# Distribution of *Wyeomyia smithii* (Diptera, Culicidae) Eggs in Pitcher Plants in Newfoundland, Canada

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**Abstract** : Egg distribution of the pitcher plant mosquito *Wyeomyia smithii* was studied in Newfoundland, Canada, in July 1979. Most eggs were laid in new pitchers and chemical stimuli specific to new pitchers were considered to play a dominant role in this selectivity. Larger pitchers had more eggs than smaller ones. The analysis of the distribution pattern of eggs suggested that a female lays eggs neither at random nor in a large batch, but tends to lay eggs in small numbers at a time. Based on the results, sampling plans for future population studies were considered. Some aspects of the ecology and evolution of mosquitoes breeding in small-container habitats, especially those associated with living plants, were discussed. The concept of "skip oviposition" was proposed.

*Wyeomyia smithii* (Coquillett) is a mosquito breeding in the water-filled pitcher of *Sarracenia purpurea*, an insectivorous plant endemic to bogs of eastern North America. In Canada this mosquito is suitable for population studies since pitchers in open bogs are easily collected, it is the only mosquito in the habitat, each population is isolated and clearly defined, each generation is distinct and the absolute number of each developmental stage from eggs to emerged adults can be counted with ease. Further, this species can easily be colonized in the laboratory. This simplicity may prove instructive in elucidating the fundamentals of mosquito populations breeding in phytotelmata, small water-collections associated with plants, which flourish in tropical environment. In this report, some factors influencing the egg distribution will be examined as a first step toward a comprehensive study on the population dynamics of this species. Based on the results, sampling plans for population studies will be considered.

## PLACES AND METHODS

Pitchers were collected at two bogs near St. John's, Newfoundland, in July 1979 (Table 1). In this region, oviposition by females which overwintered as third instar larvae starts in early July and becomes increasingly active in middle–late July. Therefore, we were able to examine egg populations at various density levels by sampling on different days as well as at two bogs. This was necessary for the analysis of the distribution pattern.

Pitchers were grouped into two age classes depending on the year of opening. Pitchers which opened last year are "old" and those which did this year are "new". Old pitchers were separable from new ones by the evidence of winter frost damage on their surface. Size, colour and hardness were not reliable for age grading because of their variability.

As eggs of *Wyeomyia smithii* are laid within the pitcher, collected plants were dissected in the laboratory and the egg number was counted under stereoscopic microscopes. Hatched eggs were included in this number. Capacity and colour of each pitcher were recorded as well as the quantity and transparency of contained water. Transparency was measured at the wave length of 620 m$u$ with Beckman's Grating Spectrophotometer.

Large samples were taken at the two bogs on 18 and 19 July. However, pitchers of Little Power Pond Bog were very small in average size, therefore examinations on factors influencing the egg number were done mostly for materials collected at Mount Scio Bog on 19 July.

Table 1.    Pitchers examined

| Place* | Date | No. (and age**) of pitchers |
|--------|------|------------------------------|
| M | 17 July 1979 | 20 (new) |
| L | 17 July 1979 | 24 (new) |
| L | 18 July 1979 | 80 (new) |
| M | 19 July 1979 | 71 (new) |
| M | 23 July 1979 | 41 (new) |
| M | 24 July 1979 | 17 (old) |
| M | 29 July 1979 | 40 (new) |
| Total | | 293 |

\* M : Mount Scio Bog. L : Little Power Pond Bog.
\*\* New : Opened this year. Old : Opened last year.

## RESULTS

*Age of pitchers*

Table 2 demonstrates a strong preference for new pitchers by gravid females. This preference minimizes the spatial overlap of the overwintered and first summer generations.

Table 2.  Relation between the age of pitchers and the mean number
(and range) of eggs

| Pitcher capacity (ml) | Opening time of pitcher | |
| --- | --- | --- |
| | This year* | Last year** |
| 11—20 | 15.66 ( 5—30) | 0.67 (0—3) |
| 21—30 | 24.88 (11—38) | 2.00 (0—9) |

\* Census date : 23 July.
\*\* Census date : 24 July.

