

Influence of container aperture size and colour on oviposition preferences in three New Zealand mosquitoes (Diptera: Culicidae)

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Abstract

This study aimed to assess some of the oviposition preferences, more specifically container aperture size and colour, of the most common mosquito species breeding in artificial containers in the North Island. Three species were recorded in the ovitraps used: the exotic *Culex quinquefasciatus* and *Aedes notoscriptus*, and the endemic *Culex pervigilans*. The latter species was rare and no inferences could be made about its oviposition preferences. No significant preferences in regards to container colour (green vs. black) were recorded for *Cx. quinquefasciatus* or *Ae. notoscriptus*. The latter displayed contrasting results in relation to container aperture size, with a major oviposition bias for medium-size containers in Wellington, in comparison to a relatively even distribution of its larvae in medium and large ovitraps in Auckland. For *Cx. quinquefasciatus* there was no significant difference between the size treatments, although it was clearly not attracted to the very small ovitraps (a pattern displayed by all species). An important observation was the rarity of native mosquito species in the anthropic habitats studied, an issue of public health significance.

Key words: mosquito, oviposition, ovitraps, container, colour, size

Introduction

Ovitraps are a widely used tool for mosquito vector surveillance and population monitoring (Bellini *et al.* 1996; Fay and Eliason 1966; Smith and Jones Jr. 1972; Tikasingh and Laurent 1981), and their use has been supported as an efficient, easy to use, cheap and sensitive technique (Fay and Eliason 1966; Leiser and Beier 1982; Madder *et al.* 1980). Nonetheless, in order to draw any conclusions regarding the presence or relative abundance of a particular mosquito species, it is necessary to have information on its oviposition preferences (Derraik and Slaney 2005).

In the North Island of New Zealand two species are highly dominant, the endemic *Culex (Culex) pervigilans* Bergroth and the exotic *Aedes (Finlaya) notoscriptus* (Skuse), with the exotic *Culex (Culex) quinquefasciatus* Say also being numerous in northern parts of the island (Hearnden *et al.* 1999; Laird 1990, 1995). The two exotic species are known vectors of a variety of human and animal diseases (Derraik 2004a), and *Cx. pervigilans* may be a vector of avian malaria (Holder *et al.* 1999; Derraik 2006). These species are of potential human and animal health significance, and this particular study aimed to assess some of their oviposition preferences.

Materials and Methods

The initial study was carried out in the Wellington Zoo (41° 20' S, 174° 46' E), chosen due to its relatively high density of mosquitoes (Derraik 2004b). Four sets of ovitraps were used for this experiment, each one consisting of three containers of different aperture sizes (2.5, 7.0 and 11.5 cm diameter) and of similar depths (6-8 cm). The ovitraps consisted of plastic containers, whose inner and outer surfaces were made rougher using 60-grit sandpaper, as rough surfaces seem to favour mosquito oviposition (Beckel 1955; O'Gower 1957a, b; Wilton 1968).

Each container was filled to within 1 cm of the rim with a sheep manure solution prepared with tap water at 5.0 g/L (dry weight), a solution found to yield large numbers of larvae of both *Ae. notoscriptus* and *Cx. pervigilans* (Derraik and Slaney 2005; Leisnham *et al.* 2004). Nutrient concentrations of nitrogen, phosphorus and potassium were approximately 0.15, 0.10 and 0.20 g/L, respectively. Even though black is the most widely used colour for ovitraps, in this particular investigation ovitraps were coated with Japanese laurel-green (Resene Paints Ltd., New Zealand) to address questions relating to a wider research project (Derraik, unpublished data).

The sets of ovitraps were placed at ground level, in full shade and randomly arranged directly against the bases of tree trunks. The latter factor has been shown to favour oviposition by container breeding species, as the presence of tree trunks adjacent to the ovitraps increases the chances of a gravid female finding them, in comparison to those not associated with trees (Jordan 1991; Jordan and Hubbard 1991). Ovitrap were checked fortnightly between February and May 2002.

