Variation of the Oviposition Preferences of *Aedes aegypti* in Function of Substratum and Humidity

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Two Aedes aegypti *(L.) populations were studied in the laboratory regarding the preference for three types of breeding sites, i.e., flasks containing only water, flasks with a plant and flasks with a stick. Each of these breeding units was placed in one cage and the choice of the oviposition sites was determined for individual females and three females per experimental unit at two humidity levels. Preference for ovipositing on the water surface was observed and varied according to experimental unit and humidity. Mean hatching of eggs in water surface was 46.6%. Experiments with three females showed a more marked difference than when only one female was used. Inter and intrapopulation variability regarding oviposition sites was observed. The discrimination between the different oviposition substrates, hatching in water surface and its implication for mosquito control are discussed.*

Key words: oviposition - humidity - *Aedes* - oviposition behaviour

Today, *Aedes aegypti* (L.) represents the main urban insect responsible for the propagation of epidemics in Brazil, with 1,672,883 cases of dengue having been reported between 1981 and 1998 (Teixeira et al. 1999), after decades of absence (Schatzmayr 2000). The ovipositing habit of this mosquito is still little understood. In the case of Brazil, the few studies on the ovipositing behaviour of this insect were conducted on a population that has already been eradicated, and the current population may be of a different origin, thus not presenting the same behaviour as observed previously. Variations have been observed in terms of life span and the number of eggs produced by females of 13 different populations of *A. aegypti* from Africa, Asia and America (Crovello & Hacker 1972). The site chosen for laying eggs at the breeding sites is of great importance for the biology of the mosquito. Most eggs have been found to be laid above the water line and only a few eggs are laid on the water surface (Young 1922, Goma 1964, Chadee et al. 1995) and the choice of oviposition site is genetically controled (Schoenig 1968).

Oviposition occurs on the water surface when the surface of the container does not enable the adherence of the female tarsi during oviposition (Christophers 1960). A study conducted in Manaus (Amazonas, Brazil) showed that non-submersed eggs did not hatch, in contrast to those on the water surface that were submersed (Young 1922). The habit to lay eggs on the water surface, as well as the occurrence of hatching without submersion has rarely been observed in *A. aegypti* (Christophers 1960). Due to the preference of the mosquito for ovipositing on rough surfaces present in breeding sites, a trap has been developed to determine the existence of adult females in the environment (Fay & Perry 1965). Today, this trap is used in several countries to determine infestation rates and to evaluate the efficacy of the control measures adopted (Reiter & Gubler 1997). Variations in the site chosen for oviposition have been observed within the species. In a study conducted in Australia, most eggs were found to be laid on the water surface instead of a porous and humid surface (O'Gower1957, 1963). However, these studies did not take in consideration the physiological age of the females. Observations made at two locations in Brazil on *A. aegypti* breeding sites in residences with plant-containing vases (unpublished data) showed a large number of eggs on the water surface, the magnitude for these variations are not clear in Brazilian *A. aegypti*.

The objectives of the present investigation were to study the oviposition behaviour of two mosquito populations in three different types of breeding sites, two of them commonly found in residences, at two different humidity levels, to determine the influence of female density on this behaviour and to evaluate the hatching response of eggs on the water surface.

MATERIALS AND METHODS

Mosquito population and laboratory rearing - The laboratory populations were formed from eggs and larvae collected at 30 residences in each of two cities (Botucatu, 22º53'9"S 48º26'42"W and Lins, 21º40'43"S 49º44'33"W) in the State of São Paulo, Brazil, respectively called B and L. The eggs and larvae obtained from ovitraps and potted plants were maintained in the laboratory at 25ºC, at a photoperiod of 12:12 h (light:dark) and 85 ± 8% relative humidity. The larvae emerging from the eggs and those obtained from the breeding sites were fed a fish ration until they reached the pupal stage. Batches of approximately 100 pupae were transferred to flasks containing 500 ml of water and each flask was placed in a cage (40 x 34 x 27 cm) for the emergence of adults. Adult mosquitoes were maintained on a 3% sugar solution and the females were fed every three days on anaesthetised mice for 30 min.

