and their daughters tested for color preference. Since oviposition site choice is not a trait expressed in males, tests on 2 filial generations were required. Nine 4-day-old females from each family were force-copulated with their brothers and tested for preference in the color of their oviposition sites. The experiments on the 2 generations were performed as described above except that there was no test for preconditioning. The 9 females were separated into individual cages, 3 of each of the 3 colors, and offered a choice between black and white oviposition jars. The numbers of eggs found in each of the jars or elsewhere in the cage were recorded every 2 days for 2 weeks. Randomizations of the oviposition jars in the cages and of the cages within the storage cabinet were used to negate any position effects.

RESULTS AND DISCUSSION

Toxorhynchites females oviposit while hovering over the oviposition site. The selection of an oviposition site seems to depend initially upon visual cues (Trimble 1979), but olfactory stimuli also appear to be important (Trimble 1979, Focks et al. 1977). Slaff et al. (1975) demonstrated that black oviposition jars were more attractive to female *Tx. r. septentrionalis* than white ones. Observations on the colony maintained at this laboratory suggested that the preference for black oviposition jars was also exhibited by *Tx. r. rutilus*. However, some eggs were found in non-black containers placed in the cage, suggesting that the colony did have some variation in its oviposition behavior.

The first question concerned the source of the eggs which were laid in non-black containers in the colony. Each female was either ovipositing a few eggs in the non-black containers or a few females utilized the non-black sites while the majority oviposited only in the black jars. The latter pattern is the one expected if females possessing a particular genotype preferred a certain oviposition site. Table 1 gives the data for 28 of the 30 control females offered both black and white jars for the duration of the tests on the parental females. The other 2 females did not oviposit. The females deposited 75.5% of their eggs into the black containers and only 5.0% into the white. Ninety-five percent of the eggs found in the white jars were oviposited by only 6 of the 28 females (21.4%); one female (White 8) oviposited all of her eggs in the white jar. Therefore, females of *Tx. r. rutilus* generally exhibit a strong preference for black containers, but a minority will oviposit in containers of other colors.

Another question was the effect of the degree of contrast between the site and its surroundings. In cages with a white interior, the black jar offered the greater contrast and the white jar blended into the surroundings. In the black cages the reverse situation was true. Control cages lined with gray paper contrasted with the jars of both colors. The data in Table 1 show that oviposition behavior of females in the white and the gray cages were similar but that females in the black cages oviposited fewer eggs in the black jar. However, instead of an increase in the number of eggs found in the white jar, as would be expected if the choice of oviposition site was affected by color contrast, more eggs were found on the floor of the cage (71.9%) or on the humidifier. A Chi-square test for independence between cage color and oviposition site, with the data from the females summed within the 9 cage color-oviposition site classes, was highly significant ($p < .001$). The total number of eggs found in the black jar was much less and the total number of eggs oviposited elsewhere in the cage was much more in the black cages than in the white and gray cages (184 compared to 541 and 492, and 217 compared to 32 and 65, respectively).

To evaluate preconditioning of the young female, 20 of the 30 females in cages of each color were initally offered only a white or a black jar. After 10 days of preconditioning, jars

Table 1. The numbers of eggs oviposited over a four-week period into each of three oviposition sites by 28 *Toxorhynchites r. rutilus* females held separately in cages of one of 3 colors and given a choice between a black and a white oviposition jar.

Cage color	Oviposition site	Number of eggs oviposited										
		Female number										
		1	2	3	4	5	6	7	8	9	10	Total
Black[1]	Black jar	6	30	30	18	27	23	12	28	10	0	184
	White jar	0	1	1	0	0	0	0	0	0	0	2
	Elsewhere	8	33	50	7	14	10	38	33	24	0	217
Gray[1]	Black jar	11	79	72	72	5	70	70	25	88	0	492
	White jar	0	0	8	0	0	0	0	0	0	0	8
	Elsewhere	1	1	1	20	2	10	12	7	11	0	65
White	Black jar	69	97	47	85	35	68	51	0	32	57	541
	White jar	6	6	0	2	0	0	13	38	5	0	70
	Elsewhere	6	2	1	1	4	4	0	12	2	0	32

[1] Only 9 females in the black and gray cages oviposited.

of both colors were placed in the cages and site choice observed. Table 2 gives the results of this test. Because the fecundity of this mosquito normally decreases with time, the results presented in Table 2 are the percentages of all the eggs laid by females undergoing a treatment which were found in one of the 3 oviposition sites during a certain treatment period. For example, among females in black cages and first given only a black jar, 43.8% of their total egg production was found in the black jar during the first 10 days.