Only a small portion of eggs or newly hatched first instar larvae of the summer generation inhabited old pitchers together with remaining individuals of the overwintered generation.

Some differences between old and new pitchers were easily visible. New pitchers often contained little or no water, while old ones were always at least partly filled. New pitchers had a fewer remains of the plant's victims than old ones. Also, the density of *Metriocnemus knabi* larvae (Diptera, Chironomidae) was much lower in new pitchers than in old ones. These differences may be more or less responsible for the lack of attraction of old pitchers to gravid females, especially the presence of water since females probably prefer empty pitchers to water-filled ones as oviposition sites (see the section *Quantity of water in pitchers*). However, this can not fully explain the concentration of eggs in new pitchers because even water-filled new pitchers had more eggs than old ones. In view of the precision of the site selection, chemical attractants and/or stimulants wholly or practically specific to new pitchers may be most important.

The following examinations were done for new pitchers.

*Colour of pitchers*

Colour of pitchers was highly variable. Some pitchers were green except for the veins and their adjacent parts, while others were entirely red. Every intermediate form between these extremes was found. Genetic, environmental and age-related factors may be responsible for this variability. For convenience, pitchers were classified into three groups, green, intermediate and red. However, no correlation was recognizable between colour and egg density.

*Capacity of pitchers*

Capacity of pitchers was highly variable, too. As for the present material, it ranged from the minimum of 4 ml to a maximum of 67 ml. Larger pitchers certainly exist and could be found in more extensive surveys. As in the case of pitcher colour, genetic, enviromental and age-related factors may be involved in this wide variation.

Fig. 1 illustrates the relation between pitcher capacity and the number of eggs per pitcher. Larger pitchers clearly had more eggs than smaller ones. The egg density
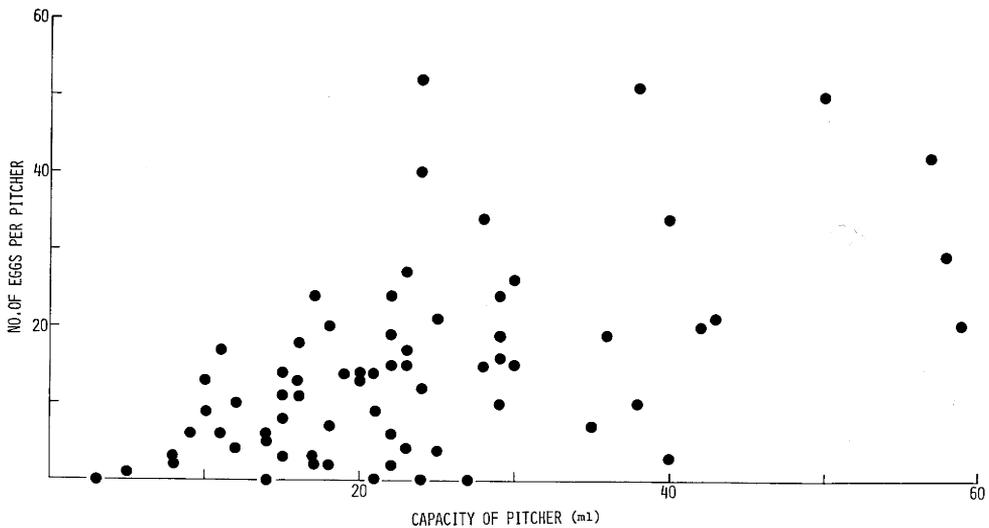
Fig. 1.   Relation between the pitcher capacity and the egg number.
          Census date : 19 July.

per ml was nearly constant irrespective of pitcher capacity at least in the range of 10 to 40 ml. The density per ml was low for pitchers smaller than 10 ml. These pitchers may be too small for gravid females to enter and/or lay eggs conveniently. Also, the egg density per ml appeared low for pitchers larger than 40 ml. This may have been temporary, however, since the mean density was still increasing on 19 July toward its peak about the end of the month.

