Colour cues for oviposition behaviour in *Toxorhynchites moctezuma* and *Toxorhynchites amboinensis* mosquitoes

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ABSTRACT: Gravid female *Toxorhynchites moctezuma* (Dyar and Knab) and *Toxorhynchites amboinensis* (Doleschall) were offered the choice of coloured (spray-painted) containers for oviposition in laboratory trials. Both species oviposited preferentially into black containers rather than into white, red, yellow, green, or blue containers. Black containers also acted as oviposition stimulants for *Tx. moctezuma* females, and black and red containers for *Tx. amboinensis* females. Absorption spectra were measured for the paints used, with red and black paints absorbing more light across most of the visible spectrum than the other colours. *Journal of Vector Ecology* 25(2): 127-135. 2000.

Keyword Index: Colour cues, oviposition, *Toxorhynchites moctezuma*, *Toxorhynchites amboinensis*.

INTRODUCTION

*Toxorhynchites* spp. mosquitoes are recognised as potential biological control agents of pest and vector mosquitoes. There have been many attempts to use them for this purpose since the beginning of this century; some have been successful but many have failed, mainly due to a lack of knowledge of the general biology of this group of mosquitoes. This includes the oviposition behaviour of these mosquitoes and the cues used to find oviposition sites, compared with those of their target species. Habitat-related olfactory compounds have been identified as important cues in the oviposition site selection of *Toxorhynchites* (Linley 1988, Linley 1989, Collins and Blackwell 1998). It is highly likely, however, that other cues are also involved, including the oviposition site colour.

Colour cues for oviposition behaviour have been identified in only two *Toxorhynchites* species: *Toxorhynchites rutilus* (subspecies: *Tx. r. rutilus* [Coquillett] and *Tx. r. septentrionalis* [Dyar and Knab]) and *Toxorhynchites splendens* (Wiedemann). *Toxorhynchites r. septentrionalis* preferred to oviposit in black rather than white containers (Slaff et al. 1975), and *Tx. r. rutilus* preferred black containers to both white and grey containers (Hilburn et al. 1983). Jones and Schreiber (1994) also found that *Tx. r. rutilus* oviposited preferentially into black containers, although in addition, they deposited a few eggs into red, blue, and brown containers. There was some discrepancy with *Tx. splendens*. In a laboratory oviposition bioassay, this species oviposited more frequently into black containers than into clear, white, yellow, green, red, or blue containers (Yap and Foo 1984). Jones and Schreiber (1994), however, found that although this species also oviposited more frequently into black containers than red, brown, green, blue, and orange containers, it did not oviposit into white or yellow containers at all. The authors attributed the discrepancy between their results and those of Yap and Foo (1984) as being due to possible differences in colour qualities between the two studies.

In a study of the influence of a number of factors on the preferred oviposition sites of some *Aedes* species, *Aedes hexodontus* (Dyar) and *Aedes aegypti* (Linnaeus) preferred to oviposit onto black rather than white surfaces (Beckel 1955); and in laboratory studies, *Aedes triseriatus* (Say) females oviposited most frequently onto dark coloured water (Wilton 1968). Williams (1962) suggested that *Ae. triseriatus* females were attracted to oviposition sites by wavelengths in the blue part of the visible spectrum and this was clarified by McDaniel et al. (1976), using coloured oviposition...
containers rather than dyed water in bioassays (eliminating unintentional olfactory stimuli), concluding that amber was more attractive than green to \textit{Ae. triseriatus}.

Oviposition behaviour experiments with \textit{Ae. aegypti} showed that they responded best to green light (550 nm) and hardly at all to red (600-650 nm) and violet (432 nm) light; they also avoided UV light (Snow 1971). Studies of the spectral sensitivity of this species demonstrated that it was most sensitive to ultra-violet and green light, and that a pigment that absorbs light at 520 nm has been isolated from the retina (Stein et al. 1979). Since \textit{Ae. aegypti} have been shown to be sensitive to only two regions of the spectrum it can be concluded that they have dichromatic vision, which would result in good contrast sensitivity but poor colour resolution. These are typical characteristics for invertebrates, which exploit low light level environments (Allan 1994). There have been only a few studies carried out on the attraction of other mosquito species to coloured targets and the data for these do not appear to have been standardised (Allan 1994).

