Incorporating density dependence into the oviposition preference–offspring performance hypothesis

Alicia M. Ellis*
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

Summary

1. Although theory predicts a positive relationship between oviposition preferences and the developmental performance of offspring, the strength of this relationship may depend not only on breeding site quality, but also on the complex interactions between environmental heterogeneity and density-dependent processes. Environmental heterogeneity may not only alter the strength of density dependence, but may also fundamentally alter density-dependent relationships and the preference–performance relationship.

2. Here I present results from a series of field experiments testing the effects of environmental heterogeneity and density-dependent feedback on offspring performance in tree-hole mosquitoes. Specifically, I asked: (i) how do oviposition activity, patterns of colonization and larval density differ among habitats and among oviposition sites with different resources; and (ii) how is performance influenced by the density of conspecifics, the type of resource in the oviposition site, and the type of habitat in which the oviposition site is located?

3. Performance did not differ among habitats at low offspring densities, but was higher in deciduous forest habitats than in evergreen forest habitats at high densities. Oviposition activity and larval densities were also higher in deciduous forests, suggesting a weak preference for these habitats.

4. The observed divergence of fitness among habitats with increasing density may select for consistent but weak preferences for deciduous habitats if regional abundances vary temporally. This would generate a negative preference–performance relationship when population densities are low, but a positive relationship when population densities are high.

5. This study demonstrates that failure to recognize that fitness differences among habitats may themselves be density-dependent may bias our assumptions about the ecological and evolutionary processes determining oviposition preferences in natural systems.

Key-words: feedback, habitat selection, ideal free distribution, mosquito, Ochlerotatus triseriatus, spatial scale

Introduction

Fretwell & Lucas’s (1970) ideal free distribution (IFD) theory has served as an important foundation for several related theories in ecology, one of which is the oviposition preference–offspring performance (P–P) hypothesis (Rausher 1983; Schriber 1983; Thompson 1988; Valladares & Lawton 1991; Nuñio & Papaj 2004). The IFD predicts how organisms should distribute themselves in a heterogeneous environment (Fretwell & Lucas 1970). It assumes that habitat suitability decreases with increasing densities of organisms (density dependence in all habitats), but that for a given density, habitats differ in their suitability (Fig. 1a). It then predicts that if organisms have perfect knowledge of their environment and behave ideally, then they should distribute themselves so that the average fitness is approximately equal in all habitats. The P–P hypothesis similarly predicts how ovipositing females should distribute their offspring in a heterogeneous environment (Rausher 1983; Schriber 1983; Thompson 1988; Valladares & Lawton 1991; Nuñio & Papaj 2004). Specifically, it predicts that when insects utilize discrete hosts or environments, and have juveniles that have limited ability to disperse, females should evolve oviposition behaviours that maximize larval growth and survival. Originally, the P–P hypothesis was formulated to explain relationships for phytophagous insects, which were not generally believed to reach densities high enough for offspring to experience competition (Jaenike 1990). For this scenario, fitness was assumed to be highest in one habitat
across all offspring densities (Fig. 1b), which would generate strong selection pressures and the evolution of oviposition specificity for a particular host or habitat.

However, more recent experimental evidence has demonstrated that the offspring of some phytophagous insects (reviewed by Jaenike 1990; Digweed 2006) and many other ovipositing organisms (e.g. mosquitoes, Livdahl 1982; Carpenter 1983; frogs, Murray 1990; dragonflies, Vanbuskirk 1993; Drosophila, Jones et al. 1996) can experience density-dependent competition. This has important implications for the relationship between the oviposition preferences of adults and the performance of their offspring. If females can respond to offspring densities in addition to resources, then a positive preference–performance relationship may result. However, if oviposition choices are not based on offspring density, a mismatch between preference and performance may occur.

Not only can density dependence influence the relationship between preference and performance, but the specific manner in which density dependence is manifest in each habitat (e.g. nonlinearity), and the relative importance of density dependence in determining offspring performance, could influence the evolution of oviposition preferences.

While many studies have examined density-dependent effects in the larvae of ovipositing organisms (Livdahl 1982; Carpenter 1983; Murray 1990; Vanbuskirk 1993; Jones et al. 1996), most have been conducted in controlled environments to isolate the effects of density dependence from the effects of environmental heterogeneity (Stiling 1980; Livdahl 1982; Grimaldi & Jaenike 1984; Edgerly & Livdahl 1992; but see Loman 2004; Digweed 2006). This is despite the fact that environmental heterogeneity not only may alter the strength of density dependence, but also may fundamentally alter density-dependent relationships and/or the importance of density dependence in determining population dynamics (Pimm & Redfearn 1988). Studies testing the effect of density dependence in the context of environmental heterogeneity may therefore have important consequences for our ability to understand relationships between life-history traits, such as oviposition preference and offspring performance, and to predict evolutionary processes generating habitat or oviposition preferences.