It was necessary to class pitchers by size before advanced analyses of the distribution pattern of eggs per pitcher could be done. Therefore, pitchers were divided into classes, each of the 10 ml range. However, pitchers of small or large size were a few, the following analyses were done for two age classes including pitchers of moderate size. One class included pitchers of 11−20 ml and the other 21−30 ml. Within each of these classes, correlation between the capacity and the egg number was practically negligible due to large variations in the egg number relative to the slope of regression.

*Quantity of water in pitchers*

On the average larger pitchers contained more water than smaller ones. To eliminate the influence of pitcher capacity, the relation between the relative amount of water to pitcher capacity and the egg number was examined. No correlation was found between the two variables (Fig. 2).

In newly opened dry pitchers most eggs were found stuck side by side on the lowest part of the inner surface. In pitchers with water, some eggs were found floating on the water surface, but many were submerged and on the lowest inside surface in the
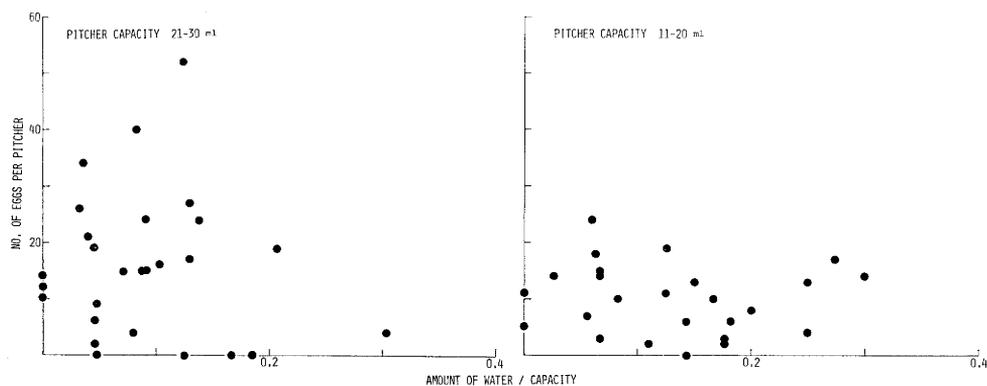
Fig. 2.   Relation between the quantity of water and the egg number.
Census date : 19 July.

same arrangement as eggs in pitchers without water, which may indicate that they were not a part of eggs laid at first on the water surface. Since it is unlikely that oviposition takes place under water, it is felt that submerged eggs were laid probably before the accumulation of water. This consideration supports the idea of the presence of chemical attractants and/or stimulants being inherent to new pitchers.

*Transparency of water*

The transparency of water in pitchers was measured as a convenient index to the level of nutrition available to mosquito larvae since water containing organic matter and micro-organisms at higher concentrations obviously had reduced transparency.   Fig.   3 illustrates that the egg number was small when transparency was very low. Pitchers with
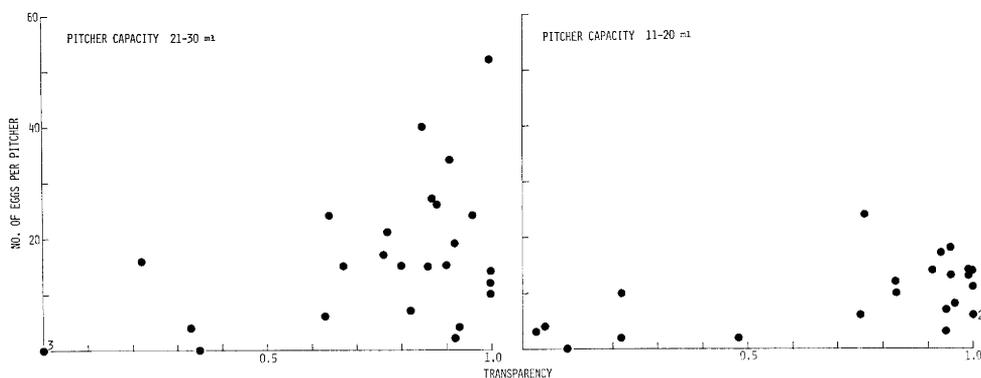