This paper reports on a laboratory study of the behavioural responses of \textit{Toxorhynchites moctezuma} (Dyar and Knab) (a neotropical species) and \textit{Toxorhynchites amboinensis} (Doleschall) (originating from the South Pacific) to oviposition containers painted standard colours (i.e., with known reflective wavelengths).

**MATERIALS AND METHODS**

**Insects**

\textit{Toxorhynchites moctezuma} (Dyar and Knab) and \textit{Toxorhynchites amboinensis} (Doleschall) were cultured at 30 ± 3°C, 73 ± 8% RH under a LD 16:8h photocycle. \textit{Aedes aegypti} (Linnaeus) were cultured as prey for the \textit{Toxorhynchites} larvae under the same conditions. One week old, mated, gravid female \textit{Toxorhynchites} spp. were used for behavioural studies.

**Behavioural Observations**

The behaviour of female \textit{Tx. moctezuma} and \textit{Tx. amboinensis} was observed in response to potential colour cues. Observations were made of groups of ten conspecific females, each group being confined in a separate cage (60 x 60 x 60 cm) for the experimental period of 20 days (there was zero mortality during this time and the insects were not changed within each cage). Ten cages contained \textit{Tx. moctezuma} and 10 contained \textit{Tx. amboinensis}. The cages consisted of steel frames with steel mesh coverings and access to each cage was via a 25 x 25 cm cotton sleeve attached to one of the sides. Humidity and water were provided by pieces of foam soaked in water, which were placed on the top of each cage. Food was provided as small pieces of foam soaked in honey, also placed on the top of each cage. The sides and top of each cage were covered in cling film to raise the humidity within the cage.

Plastic cups (7 cm diameter) were used as oviposition containers in this study. The cups were filled with tap water, which was renewed daily. They were painted on the outside and left outside to weather for seven days before use. Six plastic cups were placed in each cage, one in each corner and two on opposite sides. Each cup was painted a different colour: black, white, red, yellow, green, or blue. The positions of the containers within the cages were exchanged randomly each day.

Behavioural observations were carried out at 21°C ± 3°C, 74.5 ± 6.5% RH under a LD 10:14 h photocycle in fluorescent light. Mosquito behaviour was observed in the ten cages each day, for a total of 20 days, with the order in which the cages were observed being varied randomly each day. During each 30 min behavioural observation period, \textit{Toxorhynchites} oviposition behaviour was quantified by the number of approaches to each oviposition container (i.e., the mosquito flying directly towards the container and to within 2 cm of the rim), the number of oviposition flights initiated over each container (i.e., a distinctive looping flight above the container), and the number of ovipositions.

Behavioural observations were recorded at the same time each day; i.e., between 1000 h and 1600 h, with the peak oviposition period for both of the test species in the laboratory occurring between 1400 h and 1600 h. In addition to these observations, the number of eggs laid in each cage were recorded for each day of the 20 day experimental period. The oviposition cups were put into the cages at 0900 h and were removed and the eggs counted at 1800 h. The cups were replaced with clean ones each day.

For each species, one replicate was represented by a series of 20 days of behavioural observations and egg counts and this was repeated once, to give a total of two, 20-day replicates.

**Paints**

Black: enamel blackboard paint, ready-mixed 2:1 with enamel thinner (St James Paint Supplies, Agra St., St James, Trinidad, W.I.). White: Gloss White Spray 'n Go decorative enamel paint (DAP Inc., P.O. Box 277, Dayton, Ohio OH45401, USA). Red: Endura Fast Drying Spray Paint (A.M.E. International Inc., P.O. Box 188, North Brunswick, N.J. 08902 USA). Yellow: Canary Yellow Touch 'n Tone (DAP Inc.). Green: Hunter Green Touch 'n Tone (DAP Inc.). Blue: Royal
Blue Touch ‘n Tone (DAP Inc.). The paints were diluted (1 part paint: 49 parts diethyl ether) and their absorption spectra measured at 20 nm intervals from 340-700 nm using a single beam visible spectrophotometer (Sanyo Gallenkamp plc UK).

**Statistical Analysis**
All daily oviposition counts and behavioural observations were calculated as the percentage of ovipositions or observations for each cage. Percentage values were transformed (arcsinš) and compared using one-way analysis of variance (ANOVA) followed by studentised range tests (Sokal and Rohlf 1969). The proportions of total approaches to oviposition containers, of approaches resulting in the initiation of oviposition flight, and of oviposition flights resulting in actual ovipositions were all compared between different coloured containers. Replicates were combined if their variances were not significantly different.