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Choice of oviposition site experiments - The oviposition sites consisted of polystyrene flasks (2.5 cm high and 3 cm wide) containing 100 ml water. The first flask contained a 10 cm high plant (*Syngonium podophyllum* Schott), the second a rectangular fiberboard stick (10 cm long x 0.5 cm wide) in an oblique position about 40 degrees relative to the water surface, and the third contained only water. The flasks were placed 10 cm apart, forming a triangle in the centre of the cage. The position of the flasks was randomly chosen by drawing lots. Engorged *A. aegypti* females taken at random from the breeding cage were placed in each cage, one per cage, and kept in the oviposition container for 72 h. After this period the flasks were removed and the eggs were counted by carefully examining each substrate to verify the presence of eggs on the water surface, on the plant stem and on the stick.

Effect of humidity on the preference of the oviposition site - Two different humidities, i.e., a high humidity of 80 ± 8% and a low humidity of 51 ± 4%, both under a temperature of 28 ± 0.2ºC, were used. Twenty replicas have been performed for each humidity and population (B and L), with a total of 80 experimental units. Each replica consisted of one female in a cage containing the three oviposition sites described above. All females were four day old and were in the first gonadotrophic cycle, and all experiments were made with F2 generation.

Three females per cage - A larger number of ovipositing females was used in an attempt to observe a possible change in the choice of the oviposition site compared to that obtained with only one female. The procedure was the same as described above, except that only the higher humidity could be tested due to the high mortality of one or more females in each experimental unit in the lower humidity condition. To compare the discrimination within each oviposition site (border x water, stem x water and stick x water) another experiment was conducted in high humidity in L population using one female per cage, being each oviposition site tested separately.

Hatching of eggs on the water surface - Eggs on the water surface were counted and carefully removed with a brush in such a way that 10 eggs remained on the water surface. A total of six jars with ten eggs each were obtained. In half the jars the eggs were submerged by shaking and the other half were left untouched. The egg-containing jars were maintained in a germination chamber (25 ± 0.2ºC and 85.2 ± 8% humidity) and observed for 15 days until emergence of the larvae.

Statistical analysis - Since the data were obtained by counting and did not present a normal distribution, non-parametric tests were used, consisting of the Friedman test followed by the Student-Newman-Keuls test for the comparison of the oviposition frequencies on the substrates and the Mann-Whitney test for the comparison between the two locations B and L. The Mann-Whitney test was also used to compare oviposition in each site (border x water, stem x water and stick x water).

RESULTS

The two populations did not differ in relation to the number of eggs laid per female either in 80% (mean = 74.9 ± 4.3) or 51% (mean = 76.0 ± 3.9) humitidy. In the first condition the percentage of eggs on the water surface was 42.9% and 57.3% for L and B respectively. At low humidity 61.4% of L and 63.2% of B eggs were laid on the surface. When three females per experimental unit were used (high humidity condition), the mean of eggs laid per experiment was 113.7 ± 17.3 in B and 232.8 ± 21.8 in L population. In these arrays 56.2% of L eggs and 67.1% of B ones were laid on water.

Females obtained from the L location and maintained at 80% humidity laid eggs under all experimental conditions studied. The preferred oviposition site was the plant stem where 45.3% of eggs were laid and the least preferred site was the border of the container having only water, representing 1% of the total eggs laid, with the two experimental conditions being significantly different. No statistically significant difference was observed between the other conditions (Fig. 1). When the females were maintained at 51% humidity, the oviposition behaviour of the L population resulted in a fall in the number of eggs laid on the stem, while, on the other hand, there was an increase in the number of eggs laid on the container with the stick, both on the water surface and on the stick itself, although this difference was not statistically significant. Only the containers having just water differed from the other containers (Fig. 1). Although the number of eggs laid on the stem surface exceeded two times that observed for the water surface containing the plant, this difference was not statistically significant.

Females of the B population at 80% humidity showed a more selective behaviour compared to the L population under the same conditions, since they did not lay eggs in the flasks that contained only water. This led to a more equitable distribution of eggs in the recipients with plant and stick, with 32.2% of eggs being placed on the water surface containing the plant and 31.8% placed on the stick. Comparison of the ovipositions made on the water surface of the flasks containing the plant and stick revealed that the females did not have a preference for oviposition in some of them, as also observed for the water surface of the two containers (Fig. 2). On the other hand, at low humidity (51%), the B population preferred to lay its eggs on the water surface of both plant and stick and also on the stick itself, with no significant difference being observed between the three conditions. Although oviposition was observed on the water surface of the plant, few eggs were laid on the stem, with a significant difference compared to the other conditions. No oviposition was observed in the containers having only water (Fig. 2).