Fig. 3.   Relation between the transparency of water and the egg number.
Numerals by points show frequencies (only when more than twice).
Census date : 19 July.

turbid water were more aged than those with clear water. The first rain water accumulation in newly opened pitchers is pure, transparency being greater than 0.9, occasionally 1.0. With the decomposition of prey, transparency decreases. When a large animals such as a slug decomposed, the water smelled foul and the transparency was less than 0.2, even zero. From those pitchers, *Wyeomyia* eggs were rarely found, while the larvae of *Blaesoxipha fletcheri*(Diptera, Sarcophagidae) were frequently encountered. It is most likely that nutritives in water are not important as stimuli inducing oviposition and even mask the effect of attractants to *Wyeomyia smithii*.

Pitchers with water of transparency below 0.2 were excluded from the following analysis of the distribution pattern since they are considered to be quite unsuitable as oviposition sites for this mosquito.

*Analyses of the distribution pattern*

The method developed by Iwao (1968) was employed (Fig. 4). Regression equations of mean crowding $(\overset{*}{m})$ on mean density $(m)$ were calculated for each of two capacity classes as follows :

| Pitcher capacity | Equation |
|---|---|
| 11—20 ml | $\overset{*}{m} = 2.7206 + 1.1750\ m$ |
| 21—30 ml | $\overset{*}{m} = 3.7580 + 1.2515\ m$ |

where the intercept is *Index of Basic Contagion* $(\alpha)$ and the slope is *Density–Contagiousness Coefficient* $(\beta)$. When each individual is distributed at random, $\alpha$ is zero and $\beta$ is unity. When $\alpha$ has a positive value and $\beta$ is larger than unity, the distribution may be mimicked by a model of aggregately distributed colonies. The case of *Wyeomyia smithii* eggs appeared to correspond to the latter, but this was not confirmed statistically except for the $\alpha$ value in 11—20 ml pitchers which was significantly larger than zero ($P < 0.05$). The deviation from random distribution might be further confirmed statistically by increasing the data. In any way, it can clearly be said that a female of this mosquito does not have a habit of laying a large number of eggs in one pitcher at once.

Fig. 5 illustrates the frequency distribution of the egg number per pitcher over a period of time. In mid–July when mean density of eggs were lower than 10, frequencies of both zero and large numbers were higher than expected from the Poisson series, a model of random distribution, but no pitchers had more than 30 eggs. This suggests that a female lays eggs neither at random nor in a large batch, but tends to lay eggs in small numbers at a time. With increasing mean density, pitchers with more than 30 eggs became found, especially among pitchers of 21—30 ml in capacity. The observed maximum was 64 eggs in a single pitcher. It is evident that such a large number of eggs were not laid by one female on one day but accumulated by many females over many days. At high density levels all pitchers were occupied by eggs, which indicates that unoccupied pitchers found earlier were not avoided due to their unsuitability as oviposition sites.

PITCHER CAPACITY 21-30 ml

PITCHER CAPACITY 11-20 ml

40

$\overset{*}{m} = 3.7580 + 1.2515m$

$\overset{*}{m} = 2.7206 + 1.1750m$

MEAN CROWDING ($\overset{*}{m}$)