**RESULTS**

**Daily Egg Counts**
For both *Tx. moctema* and *Tx. amboinensis* females there were statistically significant differences between the mean percentages of ovipositions in containers of different colours. Both species oviposited more frequently in black containers than in containers of any other colour and for *Tx. amboinensis*, significantly more ovipositions occurred into red containers than into white, yellow, green or blue containers (Figure 1).

**Behavioural Observations: *Tx. moctezuma***
There was a statistically significant difference in the percentages of total approaches by *Tx. moctezuma* females to different coloured containers, with more approaches made to black containers than to containers of any other colour. Of these approaches, higher proportions of approaches resulted in oviposition flights for black (replicates 1 and 2) and red (replicate 2) containers than for the other test colours. Black containers also initiated significantly greater ovipositions from these oviposition flights, resulting in a higher percentage of total ovipositions into black containers than into containers of any other colour (Figure 2).

**Behavioural Observations: *Tx. amboinensis***
There was a statistically significant difference in the percentages of total approaches by *Tx. amboinensis* females to different coloured containers, with black and red containers being favoured. These two colours also initiated significantly more oviposition flights from these approaches than the other colours, with black containers being most active in this respect. The difference in the percentages of oviposition flights resulting in ovipositions for different coloured containers was also significant for *Tx. amboinensis*, with red (replicate 1) and red and black (replicate 2) containers receiving the greatest numbers of ovipositions. Finally, the difference in the percentages of the total ovipositions into different coloured containers was statistically significant with this species, with a higher percentage of the total ovipositions during behavioural observations occurring into black containers, than into containers of any other colour (Figure 3).

**Absorption Spectra of Paints**
The absorption spectra of the paints used on the oviposition containers were measured as percentage transmission. Red and black paints had the lowest levels of transmission (and hence highest absorption) across the majority of the visible spectrum (Figure 4).

**DISCUSSION**
In the present study, container colour was important in influencing the oviposition container choices of both *Tx. moctezuma* and *Tx. amboinensis* females in the laboratory. *Toxorhynchites* spp. mosquitoes have a relatively complex behavioural repertoire leading to egg laying, (Linley 1989, Collins and Blackwell 1998) and this study aimed to investigate the influence of oviposition container colour on the separate stages of this behaviour. All four stages observed were significantly influenced by black-coloured containers, with only red-coloured containers having any additional influence on the mosquitoes’ behaviour. Neither of the two test species of *Toxorhynchites* mosquito were attracted to containers painted blue, green, yellow, or white.

The red and black paints used in this study absorbed more light across most of the visible spectrum than the other colours and although the present transmission data cannot be related directly to spectral reflectance (i.e., the amount of reflected light available for the mosquitoes to see), it is perhaps possible to liken it to an index of what these mosquitoes have available to see under natural light conditions. This would suggest that the critical wavelength band for *Tx. moctezuma* and *Tx. amboinensis* was 300 - 600 nm, where they were able to differentiate between red/black and the other colours. However, further studies of the visual physiology and ecology of these species are required to confirm these
Figure 1. Mean % (± 95% CI) eggs from (a) *Tx. moctezuma* and (b) *Tx. amboinensis* in different coloured containers (■ = replicate 1, n = 20; □ = replicate 2, n = 20). For both species, differences between the mean percentages of ovipositions into containers of different colours attain statistical significance (*Tx. moctezuma*: $F_{5,234} = 69.149$, $P < 0.01$; *Tx. amboinensis*: $F_{5,234} = 87.355$, $P < 0.01$). *Significantly greater than other means within the same replicate (P < 0.05); # significantly greater than other means within the same replicate, except those followed by * (P < 0.05).
Figure 2. Behavioural observations (means ± 95% CI) of *Tx. moctezuma* females with different coloured containers. (a) % approaches to oviposition containers ($F_{(5,234)} = 72.846$, $P < 0.01$, replicates combined); (b) % approaches to oviposition containers resulting in oviposition flights (replicate 1: $F_{(1,114)} = 65.198$, $P < 0.01$; replicate 2: $F_{(1,114)} = 127.757$, $P < 0.01$); (c) % oviposition flights resulting in ovipositions ($F_{(5,234)} = 23.018$, $P < 0.01$, replicates combined) and (d) % ovipositions ($F_{(5,234)} = 171.975$, $P < 0.01$, replicates combined). Filled bars = replicate 1, $n = 20$; Open bars = replicate 2, $n = 20$. *Significantly greater than other means within the same replicate ($P < 0.05$); †significantly greater than other means within the same replicate, except those followed by * ($P < 0.05$).
Figure 3. Behavioural observations (means ± 95% CI) of *Tx. amboinensis* females with different coloured containers. (a) % approaches to oviposition containers (F5,234 = 74.238, P < 0.01, replicates combined); (b) % approaches to oviposition containers resulting in oviposition flights (replicate 1: F5,114 = 42.302, P < 0.01; replicate 2: F5,114 = 49.171, P < 0.01); (c) % oviposition flights resulting in ovipositions (replicate 1: F5,114 = 8.554, P < 0.01; replicate 2: F5,114 = 13.079, P < 0.01) and (d) % ovipositions (F5,234 = 45.805, P < 0.01, replicates combined). ■ = replicate 1, n = 20; □ = replicate 2, n = 20. *Significantly greater than other means within the same replicate; †significantly greater than other means within the same replicate, except those followed by *; +significantly greater than other means within the same replicate, except those followed by * or †.
Figure 4. Absorption spectra of paints used to coat the oviposition containers (measured as percentage transmission), (a) black, white, and yellow; (b) red, green, and blue.
hypotheses.