When the black jar was present during the first 10 days of oviposition, an average of 79.7± 9.8% of the eggs were observed (Table 2). Conversely, when the black jar was absent, only 15.6 ± 2.3% of the eggs found in the white and gray cages were produced. In the black cages without a black jar, 82.1% of the eggs were found on the floor or on the humidifier. That number of eggs found in places other than the oviposition jar was almost twice that observed when the females in the black cages were given a black jar.

When females initially given only black jars were offered a choice between black and white jars, the proportion of eggs found in the black jars did not differ from those found in the controls during the same period. However, in the black and gray cages the percentages of eggs found in the white containers when they became available were greater than those in the controls. After being given a choice, 22.7% (5 of 22) of the females preconditioned to the black jar oviposited more eggs into the white jar and another 22.7% (5 of 22) oviposited into both jars although they laid more eggs into the black jars.

Among females initially offered only the white jar, during the choice period about 4 times the number of eggs were laid into the black jars by females in the white and gray cages than among their respective controls. Although oviposition rate was not reduced during the preconditioning period in the black cages, there was a three-fold difference between the treatment and control females during the choice phase. Of the females that were preconditioned

Table 2. The percentages of the total egg productions of 78 *Toxorhynchites rutilus rutilus* females, held separately in cages of 3 colors, that were found in 3 oviposition sites during a 10-day preconditioning to one colored jar followed by a 10-day period of choice between jars of both colors.

Cage color	Oviposition site	First presented black jar		Control		First presented white jar	
		Before	After	Before	After	Before	After
Black	Black jar	43.8	4.2	39.0	4.4	—	13.5
	White jar	—	4.2	0.3	0.3	0.3	0.6
	Elsewhere	45.8	2.0	53.9	2.2	82.1	3.5
Gray	Black jar	55.8	21.2	67.0	20.2	—	76.6
	White jar	—	9.2	1.7	0	7.1	3.5
	Elsewhere	11.7	2.2	9.9	1.3	6.9	5.9
White	Black jar	73.2	15.7	67.7	16.7	—	72.8
	White jar	—	2.5	5.1	5.8	15.2	1.5
	Elsewhere	2.2	6.4	0.9	3.7	2.0	8.5

to a white jar, 67.9% did not oviposit when only a white jar was present, and 17.9% had used the white jar at first but switched to the black jar when given a choice. Interestingly, 97.0% of the eggs found in the white jars among the treatment classes were produced by 46.9% of the females.

These tests on the parental females strongly suggest that oviposition behavior in *Tx. r. rutilus* was elicited by the color black. This behavior was not greatly altered by the degree of contrast between the oviposition site and its surroundings except that the female might have had difficulty orienting on target when both the site and its surroundings were black. The response to black was not altered by an extended period of stimulation only by an alternative site color. Instead, the females appeared to wait until the proper stimulus was available.

There were differences between females in their acceptance of the white oviposition sites. Thus, the variability necessary for breeding a strain of *Tx. r. rutilus* with a broader range of oviposition site preferences appeared to exist. To determine if the selection of the white jar was an inherited trait, families of 10 selected females (4 that had oviposited only in the black jar, 1 that had selected only the white jar, and 5 that had used both) were reared through 2 generations and 9 females of each generation were tested for preference of site color.

To test the relationship between the mother and her daughters, each female was classified as having laid all of her eggs in the black jar or having laid some in the white jar. The data were submitted to a Chi-square test for independence between mothers and daughters (Snedecor and Cochran 1967), and the resulting Chi-square value was not significant. A similar test on the mothers and daughters of the next generation also yielded nonsignificant results, as did a three-way test for independence of the color preference of the F_2 females and those of their mothers and grandmothers using the method in Sokal and Rohlf (1969).

These results appeared to indicate that the selection of a white oviposition site exhibited by a female was independent of any preference for white exhibited by her mother and therefore, not strongly inherited.