20

10                    20                    30

10                    20                    30

MEAN DENSITY PER PITCHER (m)
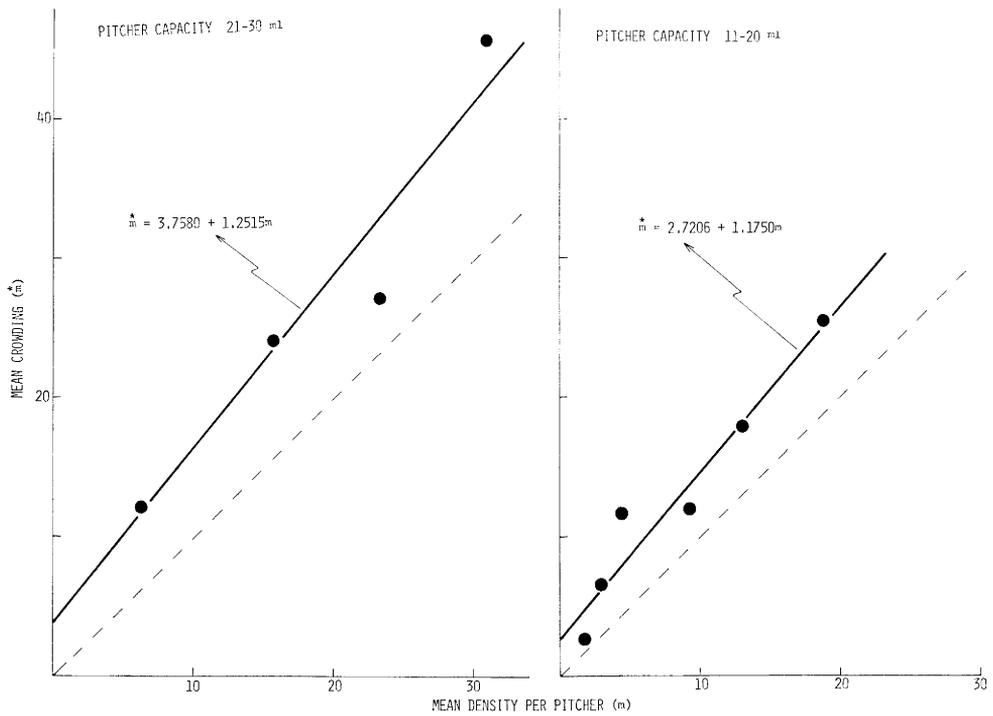
Fig. 4. Relation between mean density and mean crowding

PITCHER CAPACITY 11-20 ml

PITCHER CAPACITY 21-30 ml

4

L 17 JULY n=12 m=1.83

4

M 17 JULY n=10 m=3.00

12

FREQUENCY

L 18 JULY n=46 m=4.43

L 18 JULY n=16 m=6.31

4

M 19 JULY n=23 m=9.26

M 19 JULY n=29 m=15.66

4

M 23 JULY n=20 m=13.10

M 23 JULY n=18 m=23.28

4

M 29 JULY n=20 m=18.85

M 29 JULY n=17 m=30.82

0        10        20        30        40        50   0        10        20        30        40        50        60

NO. OF EGGS PER PITCHER
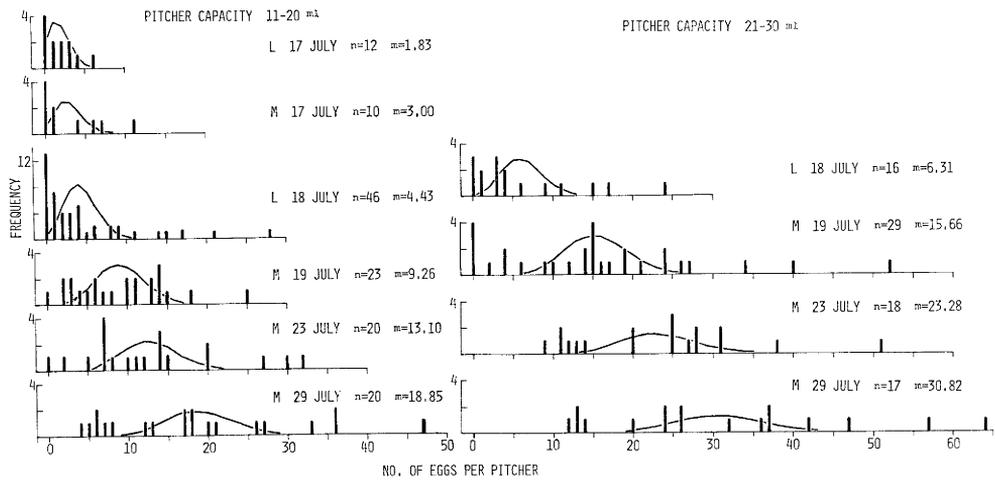
Fig. 5.  Frequency distributions of the egg number per pitcher.
         Vertical bar : Observed frequency. Curve : Poisson distribution.
         M : Mount Scio Bog. L : Little Power Pond Bog.