As with female *Tx. splendens* (Yap and Foo 1984) and *Tx. r. septentronialis* (Slaff et al. 1975), *Tx. moctezuma* females in this study were attracted to black containers rather than to white, red, yellow, green, or blue containers. The oviposition preferences of *Tx. amboinensis* females, however, were most similar to those of *Tx. r. rutilus* females, which oviposited preferentially into black containers and to a lesser extent into red, blue, and brown containers (Jones and Schreiber 1994). Here, *Tx. amboinensis* females oviposited preferentially into black containers and also into red rather than white, yellow, green, or blue containers. The present behavioural observations made it possible to separate oviposition attractants and stimulants, defining a visual oviposition stimulant as a colour for which higher proportions of approaches to containers resulted in oviposition flights, and higher proportions of oviposition flights resulted in ovipositions. Hence, in this study, black containers, as well as acting as attractants, also acted as oviposition stimulants for *Tx. moctezuma* females, and black and red containers for *Tx. amboinensis* females.

The females of many *Toxorhynchites* species, including those of *Tx. moctezuma* and *Tx. amboinensis*, oviposit into tree holes, bromeliads, nut shells, and artificial containers (Steffan and Evenhuis 1981). These containers, which become the habitats of the hatching larvae, usually contain decaying plant material and the more plant material present, the greater the potential of the container to support the prey of the *Toxorhynchites* spp. larvae. It would, therefore, be advantageous for *Toxorhynchites* spp. females, and females of other culicine genera, to use visual cues to identify potential oviposition sites. Since the natural oviposition sites most often chosen by *Toxorhynchites* spp. females are dark, either black or brown in colour, it can be hypothesised that these colours are most likely to act as oviposition attractants or stimulants. This hypothesis is supported by the current data, in addition to data from previous studies on other culicine species. For example, *Ae. hexodontus*, *Ae. aegypti*, and *Ae. triseriatus* were all attracted to black rather than white containers (Beckel 1955, Wilton 1968). *Ae. triseriatus* to blue containers (Williams 1962, McDaniel et al. 1976), and *Ae. aegypti* to green rather than red, violet, or blue containers (Snow 1971). In addition to colour cues acting as oviposition attractants and stimulants, it has been established previously that habitat-related volatiles act as chemo-attractants for ovipositing *Tx. moctezuma* and *Tx. amboinensis* females (Collins and Blackwell 1998) and there is further evidence for an additive effect on oviposition between chemical and colour cues (Collins, unpublished data).

These data contribute to the growing body of data on mosquito oviposition and the influence of external cues on this behaviour, in addition to providing baseline data for further investigations of the visual ecology of *Toxorhynchites* spp. They also have potential implications for incorporating these predatory mosquitoes into systems of biological control for vector species, since they could allow oviposition cues to be matched to those for target species and incorporated into oviposition traps and possibly increase the efficiency of adult release programmes, through encouraging released adult female *Toxorhynchites* spp. mosquitoes to remain in a target area.

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