Following the results from the above study, another experiment was carried in West Auckland, whose annual mean temperature c.2.3°C warmer than Wellington's (NIWA 2004) is likely to be more favourable for mosquitoes. Ovitrap were set at two urban sites (the Auckland Zoological Park and a house backyard in an urban suburb - Kelston) and one semi-rural site (Landsend, an exotic plant nursery). Approximate coordinates for the individual sites were: Zoo (36° 52' S, 174° 42' E), Kelston (36° 53' S, 174° 38' E) and Landsend (36° 56' S, 174° 36' E). As previously, anthropic habitats were used for this experiment to maximize ovitrap utilization by the target species. Two sets of plastic ovitraps following the design used in Wellington were placed at each site. For this study however, each site contained a set of the previously used green ovitraps and another of black ovitraps to assess colour preferences. Sets were arranged side-by-side in a random order at the base of tree trunks, in full shade, and all containers were fortnightly checked between February and April 2003.

Note that larval instead of egg counts were made, as described by Derraik & Slaney (2005). Each time a container was checked for mosquito larvae, it was emptied and the solution replaced. All contents were taken to the laboratory, where larvae were counted and identified using a key to the New Zealand Culicidae (Snell 2005). Many larvae were reared to adults, with voucher specimens of both stages sent to Amy Snell (Wellington School of Medicine and Health Sciences, University of Otago) for confirmation and/or identification. Oviposition preferences for both container colour and aperture size were analysed based on presence/absence (number of larva-positive ovitraps) and larval density (number of larvae per cm²). Data were analysed using a generalized linear model, and abundance variables were $\sqrt{+ 0.5}$ transformed to stabilise the variance.

Results

The only mosquito species collected in the 60 ovitraps inspected at the Wellington Zoo was the exotic *Ae. notoscriptus*, of which 558 larvae were recorded in 15 larva-positive containers. Larvae were collected in ovitraps within the first four fortnights, but no larvae

were collected in the last fortnight, when the presence of mosquitoes dwindled throughout the Zoo. The number of larvae collected in the small, medium and large ovitraps were 9, 430 and 119, respectively. *Aedes notoscriptus* displayed a strong and significance preference for ovipositing in medium-size containers ($P < 0.001$), with 13 out of 20 (65%) of the latter being larva-positive, in comparison to 1/20 (5%) for both the small and large ovitraps (Fig. 1). Mean larval-densities were 0.09 (SE = 0.09), 0.56 (SE = 0.15) and 0.06 (SE = 0.06) per cm² for small, medium and large ovitraps, respectively ($P < 0.001$; Fig. 1).

The study in West Auckland yielded a total of 2437 culicid larvae, with 47 of the 90 (52%) ovitraps being larva-positive. The larvae represented three species: the exotic *Cx. quinquefasciatus* (1698) and *Ae. notoscriptus* (692), and the endemic *Cx. pervigilans* (47). Note that 98% of the larvae comprised exotic species. The species were not evenly distributed among the sites, with *Cx. pervigilans* only being found at Landsend, where 75% of *Ae. notoscriptus* were also recorded. In comparison, 91% of all *Cx. quinquefasciatus* larvae were recorded in the urban backyard at Kelston. No mosquito larvae were recorded in any of the 30 small containers inspected. As a result, to reduce statistical noise the latter data were excluded from the subsequent analyses, which were therefore based on medium and large ovitraps (a total of 60).

Aedes notoscriptus was found to be the most widespread species, although not the most abundant, being recorded in 39 of the 60 ovitraps (65%). *Culex quinquefasciatus* was recorded in 21 ovitraps (35%) and *C. pervigilans* in only 3 traps (5%). No statistical analyses were carried out on data for *Cx. pervigilans* due to its rarity.

No significant oviposition preferences for container colour were recorded for *Cx. quinquefasciatus* or *Ae. notoscriptus* in relation to presence/absence or mean larval density (Fig. 2; all $P > 0.560$). The mean larval density per cm² for *C. quinquefasciatus* was very similar in both colour treatments: 0.47 (SE = 0.17; 733 specimens recorded) and 0.45 (SE = 0.20; 965) for black and green containers, respectively ($P = 0.706$; Fig. 2). Ovitrap occupancy rates were also similar, with *Cx. quinquefasciatus* being present in 11 (37%) and 10 (33%) ovitraps (Fig. 2). The density (and total number) of *Ae. notoscriptus* larvae was higher in green ovitraps than in black ones, 0.19 (SE = 0.06; 422 specimens) and 0.13 larvae/cm² (SE = 0.04; 270), respectively, but not statistically different ($P = 0.561$; Fig. 2). However, the species' occupancy rate of ovitraps was nearly equal, 19 (63%) and 20 (67%), respectively (Fig. 2).