*Number of samples required for estimating the density with a fixed precision level*

The sample size ($q$) necessary for estimating the mean density of eggs per pitcher with a certain precision level ($D$ = standard error/mean) was calculated following Iwao and Kuno (1968) :

$$q = \frac{t^2}{D^2} \left(\frac{\alpha+1}{m} + \beta-1\right)$$

where $t$ is Student's $t$.

Fig. 6 illustrates an example where pitcher capacity is $11-20$ ml and $t=1$. From this we can determine the sample size appropriate to a particular purpose at each density level.   It is unlikely that the distribution of larvae or pupae is more aggregated than eggs, considering probable density-dependent mortality during post-embryonic development.   Therefore, sampling plans for eggs may also be safely applied to later developmental stages. Except for populations at very low density



Fig. 6.   Sample size required for estimating the population density of eggs with a certain value of $D$ (= standard error/mean).

levels, 20 pitchers for each capacity class taken weekly may be sufficient to obtain parameters which demonstrate the general attributes of the entire population.

## DISCUSSION

*Chemical stimuli as important factors to maintain special associations between mosquitoes and plants*

Most eggs of *Wyeomyia smithii* were found in new pitchers which had opened this summer and chemical stimuli specific to new pitchers were considered to be most responsible for this selectivity. Istock et al. (1975) confirmed experimentally that the gravid female of this mosquito locates the pitcher of *Sarracenia purpurea* by chemical attractants and also suggested that she can probably discriminate young pitchers from old ones. It was observed in this study that eggs were preferably laid in newly opened pitchers before water is accumulated. This agrees with earlier observation of Smith (1904) and Fish and Hall (1978). This preference for newly opened pitchers as oviposition sites appears highly adaptive to very small but continuously reproduced pitcher plant habitats since it minimizes such ill effects of overcrowding as food shortage, accumulation of overcrowding factors and cannibalism*. Indeed, each pitcher is usually inhabited by only two succesive larval

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* The larva of *Wyeomyia smithii* is not predacious, but cannibalism probably occurs widely among filter-feeding mosquito larvae (Mogi, 1978)

instars of *Wyeomyia smithii* (Fish and Hall, 1978).

A close association of mosquitoes with a species or a group of water-holding living plants is common especially in the tropics. Although almost nothing is known about factors which maintain these close associations, chemical cues may play a dominant role as in the case of *Wyeomyia smithii* and its host plant *Sarracenia purpurea*. They may be called kairomones in their ecological effect. As some vector mosquitoes of human diseases breed exclusively in water collections associated with particular living plants, it is of practical importance as well as biological interest to identify these attractants.

*Evaluation of the role of habitat size in adaptive radiation of mosquitoes associated with living plants*

In *Wyeomyia smithii*, eggs were laid in proportion to pitcher size, no distinct preference being recognized for a certain size of pitchers. This agrees with an observation that larger pitchers contained more larvae of this mosquito than smaller ones (Paterson, 1971). A simple positive correlation between the habitat size and the egg number was also found in *Wyeomyia vanduzeei* and *W. medioalbipes* breeding together in the bromeliad *Tillandsia utriculata* of Florida (Frank et al., 1977). It would be of interest to know whether the same relation holds true in warmer regions where more species with similar ecological requirements are sympatric. In Jamaica, for instance, 1 *Culex* and 3 *Aedes* (*Howardina*) species breed exclusively in bromeliads besides 10 *Wyeomyia*. All these other than 2 *Wyeomyia* species common to Florida* are endemic to Jamaica and it is of particular interest that 6 *Wyeomyia* belonging to the Hirsuta group were considered to have been derived from a common ancestor through adaptive radiation in that island. Close associations between the species of the Hirsuta group and the respective bromeliad genera were also suggested (Belkin et al., 1970). It is unlikely, however, that habitat size is a major factor involved in that selectivity, as intraspecific variation in bromeliad size is extremely large due to constant growth during their very long lives. A preference for a special size, if any, may be apparent and yet prove to be attributable to other factors on closer examinations. In Trinidad, *Anopheles homunculus* concentrates in small species of bromeliads in the overlap zone with *A. bellator*, but microclimate and interspecific competition were considered to be responsible for this apparent "preference for small bromeliads" (Pittendrigh, 1950). This consideration agrees with the above emphasis on chemical cues as a key factor which restricts a mosquito species to a particular plant. It may be worth adding that simple situations in colder regions are often helpful for analysing more complex phenomena in tropical environment.

