

REPORT

Predation-dependent oviposition habitat selection by the mosquito *Culiseta longiareolata*: a test of competing hypotheses

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Abstract

We investigated the mechanism underlying oviposition habitat selection (OHS) in the mosquito *Culiseta longiareolata*. The putative outcome of a trade-off between the risk of predation and detrimental density dependence, OHS in this species presents an opportunity to test two competing alternatives: (1) a polymorphic scenario, in which a fixed proportion of females constantly avoid ‘predator pools’, while the remainder oviposits at random; and (2) a monomorphic scenario, in which all females oviposit in predator pools with a certain probability. We present a conceptual framework that demonstrates how a simple experimental design – whereby predator incidence in artificial pools is alternated between 0.25 and 0.75 – can distinguish between, or refute, the two scenarios. Given the proportional use, by ovipositing females, of predator-free pools observed under each treatment, and a bootstrap estimate of the ratio of daily oviposition rates, we find the monomorphic scenario twice as likely as the polymorphic.

Keywords

Culiseta, mechanisms, *Notonecta*, oviposition habitat selection, predation.

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INTRODUCTION

In organisms with no parental care or juvenile dispersal, offspring survival and growth should strongly depend on the quality of the habitat in which they are deposited. When potential habitats vary in their inherent suitability for juveniles, we would thus expect adults to choose those habitats that are most favourable. As more females choose the same sites, however, habitat quality in these sites may begin to decline due to detrimental density-dependent effects. Evolutionary theory predicts that such trade-offs could lead to variation in habitat utilization, in accordance with the relative ‘quality’ of alternative habitats (Brown 1998; references therein). Here we attempt to identify the mechanism responsible for the variation in habitat selection, observed in the mosquito *Culiseta longiareolata*.

Female *C. longiareolata* avoid ovipositing in pools that contain the backswimmer *Notonecta maculata*, which preys on its larvae. Faced with a choice between an equal number of ‘predator’ and ‘predator-free’ pools, *c.* 90% of females oviposit in the pools that lack the predator (e.g. Blaustein *et al.* 1995; Blaustein 1998). This incomplete avoidance of predator pools has been suggested to reflect an adaptive response to a trade-off between the risk of predation and

the negative effect of larval density on larval survival and development (Spencer *et al.* 2002; Kiflawi, Blaustein & Mangel *unpubl.*).

Contrary to expectations from an ‘ideal free’ scenario (*sensu* Fretwell & Lucas 1970), recent findings indicate that *C. longiareolata* females do not adjust their use of predator pools based upon *relative* habitat quality; at least not when the alternatives are found within a limited spatial scale (Kiflawi, Blaustein & Mangel *unpubl.*, see Appendix). Although ovipositing females may be responding to habitat heterogeneity across larger spatial scales, other mechanisms (adaptive or otherwise) may also explain incomplete avoidance of predator pools. Here we attempt to differentiate between two such alternatives, which we term the ‘monomorphic’ and ‘polymorphic’ scenarios.

Female *C. longiareolata* may have been selected to utilize each pool type with a specific, monomorphic, probability; or, conversely, females may always prefer predator-free pools, but share an equal probability of failing to detect the predator. On the other hand, a polymorphism may exist (either genetic or state-dependent), by which some proportion of females avoids predator pools while the remainder oviposits at random. We seek to differentiate between these two scenarios by analysing the differences in oviposition

patterns observed when predator incidence (i.e. the proportion of predator pools) in an array of artificial pools is switched from 0.25 to 0.75.

The rationale behind our experimental design stems from the significant drop in daily oviposition rates, observed when the overall habitat 'quality' of experimental arrays is lowered (see Appendix). Below we present a theoretical evaluation of the ability of this simple design to differentiate between the competing scenarios, as well as to refute them entirely. We then proceed to compare the likelihood of the two scenarios, given the observed proportional use of predator-free pools under the two treatments.