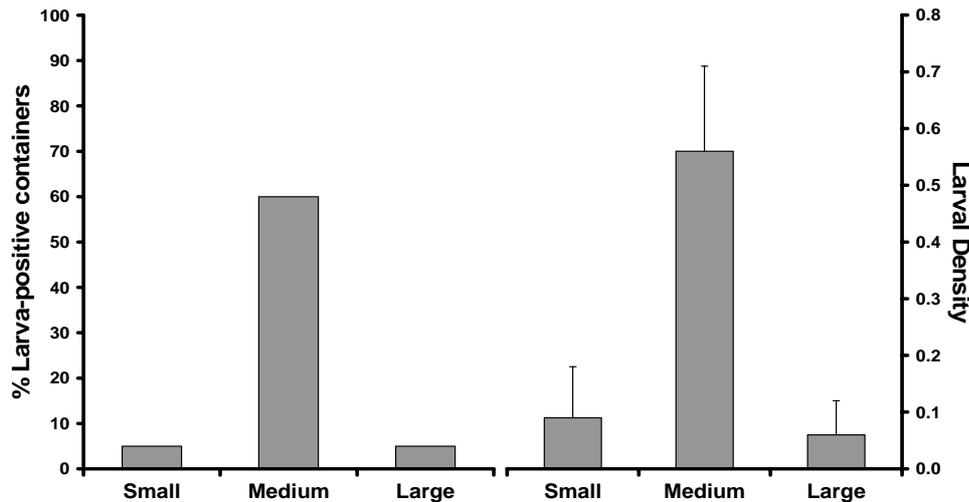


Figure 1. Percentage occupancy rate and mean larval density in ovitraps for *Aedes notoscriptus* recorded in the Wellington Zoo. Note that n = 20 for each treatment. Error bar represents standard error for the mean.

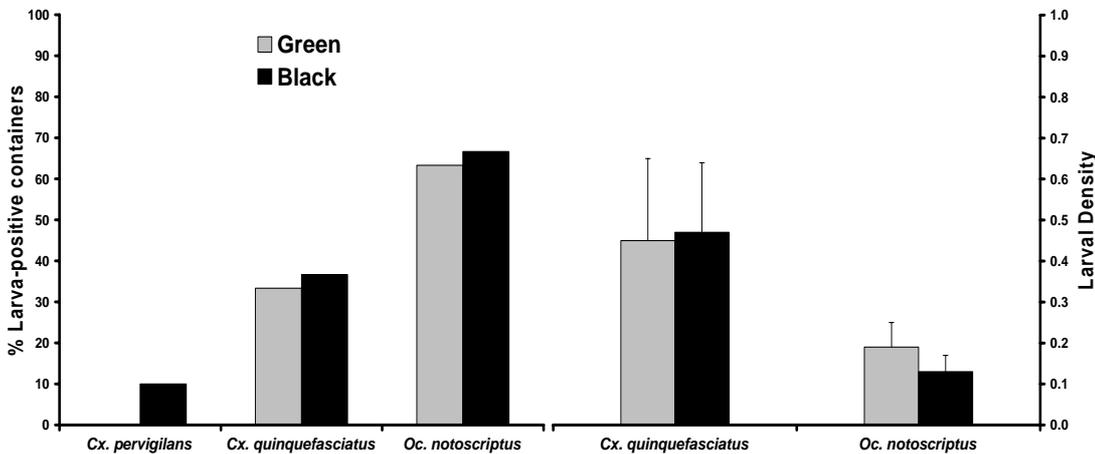


Figure 2. Larval density per cm² and percentage occupancy rate of ovitraps for *Culex pervigilans*, *Culex quinquefasciatus* and *Aedes notoscriptus* recorded in West Auckland, according to container colour (green vs. black). Note that data for the small containers were not included as they were all negative for mosquito larvae, and, consequently, for each species n = 30 for each treatment. Density for *Culex pervigilans* was not included due to the species' rarity. Error bar represents standard error for the mean.