The analysis of preference for oviposition substrate using three females in each experimental unit showed that L females laid most eggs in the flasks containing the plant and stick both on the solid and water surfaces, with no statistical difference being observed between substrates. A significant difference was observed between these substrates and the flasks containing only water, with much less eggs being laid in the later (Fig. 3). B females oviposited preferentially on the water surface of the flasks containing the plant and stick, and on the stick itself, with no significant difference being observed between substrates. The flask containing only water, including both surface and border, was the least preferred substrate, differing significantly from the other substrates (Fig. 3). The plant stem presented the second smallest number of eggs laid by B females, also differing significantly from the other substrates. Comparing the L and B populations regarding the sites used for oviposition, L females laid more eggs on the stem than B females. On the other hand, B females showed a higher preference for laying their eggs on the water surface of the flask containing the plant and stick and also on the stick itself than L females. No significant difference in the number of eggs laid on the water surface of the flask containing the stick and flasks containing only water was observed between the two populations (Fig. 3). However this result should be analyzed with caution, because the number of eggs laid by B was very low in this survey using three females.

In experiments with only one female, it should be noted that there was an intrapopulation difference in behaviour. Within the two populations some females laid their eggs only on the water surface and others laid their eggs on the non-liquid surface of the stick, plant and border. The discrimination between the oviposition sites also seemed to be associated with the level of humidity. At 80% of humidity in the L population, 17.6% of females laid their eggs only on the liquid surface, 29.4% laid eggs on the

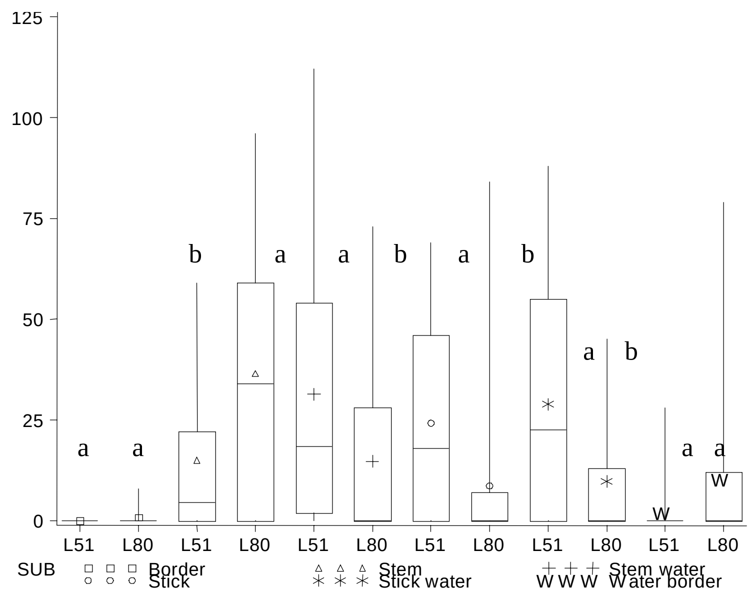

Fig. 1: boxplot of the number of eggs laid on different substrates (SUB) by *Aedes aegypty* females obtained from Lins at 80 and 51% humidity. Values for each substrate followed by the same letter did not differ significantly ($p > 0.05$).

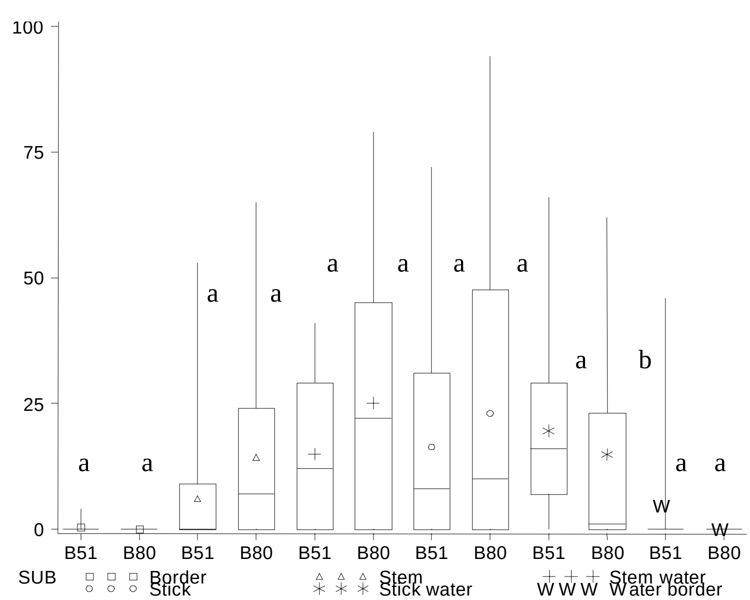

Fig. 2: boxplot of the number of eggs laid on different substrates (SUB) by *Aedes aegypty* females obtained from Botucatu at 80 and 51% humidity. Values for each substrate followed by the same letter did not differ significantly ($p > 0.05$).