MATERIALS AND METHODS

Study organism

The mosquito *Culiseta longiareolata* Macquart (Diptera: Culicidae) is common and abundant in temporary pools in the Middle East and Africa (van Pletzen & van der Linde 1981). Oviposition is nocturnal and involves, with rare exceptions, the deposition of all of a female's eggs in a single 'egg raft' (van Pletzen & van der Linde 1981). The larvae are extremely vulnerable to predation by the backswimmer *Notonecta maculata* Fabricius (Hemiptera: Heteroptera), which is also common in temporary pools (Ward & Blaustein 1994; Blaustein 1998). Larval density has a significant effect on adult body size (Kiflawi, Blaustein & Mangel *unpubl.*), which, in many mosquito species, is positively correlated with longevity and female fecundity (e.g. Bradshaw & Holzapfel 1992; Lyimo & Takken 1993; Ameneshewa & Service 1996). Given the high adult mortality rates of Culicidae in the wild (*c.* 0.2 day⁻¹, Service 1993), and the lengthy gonotrophic cycle of *C. longiareolata* (means: *c.* 4–11 days, Al-Jaran & Katbeh-Bader 2001), females of the species are unlikely to oviposit more than once during their lifetime (Spencer *et al.* 2002).

Experimental set-up

We set up a rectangular array of 18 artificial pools (0.6 × 0.4 × 0.15 m plastic tubs) in the Mt. Carmel Nature Reserve, Israel. Each tub was separated by *c.* 50 cm from its two flanking neighbours. Water level in the pools was established and maintained at 13 cm using aged tap water. An initial inoculate of re-suspended sediment and algae was introduced into each of the 18 tubs. A canopy consisting of a shading net (mesh density: 90%) was placed *c.* 2 m above the entire array. Maximum water temperatures among the pools ranged from 24 to 26 °C. Three *Notonecta maculata* nymphs (instars IV & V) were introduced into each of nine of the pools, such that predator (P+) and predator-free (P-) pools alternated around the array.

To manipulate predator incidence, we used plastic covers to seal six of the nine P+, or P-, pools, thereby setting predator incidence at either 0.25 or 0.75, respectively. The covers were placed during the afternoon preceding an experimental night, and removed the following morning when we counted and removed the egg rafts oviposited in each of the exposed pools. In the period spanning March 23 and May 17, 2001, we monitored oviposition for a total of 22 nights, alternating treatments (predator incidence) across consecutive nights by shifting, clockwise, the covers from one pool to its neighbour. Dead and metamorphosed *N. maculata* nymphs were replaced within 12 h.

Rationale

Our data provides two pieces of information that are independent of each other: (1) the ratio (*R*) of the daily average number of egg rafts oviposited in the 12 exposed pools, under each of the two treatments; (2) the proportion (*C*) of egg rafts oviposited in predator-free pools on nights that the array was biased in favour of either predator or predator-free pools. We can thus use the first piece of information (*R*) to identify the relevant parameters necessary, under each scenario, to calculate the expected values of the second (*C*).

The polymorphic scenario

We assume a polymorphism between a selective morph, with a frequency of *S* and which oviposits in predator-free (P-) pools only, and a non-selective morph that oviposits at random.

Let *w* equal the proportion of predator (P+) pools, or the probability of alighting on a predator pool. Assume also that non-selective females oviposit in the first pool they visit, while selective females oviposit the first time they visit a predator-free pool (i.e. only selective females visit more than one pool, but successive visits are independent). A proportion *Sw* of all females fails to oviposit by the end of their first visit. By the *v*'th visit only *Sw^v* females would have failed to oviposit. In other words, by the *v*'th visit 1 - *Sw^v* of all females are expected to have oviposited, of which (1 - *S*)*w* in predator pools. Hence, the proportion of females that are expected to oviposit in P- pools after *v* visits is:

$$C = [(1 - Sw^v) - (1 - S)w] / (1 - Sw^v) \quad (1)$$

Given predator incidence *w*, the daily average number of egg rafts expected with *n* females visiting the array is: $E = n(1 - Sw^v)$. Since $E_{w=0.25}$ may be expressed as a multiple (*R*) of $E_{w=0.75}$, the frequency of the selective morph may be calculated as

$$S = (R - 1) / (0.75^v R - 0.25^v) \quad (2)$$

The monomorphic scenario

We assume females oviposit in P+ pools with a probability m , and with the probability of ovipositing in P- pools taken as one.