Mosquitoes that oviposit in water-filled tree holes are an ideal organism for testing the combined roles of density-dependent feedback and heterogeneity in determining the preference–performance relationship. Tree-hole mosquitoes appear to exhibit both habitat selection (Service 1971; Lounibos 1981; Beier, Berry & Craig 1982; Barker et al. 2003a, 2003b; Ellis 2007) and oviposition site selection (Edgerly et al. 1998; Kitron, Webb & Novak 1989), and often experience intense density-dependent competition for resources in the larval stage (Fish & Carpenter 1982; Livdahl 1982; Edgerly & Livdahl 1992; Lounibos, Nishimura & Escher 1993). Moreover, studies have demonstrated that the strength of density-dependent competition among larvae may be influenced by environmental factors including the quantity (Livdahl 1982; Clements 1992) and quality (Lounibos et al. 1993; Yanoviak 1999) of leaf litter resources in the aquatic habitat. Finally, tree-hole mosquito systems are logistically tractable (Kitching 2000) and can be manipulated to test the influence of oviposition, density dependence and environmental factors on offspring performance in natural populations.

Here I explore the combined effects of offspring density and environmental heterogeneity on the performance of the eastern tree-hole mosquito, *Ochlerotatus triseriatus* (Say) (formerly *Aedes triseriatus*, see Reinert 2000). *Ochlerotatus*
O. triseriatus is one of the most common species throughout the USA (Means 1979), and is the most abundant tree-hole mosquito in the vicinity of Hanover, New Hampshire, where this study was conducted. Ochlerotatus triseriatus oviposits in water-filled tree holes and artificial containers, and has larvae that are filter-feeders and browsers of leaf detritus and microbes (Means 1979). In New Hampshire, it is primarily univoltine and experiences little or no predation in the larval stage (Barrera 1988).

Previous studies with O. triseriatus have demonstrated that larvae experience intense density-dependent competition for resources, including leaf detritus (Kitching 2000), and that adults may exhibit habitat selection (Sinsko & Craig 1979; Beier et al. 1982; Nasci 1982; Ellis 2007) and oviposition preferences (Wilton 1968; Bradshaw & Holzapfel 1988; Edgerly et al. 1998). Specifically, adults tend to avoid open habitats (Nasci 1982; Ellis 2007) and are often found in forested areas with dense understorey vegetation (Service 1971; Beier et al. 1982; Ellis 2007; Ellis 2008). They may select oviposition sites based on physical characteristics (Wilton 1968; Bradshaw & Holzapfel 1988), chemical cues (Wilton 1968; McDaniel et al. 1976) or conspecific density (Edgerly et al. 1998). Research has also demonstrated that the quality (Lounibos et al. 1993; Yanoviak 1999) and quantity (Livdahl 1982; Clements 1992) of leaf litter resources may influence the strength of density dependence in the larval stage. Maple leaves, for example, tend to reduce competition and generate higher mosquito growth than beech or oak, which decay much more slowly (Fish & Carpenter 1982; Barrera 1988; Yee & Juliano 2006).

Although studies have suggested that O. triseriatus prefers forest habitats with a dense understorey (Service 1971; Beier et al. 1982; Clements 1992; Ellis 2007; Ellis 2008), most have not adequately demonstrated differences in performance between habitats that may generate the selective pressures necessary for the evolution of these behaviours. Moreover, although several researchers have tested the effects of resource type (litter type) in the larval habitat on density-dependent relationships and offspring performance (Fish et al. 1982; Lounibos et al. 1993; Dieng et al. 2002), few have tested the combined effects of litter type in the oviposition site and regional habitat characteristics on the dynamics of natural populations.

The purpose of this study was to examine the combined effects of density, habitat and litter type on the performance of O. triseriatus. The design was based on a previous study demonstrating that O. triseriatus tends to move to, and oviposit more often in, forest habitats that have dense understorey vegetation (<1 m) (Ellis 2008). Building on this research, I sought to evaluate differences in performance among deciduous forest stands with dense understorey vegetation, and evergreen forest stands with very little understorey vegetation. Because the type of leaf litter resource in the larval habitat (litter type) may influence offspring performance (Fish et al. 1982; Lounibos et al. 1993; Dieng et al. 2002), and may differ among deciduous and evergreen habitats, I also tested the influence of leaf litter on performance to tease apart effects of regional habitat characteristics from the effects of leaf litter type. The specific objectives were to: (1) establish patterns of oviposition and colonization in relation to resource type (pine needles, maple leaves) and habitat type (deciduous forests, evergreen forests); (2) compare offspring performance among habitats (deciduous forests, evergreen forests) and among oviposition sites with different resources (white pine needles, maple leaves); and (3) test the interactive effects of density, resource type and habitat on performance and population growth. Results were then used to provide information on the potential roles of density dependence and environmental heterogeneity in determining the preference–performance relationship and the evolution of oviposition preferences.

Methods

Three studies were conducted. An observational study was used to determine patterns of oviposition among habitats and between oviposition sites with different types of leaf litter. Patterns of colonization and larval density among habitats and oviposition sites with different resources were then monitored. Results from this second study were used to establish the natural range of larval densities generated by oviposition in the field. This information was used to determine treatment levels for the final study, which was an experiment examining the effects of larval density, habitat and resource type on performance.