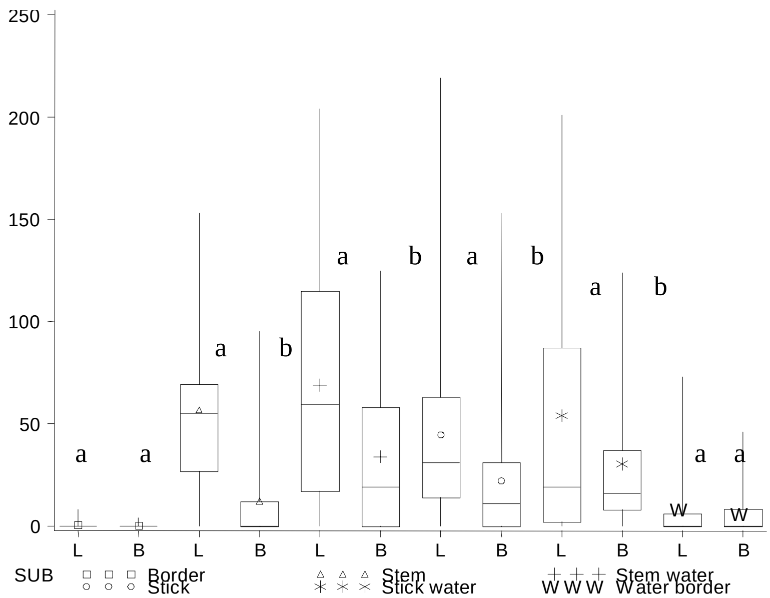

Fig. 3: boxplot of the number of eggs laid on different substrates (SUB) by three *Aedes aegypty* females obtained from Lins and Botucatu at 80% humidity. Values for each site followed by the same letter did not differ significantly (p > 0.05).

non-liquid surfaces, and the remaining ones laid their eggs on both surfaces. In the B population, 10% of the females preferred to lay their eggs on the water surface and 15% on the non-liquid surface. When these females were submitted to 51% of humidity, a higher diversification of the sites chosen for oviposition was observed, which led to a reduction in the number of L females preferring to lay eggs on the liquid surface as the only oviposition site (5.6%) and to a 20% increase in the number of B females preferring to lay eggs on water. This diversification of oviposition sites disappeared when the females were placed in contact with equal sites for oviposition. Having no choice, the females laid most of their eggs on the substrate and preferred water only when this was the only alternative offered (Fig. 4).

Mean hatching of eggs obtained from the water surface after 15 days was 53.3% for eggs that were submersed and 46.6% for those that were kept on the surface, with this difference being nonsignificant (P > 0.2302).

DISCUSSION

The present results show that the *A. aegypti* populations from the two cities of Brazil studied here lay eggs on both the water surface and on adjacent substrates. The capacity to lay eggs on the water surface, together with the fact that these eggs hatched, favours the development of this insect even during dry periods. Oviposition on the water surface permits the cycle to develop faster, favouring the rapid colonization of breeding sites since no further addition of water is required, in contrast to eggs laid in wet surface above the edge. During this period, eggs laid just above the edge of water do not hatch until the rainy season. Delayed hatching is associated with a decrease in the viability of eggs. According Silva et al. (1998), after two months, 74% of mortality occurred in eggs in laboratory in Brazil, number close by the mean of 66% obtained in other experiments at the same period (Christophers 1960). Another important aspect is that predation by ants and cockroachs is avoided when the eggs are in the water. Sometimes we could see some eggs being predated by these groups of insects in the field. Oviposition on the water surface may be due to the adaptive selection observed among these populations in response to the environmental conditions they are exposed to, since the regions where the populations were collected present a period of the year with low rainfall, low humidity and a temperature favourable to the evolution of the mosquito.

A correlation between the type of substrate and the site of oviposition has been observed. In the laboratory, oviposition on the water surface has been shown to be dependent on the oviposition substrate used, i.e., when the oviposition surface consisted of a piece of wood, only 1.2% of eggs were laid on the water surface, but when it was glass, this percentage increased to 89.1% (Thirapatsakun et al. 1981). The present results can not be attributed to laboratory artefacts because first, we observed a large number of eggs on the water surface in the field; second, we allowed the female to choose the substrate, and, third we individualized each female of the experiment, avoiding saturation of the preferred oviposition site with eggs. This allowed us to verify the choice of each female without interference from another female competing for the same site, as postulated by Corbet and Chadee (1993).