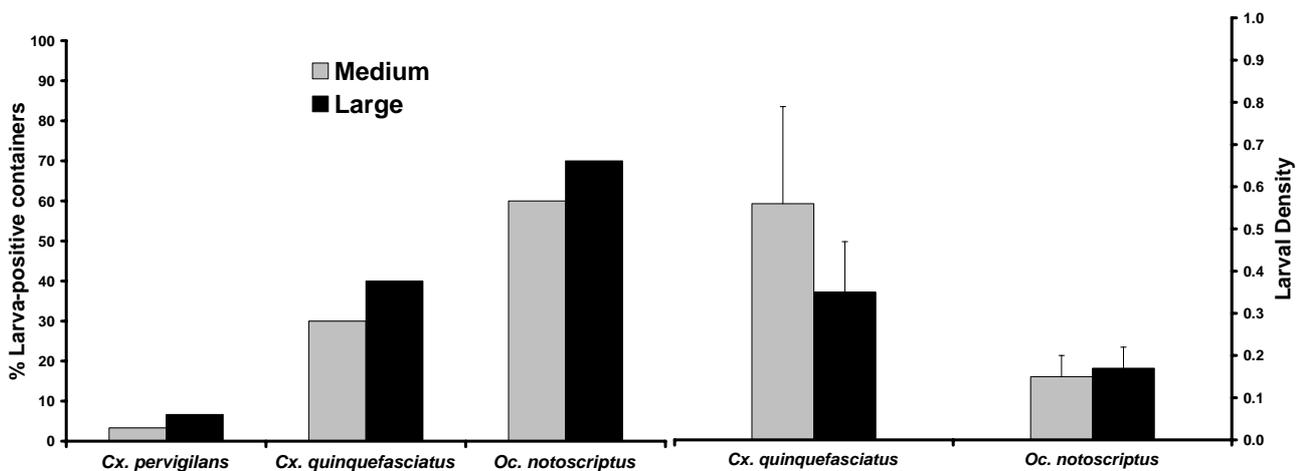


Figure 3. Larval density per cm² and percentage occupancy rate of ovitraps for *Culex pervigilans*, *Culex quinquefasciatus* and *Ochlerotatus notoscriptus* recorded in West Auckland, according to container size (medium vs. large). Note that data for the small containers were not included as they were all negative for mosquito larvae, and, consequently, for each species n = 30 for each treatment. Density for *C. pervigilans* was not included due to the species' rarity. Error bar represents standard error for the mean

In relation to container aperture size, as mentioned earlier, all species recorded in West Auckland were clearly not attracted to the small ovitraps in which no culicids were recorded. When larval densities and occupancy rates were compared between medium and large ovitraps, no significant differences were observed (Fig. 3). For *Ae. notoscriptus* 18 (60%) medium and 21 (70%) large ovitraps were larva-positive ($P = 0.414$), with mean larval densities equal to 0.15 (SE = 0.05; 164 larvae) and 0.17 (SE = 0.05; 528), respectively ($P = 0.599$; Fig. 3). *Culex quinquefasciatus* larvae were present in 9 (30%) medium-size and 12 (40%) large containers ($P = 0.365$), with mean larval densities being 0.56 (SE = 0.23 ; 594 larvae) and 0.35 (SE = 0.12; 1104), respectively ($P = 0.838$; Fig. 3).

No triple infestations were recorded in any ovitraps, but *Ae. notoscriptus* was recorded in double infestations with both *Cx. pervigilans* (twice) and *Cx. quinquefasciatus* (15 times). *Aedes notoscriptus*, being the most commonly recorded species, singly occupied 23 ovitraps, while single infestations occurred only once for *Cx. pervigilans* and in 6 occasions for *Cx. quinquefasciatus*.

Discussion

Despite the limitations of this study, the results indicated that the standard practice of using black ovitraps is not necessarily justifiable, as both *Ae. notoscriptus* and *Cx. quinquefasciatus* showed no significant discrimination between green and black containers. The number of *Ae. notoscriptus* larvae collected in green containers was actually 56% higher than in black ones. At least for *Cx. quinquefasciatus*, the results from this study are partially supported by another experimental work, which found the species to have no preference for black containers over dark green when ovipositing (Frank 1985). Some species have been shown to prefer to oviposit in black containers in comparison to other colours (Dhileepan 1997; Frank 1985), but these would still oviposit in other container colours. Jones & Schreiber (1994) for instance, demonstrated that though *Toxorhynchites (Toxorhynchites) splendens* (Wiedemann) preferred to oviposit in black containers, substantial oviposition was also recorded in green, blue and orange containers. According to Hilburn *et al.* (1983) and Jones & Schreiber (1994) oviposition in recipients with other colours would be likely to increase in the absence of adjacent black containers. Nonetheless, other species such as bromeliad-inhabiting *Wyeomyia* species actually “showed an aversion to black” (Frank 1985, p.28).