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* *Wyeomyia medioalbipes* of Frank et al. (1977) is identical with *W. mitchellii* of Belkin et al. (1970)

*Skip oviposition adaptive to small habitats*

Little is known about the actual process of oviposition in nature of mosquitoes which lay eggs singly. In *Wyeomyia smithii*, it was suggested from the analysis of the egg distribution that eggs are laid neither at random nor in a large batch, but in small numbers at a time. This may be explained by either or both of the following.

(A) Only a portion of a female's mature eggs are laid at a time. A short flight which may be followed by rest, feeding and other activities precedes the next partial oviposition.

(B) Follicles develop asynchronously and a female has only a small number of mature eggs at a time.

Although available observations on the reproductive biology of this species (Price, 1958; Wallis and Frempong–Boadu, 1967; Istock et al., 1975) are insufficient to give precise details of the oviposition process in nature, we suspect that both processes may be involved to some extent. Autogeny of the species complicates the problem.

In anautogenous mosquitoes, ovarian development beyond the resting stage is synchronized by blood meals. In this case the partial oviposition observed in the field may be attributed mainly to the characteristic behaviour (A). An example is *Aedes albopictus* which breeds in tree holes and artificial containers in or near residential areas. Makiya (1976), who analysed the distribution pattern of the species eggs laid in ovitraps, detected according to the Iwao's method a colony of several eggs as the basic component in the population. This number is much smaller than that of mature eggs following normal feeding. It was also reported that the gonotrophic cycle of this species in the field was rather regular and approximated the duration from blood feeding to egg maturity observed in the laboratory (Mori and Wada, 1977). Therefore, a female of this species probably distributes an egg batch among several containers within one or two days when a number of suitable containers are accessible.

This type of behaviour may be common to many small–container breeders which lay eggs singly, and is adaptive to small habitats in the sense that it lessens both unoccupied collections of water and intraspecific competition. In view of its ecological significance and probable wide occurrence among mosquitoes breeding in small–container habitats, it may be convenient to coin a simple term for this type of behaviour. "Skip oviposition" is proposed.

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*Wyeomyia smithii* (双翅目，蚊科) の卵の分布
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カナダのニューファンドランドで食虫植物 *Sarracenia purpurea* のツボ状の葉 (pitcher) の中に溜った水に発生する *Wyeomyia smithii* の卵の分布を調べた．多くの卵は若い pitcher に産みつ

けられ，この選択に際しては若い pitcher に固有の化学的刺激が重要な役割をはたしていると考えられた．大きな pitcher ほど多数の卵を産みつけられていた．pitcher あたりの卵の分布様式を検討した結果，雌成虫は一卵ずつ独立に産んでゆくのでもなければ，多数の卵を一個の pitcher に産んでしまうのでもなく，いくつもの pitcher に少数ずつ分けて産んでゆく傾向があることがわかった．これらの結果にもとづき，この蚊の個体群動態を研究するための pitcher のサンプリング法についてもふれた．この調査で明らかになった諸点は，熱帯に多い，特定の植物に溜った水から発生する蚊の性質と基本的に共通すると考えられたので，考察では，それらの蚊の生態や進化についてもふれ，その中で skip oviposition という概念を提出した．生物相の単純な寒地での知見は，生物相の豊かな熱帯でのより複雑な現象を分析するための一助になると思われる．