Assuming that the probability of alighting on a P+ pool is independent of the number of pools already visited within the array, the proportion of females expected to oviposit after v visits is $1 - [w(1 - m)]^v$. Of these, the proportion expected to oviposit in P- pools is:

$$C = (1 - w) \sum_{i=1}^v [w(1 - m)]^{i-1} / 1 - [w(1 - m)]^v \quad (3)$$

The first term of the numerator is the probability of alighting on a P- pool, whereas the term being summed is the probability of alighting on, and failing to oviposit in, P+ pools for the first $v - 1$ visits. Given the ratio of the per-treatment daily average oviposition rates,

$$R = \{1 - [0.25(1 - m)]^v\} / 1 - [0.75(1 - m)]^v$$

the probability of ovipositing in P+ pools is

$$m = 1 - [(R - 1) / (0.75^v R - 0.25^v)]^{1/v} \quad (4)$$

Comparing eqns 1 and 3 we find that the two scenarios produce identical predictions if females were to visit the array only once (note from eqns 2 and 4 that when $v = 1$, S and $(1 - m)$ are interchangeable). Were females to visit the array more than once before they either oviposit or leave the array having failed to do so, a discrepancy between the two scenarios is possible; a discrepancy that may enable us to differentiate between the competing scenarios. Moreover, from eqns 2 and 4 it is clear that ‘observed’ values of $R > 3$ would not be compatible with either scenario as they would produce expected values of $S > 1$ and $m < 0$, respectively. The same holds for $R < 1$, which would result in $S < 0$ and $m > 1$. In other words, our experimental results could either falsify both scenarios, or enable us to evaluate which is the more likely (Table 1, Fig. 1). The likelihood of each scenario given the number of egg rafts oviposited in total (N), and in predator-free pools only (K), under each treatment is:

$$L(K_{w=0.75}, N_{w=0.75} | C_{w=0.75}) \times L(K_{w=0.25}, N_{w=0.25} | C_{w=0.25}) \quad (5)$$

where $L(K, N | C)$ is the binomial probability of K , and C is the expected proportional use of predator-free pools under the appropriate scenario (eqns 1 & 3).

The outcome of the proposed analysis depends heavily on our estimation of R . A ratio based on the *observed* daily average oviposition rates ($E_{w=0.25} / E_{w=0.75}$) may become confounded by outliers (i.e. days with exceptionally low or high oviposition), especially since the total number of days is small. Hence, we conducted the analysis using a bootstrap estimate of R , based on 500 iterations in which we resampled (with replacement) the 9 days with $w = 0.25$ and the 13 days with

Table 1 (a) The probability of ovipositing in predator pools (m , the Monomorphic scenario) and the frequency of the selective morph (S , the Polymorphic scenario), expected for various combinations of v (the number of visits to the array) and R (the ratio of the daily average oviposition rates). (b) The corresponding proportion of females expected to oviposit in predator-free pools (C) when predator incidence (w) is 0.75; see text for details. Empty cells correspond to combinations of R and v that would produce nonsensical values of S and m

$v \setminus R$	1.4	1.6	1.8	2.0	2.2	2.4
(a)						
Monomorphic						
1	0.50	0.37	0.27	0.20	0.14	0.10
2	0.26	0.15	0.08	0.03		
3	0.11	0.03				
4	0.02					
Polymorphic						
1	0.50	0.63	0.73	0.80	0.86	0.90
2	0.55	0.72	0.84	0.94		
3	0.70	0.91				
4	0.91					
(b)						
Monomorphic						
1	0.40	0.48	0.55	0.63	0.70	0.78
2	0.56	0.68	0.80	0.92		
3	0.75	0.92				
4	0.94					
Polymorphic						
1	0.40	0.48	0.55	0.63	0.70	0.78
2	0.51	0.64	0.78	0.91		
3	0.68	0.89				
4	0.91					

$w = 0.75$. The frequency distribution of resampled R 's served to evaluate how probable are data sets that would have resulted in the falsification of the two scenarios (i.e. $R < 1$ or $R > 3$). The average value of resampled R 's ($1 \leq R \leq 3$) served as our bootstrap estimate.

While our approach is somewhat indirect, logistic and practical difficulties precluded direct behavioural observations within, for example, artificial enclosures. These include: (1) obtaining gravid females in the absence of a breeding colony; (2) providing females with enough pools of adequate size, in an area of ‘manageable’ size; (3) tracking individuals in the dark, even within small spatial confines; and (4) interpreting behaviour that is elicited by the artificial nature of the enclosures (e.g. females ‘resting’ on the sides of the enclosure), etc.