The behaviour of oviposition on aquatic surfaces may compromise the surveys carried out to determine the existence of adult mosquitoes using the egg trap developed by Fay and Perry (1965), unless eggs and/or larvae present in the water of the trap are counted in addition to those present on the stick, since otherwise an error may occur

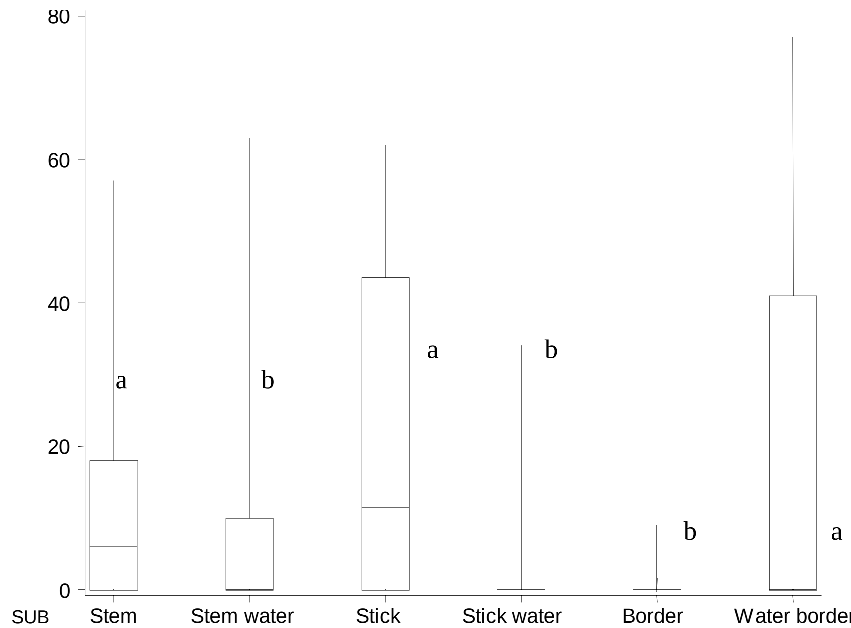

Fig. 4: boxplot of the number of eggs laid on different substrates (SUB) obtained from Lins at 80% humidity (comparisons made in each of the oviposition site) when only one type of recipient was offered. Values for each site followed by the same letter did not differ significantly ($p > 0.05$).

in the calculation of the infestation rates, considering as negative what would be positive. However, as observed for the populations studied here, most females laid eggs on both the water surface and the substrates, and few females showed only one behaviour, even at different humidities, indicating that most females of the two populations presented both oviposition characteristics and distributed their eggs at different sites, although the number of eggs laid at each site varied. For example, the number of eggs laid by L solitary females (80% of humidity) on the stem surface exceeded more than two times the number of eggs laid on the water surface surrounding the plant; however, statistical analysis did not reveal any difference between the two conditions. This observation is associated with the large behavioural variability observed for the females tested, i.e., two females laid all their eggs only on the stem, while five females did not lay any egg on the stem, leading to a very wide variability which did not permit a differentiation between this substrate and the others. This fact suggest the occurrence of a comportamental plasticity influenced by environmental conditions. This plasticity seems to be linked to the type of breeding site offered. The L population preferred to lay more than 80% of its eggs on the stick and on the stem when they had no other choice of breeding site, but laid 97% of their eggs on the water rather than on the border of the container when only this option was offered (Fig. 4). Previous studies (O'Gower 1957) showed the maintenance of oviposition on the water surface even when there is a change in the texture of the substrate of oviposition and water reflectance. However, the cited author worked with a non determined age of females and there is no information about variation in the number of females tested; also, most of the comparisons were made pairwise. So, it is not possible to make any comparison in relation to the effect of number of females in the behaviour of oviposition based in this study.

Humidity was another factor which seemed to have influenced the oviposition behaviour of the populations studied here, so that L individuals showed a behaviour closer to that of B individuals. At high humidity, L females laid 57% of the eggs on dry substrates, while at lower humidity this proportion dropped to 38.7%, resulting in the preference of the females for oviposition on the liquid surface. The same tendency was observed for B females but at a lower proportion. The variation in oviposition behaviour observed in the laboratory suggests that it may also occur in nature, and implies that oviposition should be analysed under different climate conditions in this country.

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