In regards to the container size preferences of *Ae. notoscriptus*, it is difficult to explain the major oviposition bias for medium-size containers observed in Wellington (Fig. 1), which contrasted to a relatively even distribution of its larvae in medium and large ovitraps in Auckland (Fig. 3). A larger experiment in the Auckland region also failed to show significant size preferences for this species in identical containers (Derraik and Slaney 2005). It is possible that Wellington’s cooler and windier climate (NIWA 2004) might have worked as selective pressures on the local *Ae. notoscriptus* population, leading to its marked preference for a particular container size. Changes in container size *per se* would only affect evaporation rates with a change in surface area to volume ratio, but it is possible that container size may affect the way the wind generates turbulence over the top of a particular recipient, which could be an important factor in Wellington’s windy environment (David Murray, University of Otago, pers. comm. 2002). Although the results of the small scale Wellington experiment have to be interpreted with caution, one ovitrap experiment with two *Aedes* species has also provided evidence for oviposition in containers with medium-size apertures (Buxton and Hopkins 1927). Nonetheless, the contrasting results observed between the different *Ae. notoscriptus* populations could be a result of phenotypic plasticity. Williams *et al.* (1999) for instance, obtained heterogeneous oviposition preferences for *Ae. notoscriptus* between different habitats, and the authors suggested that oviposition behaviour could be ultimately determined by the species environment, in particular habitat and season.

For *Cx. quinquefasciatus*, we observed no significant difference between the size treatments (apart from the clear lack of attraction to the very small ovitraps). Becker (1995) obtained evidence that this species preferred to oviposit in containers with the largest aperture size (c.20-200 cm diameter), which better reflect *Cx. quinquefasciatus* preferred breeding habitats (Belkin 1962, 1968). In relation to our results, it is possible that the differences between container aperture sizes were not large enough to detect an oviposition bias. Nonetheless, as it happened for *Cx. pervigilans*, *Cx. quinquefasciatus* larvae were also rare in ovitraps in the much larger oviposition experiment (Derraik and Slaney 2005). In this study, although *Cx. quinquefasciatus* was the most abundant species in ovitraps in Auckland, 91% of the specimens were present at a single site. Derraik & Slaney (2005) concluded that ovitraps are not suitable for population monitoring of this species, although *Cx. quinquefasciatus* may indeed utilize such ovitraps to a greater extent in areas where it occurs at high densities.

The absence of *Cx. pervigilans* from the ovitraps in Wellington was surprising, as the species was recorded at the site both as adults (Derraik *et al.* 2003) and abundantly as larvae in artificial containers (Derraik 2004b). Our initial assumption was that the green colour might have inhibited oviposition by this species, which became one of the motivating factors for the subsequent Auckland experiment. However, in the latter study *Cx. pervigilans* was also rarely present in ovitraps. The fact that *Cx. pervigilans* made up a mere 2% of all culicid larvae recorded in the Auckland region was surprising, since it is New Zealand's most abundant and widespread species (Hearnden *et al.* 1999; Laird 1990). The manure solution adopted in this study is not likely to have been a factor, as it had previously yielded numerous *Cx. pervigilans* larvae (Leishnam *et al.* 2004). Moreover, this species is known for its wide tolerance to water quality levels, capable of breeding in both clean and contaminated waters (Graham 1929). Belkin (1968) however, pointed out that *Cx. pervigilans* is not a container breeder *per se*, so the container aperture sizes used in this study might have been the main factor inhibiting oviposition by the species. In fact, it is clear that this species does not readily utilize ovitraps, as also indicated by a larger oviposition experiment simultaneously carried out in the Auckland region (Derraik and Slaney 2005).

One of the most interesting observations from this study was the near absence of native mosquito species from ovitraps in the anthropic habitats studied, findings corroborated by other studies (Derraik and Slaney 2005; Derraik *et al.* 2005). These results are of public health relevance as both *Ae. notoscriptus* and *Cx. quinquefasciatus* are known disease vectors (Derraik 2004a), and in such environments contact with human hosts is obviously maximized. There is consequently increasing evidence that native mosquito species underutilize breeding containers in modified habitats to a large extent. This apparent underutilization of breeding containers by native mosquitoes, as previously proposed by Laird (1990), may actually facilitate the establishment of new invading exotic vectors. Currently, the exotic *Ae. notoscriptus* appears to have become the main species exploiting both artificial and natural containers, not only in anthropic environments, but also in disturbed patches of indigenous forests.

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