RESULTS

Overall, 99 egg rafts were oviposited in our experimental array. Over the 13 nights in which predator incidence was

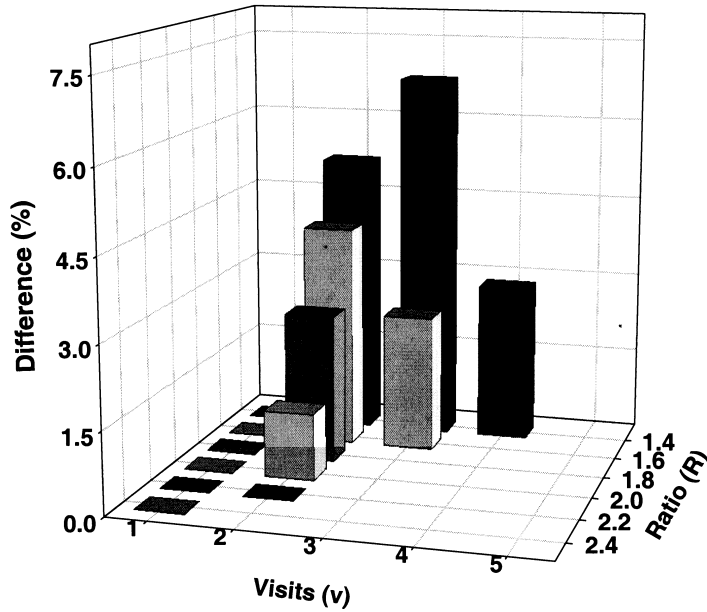


Figure 1 The difference between the two scenarios in their prediction of female use of predator-free pools (C), expressed as a percentage of all ovipositing females. Differences were calculated for various combinations of v (the number of visits to the array) and R (the ratio of the daily average oviposition rates), and predator incidence of 0.75. In cases where a difference exists, the proportional use of P-pools predicted by the monomorphic scenario exceeds that predicted by the polymorphic scenario. Empty cells correspond with combinations of R and v that would produce nonsensical values of S and m .

biased in favour of predator pools ($w = 0.75$), 35 of the 42 egg rafts collected were found in predator-free pools, i.e. observed $C = 0.83$. Over the 9 days in which predator incidence was biased in favour of predator-free pools ($w = 0.25$), all of the 57 egg rafts collected were found in predator-free pools, i.e. observed $C = 1.00$. The daily average oviposition rate (i.e. the total number of egg rafts collected under each treatment, divided by the number of nights it was offered) was 3.2 ± 2.2 and 6.3 ± 2.5 , respectively (two sample t -test for the comparison of means: $t = 2.76$, $P_{2 \text{ tail}} = 0.012$).

Outright rejection of both scenarios ($R \geq 3$ or $R \leq 1$) occurred in only 3% of the resampled data sets (Fig. 2). With the bootstrap R equalling 1.84 (± 0.4), both scenarios are plausible, assuming that individual females make no more than two visits within the array (Table 1). With these values of R and v , our ability to distinguish between the two scenarios is somewhat low (Fig. 1), but possible; as is shown below.

The polymorphic scenario

Solving for S (eqn 2) with the number of visits (v) set at 1 and 2 yields 0.74 and 0.86 as the frequency of the selective morph, respectively. Values of v greater than 2 would produce values of S greater than 1 (Table 1). Substituting these values of S in eqn 1 gives the proportion of females expected to oviposit in P-pools: for $v = 1$: $C_{w=0.75} = 0.57$ and $C_{w=0.25} = 0.92$. For $v = 2$: $C_{w=0.75} = 0.80$ and $C_{w=0.25} = 0.96$, which is closer to the observed values. The likelihood (L) of the experimental results with $v = 2$ is given by eqn 5:

$$L(35, 42|_{C=0.80}) \times L(57, 57|_{C=0.96}) = 0.017$$

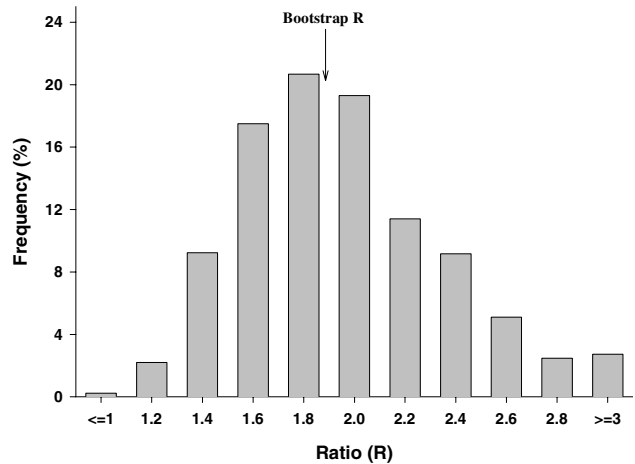


Figure 2 The frequency distribution of the ratio of daily average oviposition rates ($R = E_{w=0.25}/E_{w=0.75}$), calculated for 500 resampled data sets. The mean resampled ratio – ‘bootstrap R ’ – was used to calculate the proportional use of predator-free pools by ovipositing *Culiseta longiarvolata*, expected under each of the competing scenarios. Only 3% of the resampled data sets would have resulted in the rejections of the both scenarios ($R \geq 3$ or $R \leq 1$).