Care must be taken in interpreting these results. It was not possible to know what contribution the male genotype made on the behavioral phenotypes of his daughters, but it was probably of some consequence. For instance, one of the parental females, that was randomly mated to a colony male, oviposited all of her eggs in the white jar. Among those 9 of her daughters that were tested, only one oviposited any eggs in the white jar; but among 9 progeny produced by inbreeding one of these daughters, 4 laid some eggs in the white container. The reverse situation was also observed: a female that exhibited an absolute preference for a black jar produced F_1 and F_2 female progeny, an increasing number of which oviposited some of their eggs into the white jar. Thus, the genomes from the males appeared to have contributed to the low covariance in oviposition site color preference between females and their progeny.

Another difficulty in interpreting the results was that among the progeny not all of the females that might have oviposited in a white jar were observed. Among the parentals only about 20% of the females oviposited in the white jar when both colored jars were presented to them, but 47% of the females oviposited in the white jar when it was the only site available. This suggested that in the choice test to which the F_1 and F_2 females were submitted about half of the females bearing phenotypes which would allow them to use alternatve oviposition sites were missed. The presence of these females that appeared to prefer black among the progeny of a female that oviposited in a white jar would have reduced the apparent heritability of the trait.

CONCLUSIONS

This investigation on the role of the color of sites in attracting female *Toxorhychites r. rutilus* to oviposit confirmed previous reports that black containers are preferred. About 21% of the females oviposited some of their eggs in a white container whether or not a black one was available, and another 26% oviposited in a white container if a black one was not available. The choice of a site was based on color and was unaffected either by the degree of contrast between the site and its surroundings or by any preconditioning of the young female to the presence of only one color of oviposition jar during the first 2 weeks of adulthood.

Despite differences in oviposition site color selection between females and an apparent similarity of behavior within certain families, there was no significant similarity between mothers and daughters. This lack of similarity could be an artifact caused by the inability to define the phenotypes of the male parents and/or to recognize certain females that would oviposit in white jars only in the absence of black jars. Although variability in oviposition behavior exists in this species, many generations of selective breeding would be required to produce strains that would be useful in release programs against pest and vector mosquitoes occurring in an urban environment.

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FIELD TRIALS ON THE USE OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST *MANSONIA* MOSQUITOES IN MALAYSIA[1]

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ABSTRACT. A suspension concentrate of *Bacillus thuringiensis* serotype H-14 formulation (Teknar®, SAN 402–1 SC) was tested against laboratory reared late third/early fourth instar larvae of *Mansonia uniformis* as well as naturally occurring *Mansonia* larvae using Hudson knapsack sprayers on small plots in swampy ditches on Penang Island, Malaysia. Six dosages ranging from 1.1 to 11.40 kg/hectare were used in two experiments. Mean dosage/response values at the 50% level for the introduced and natural population were 0.66 and 1.19 kg/hectare, respectively, whereas, the mean dosage/response values at 95% level were11.02 and 25.98 kg/hectare for the introduced and natural population, respectively. Higher dosages of the *B. thuringiensis* H-14 formulation were needed to achieve control of the *Mansonia* larvae when compared with other vector mosquitoes. The heterogeneity of the response of *Mansonia* population towards *B. thuringiensis* H-14 was also observed. The comparable dosage/response values for introduced and natural populations suggest that caged introduced populations can be used as a bioassay method for *Mansonia* larvae in the field.

INTRODUCTION

There has been a general lack of information concerning larvicidal tests against *Mansonia* using either conventional insecticides or biological control agents. Laboratory tests on the susceptibility of *Mansonia* larvae using conventional insecticides were conducted earlier (Yap et al. 1968, Yap and Sulaiman 1976). Among the biological control agents, *Bacillus thuringiensis* serotype H-14 (de Barjac 1978) has been shown to be a highly effective biological insecticide against vector mosquitoes and simuliid blackflies (Anonymous 1981, 1982). To date, most reports on laboratory and field evaluations of *B. thuringiensis* H-14 have involved *Aedes, Culex* and *Anopheles* mosquitoes. In a comparative laboratory study, the susceptibility of *Ma. indiana* Edwards and other mosquito larvae to a few formulations of *B. thuringiensis*

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