The monomorphic scenario

Solving for m (eqn 4) with $v = 2$, yields $m = 0.07$ which, when substituted in eqn 3, gives the proportion of females expected to oviposit in P-pools: $C_{w=0.75} = 0.81$ and $C_{w=0.25} = 0.98$.

The likelihood of the experimental results under this scenario is:

$$L(35, 42|_{C=0.81}) \times L(57, 57|_{C=0.98}) = 0.035.$$

Comparing the likelihoods of the two scenarios shows that the monomorphic scenario is approximately twice as likely as the polymorphic scenario. Were we to conduct the above analysis using the observed value of R (1.97), we would have found the former 1.3 times as likely as the latter. In general, because the proportional use of predator-free pools (C) predicted by the monomorphic scenario is consistently higher than that predicted by the polymorphic (Table 1b), the former will consistently offer the more likely explanation of any observed values of C that exceed the expected.

DISCUSSION

One of the main goals of evolutionary biology is an understanding of the causes of phenotypic variation; variation that may be realized at both the population and the individual level. Adaptive variation in behaviour may be due to heterogeneity in conditions (of the environment and/or of the individual), or it may result from mixed evolutionary stable strategies (ESS) played under conditions that are identical for all individuals. Mixed ESS, in turn, can be achieved in one of two ways (Parker 1984): first, through genetic polymorphism, with genotype frequencies corresponding to the ESS strategy frequencies; second, through genetic monomorphism with individuals playing strategies at random, but at ESS probabilities.

A number of recent studies have shown that in some insect species, oviposition habitat selection in response to predation does not result in complete predator avoidance (Blaustein *et al.* 1995; Grostal & Dicke 1999; Stav *et al.* 1999; Resetarits 2001). In *C. longiareolata*, to the extent that the observed variation in oviposition behaviour is adaptive (Spencer *et al.* 2002), our findings would suggest that it is driven by a mixed strategy, played by all females.

The evolutionary advantage of monomorphic mixed ESS's, over polymorphic ones, is explained, theoretically, by negative frequency-dependence. In a finite population, random fluctuations away from the ESS frequency of pure strategists (i.e. in a polymorphic scenario) would lead to a mixed strategist having a higher fitness than the population mean, facilitating its ultimate fixation (Hines 1987; Bergstorm & Godfrey-Smith 1998). Conversely, when the ESS frequency of one pure strategist is low, demographic and environmental stochasticity may cause its extinction, leaving an otherwise selectively neutral mixed strategist with an advantage over the remaining pure strategist (Vickery 1988; Bergstorm & Godfrey-Smith 1998; but see Maynard-Smith 1988).

Our monomorphic scenario, however, is equally compatible with maladaptive behavioural variation, whereby all

females play a pure strategy of predator avoidance but, on occasion, fail in its detection. Indeed, Spencer *et al.* (2002) have shown that given the current estimates of life-history parameters for *C. longiareolata*, females should (on game-theoretical grounds) show complete avoidance of predator pools. Hence, in conclusion, we suggest that to the extent that OHS in *C. longiareolata* is an adaptive response to the trade-off between the risk of predation and negative density-dependent effects, all females follow a single, simple behavioural 'decision rule' that is responsible for the lack of complete predator avoidance. Otherwise, the observed variation in behaviour may well be random, but potentially selectively neutral.

APPENDIX

Using artificial pools, arranged in an array spanning $c. 3 \times 8$ m, we presented wild *C. longiareolata* females with a choice between two of three treatments: predator-present (P), predator-free with low conspecific larval density (L), predator-free with high larval density (H). As expected, the majority of females oviposited in low-density pools rather than in the predator or high-density alternatives. However, we found no evidence to suggest that females increase their use of predator pools when faced with the high-, rather than the low-density alternative. In fact, we recorded a significant drop in overall oviposition rates during nights offering the P-H combination, as compared with nights offering the P-L combination. It would seem therefore that, at the level of the individual female, the response to predators and larval densities are independent of each other; at least at the spatial scale we examined.

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