FIELD SITES

The studies were performed in two regions, Trescott Hill and Boston Lot (17 km apart), around Hanover, NH, USA. Each had two adjacent habitat types: evergreen forest stand with little understorey vegetation, and deciduous forest stand with dense understorey vegetation of approximately equal size (0.006 km²). Forest habitats in both regions were similar in size, dominated by beech (Fagus grandifolia), maple (Acer saccharum), oak (Quercus rubra), pine (Pinus strobus) and hemlock (Tsuga canadensis), and had similar amounts of human usage. Containers simulating tree holes were placed within a ~0.02-km² area of each region that, according to previous studies, encompasses an area in which dispersal is not limiting for O. triseriatus (Beier et al. 1982; Ellis 2008).

OVIPOSITION

Patterns of oviposition were examined in 2004 using artificial oviposition traps deployed in a nested experimental design at Trescott Hill with two habitats (deciduous forest, evergreen forest) and 10 replicates of each of two litter types (maple leaves, pine needles) within each habitat (40 oviposition traps). Oviposition traps were constructed from black plastic cups (~473 ml, Plum Party, Long Island City, NY, USA) that were lined with brown paper towels (Georgia-Pacific, Envision multifold one-ply hand towels, Atlanta, GA, USA). Two overflow holes in each trap prevented water volumes greater than 150 ml.

To examine the effects of leaf litter on performance, I selected the most dominant tree species in each of the focal habitats (sugar maple and white pine). Equal weights of dried maple leaves (Acer saccharum) from the deciduous habitats in each region were pooled, mixed well and crushed by hand (Srivastava & Lawton 1998; Daugherty & Juliano 2002). The same procedure was performed with white pine (Pinus strobus) needles from the evergreen stands in both regions. Containers received ~1.1 g maple leaves or pine needles and 150 ml water (maintained throughout the experiment), consistent with amounts quantified in natural tree holes (Kitching 1983).
Oviposition traps were placed on trees along a transect through the forested habitats. Adjacent traps were >50–70 m apart in all habitats. Two traps, one for each litter type, were placed on each tree >1.5 m above ground with traps directly next to each other on a randomly selected compass orientation. This height is consistent with the height at which larvae of *O. triseriatus* have been found in oviposition traps in other studies (Scholl & DeFoliart 1977; Sinsko & Grimstad 1977). Oviposition traps were filled with 150 ml tap water and sampled once every week for 9 weeks, from 20 June to 30 August 2004, by removing the paper towels and inspecting traps for loose eggs. Fresh towels were inserted immediately, and the eggs oviposited on towels were counted in the laboratory using a dissecting microscope.

Data were analysed using repeated measures ANOVA in PROC GLM in SAS ver. 9.1 (SAS Institute, Inc., Cary, NC, USA) to test for the effects of habitat, tree within habitat, litter, and habitat × litter interaction on the number of eggs laid per trap. No data transformations were needed to meet assumptions.

**SPATIOTEMPORAL PATTERN OF LARVAL DENSITY**

Colonization dynamics and spatiotemporal patterns in larval density were examined in 2003 and 2004. Artificial containers were deployed in a nested experimental design. At each site, which represents a block in the design, two habitats were delimited: deciduous forest and evergreen forest. Habitat plots were adjacent to one another. Ten replicates of each litter type were established within each habitat in Trescott Hill (40 containers) and five replicates in each habitat in Boston Lot (20 containers). This study was continued through 2004 to include dynamics of overwintering mosquitoes, but only five of the original 10 replicates of each litter type in each habitat were continued at the Trescott Hill site (the other five replicates were used for a separate study). Containers were also placed in adjacent open-field habitats in each region; however, consistent with previous studies (Nasci 1982; Barker *et al*. 2003), very few mosquitoes oviposited in them (no eggs were laid in oviposition traps, and few larvae were observed in water-filled containers located in open fields; Ellis 2007). The open-field treatment was therefore removed from the study.

Black plastic containers (diameter =15 cm, depth =10 cm, volume =1.8 l) were used to simulate tree holes and examine larval dynamics (Service 1993). A small overflow hole was cut into the container to prevent volumes greater than =1.5 l. Containers were covered with 6-mm mesh plastic netting (US Netting Co., Erie, PA, USA) to exclude large organisms and additional detritus. Leaf litter was collected as described above and added at the same water: litter ratio (11 g l⁻¹). No leaf litter was added between the 2003 and 2004 field seasons.

Containers were placed on trees along a transect through the forested habitats with adjacent containers 50–70 m apart. Two containers, one for each litter type, were placed on each tree >1.5 m above ground. Containers were placed directly opposite each other on the tree with a randomly selected compass orientation (e.g. one on the north side, the other on the south side), and filled with 1 l tap water (maintained at =1 l throughout the experiment).

The containers were examined with replacement once every 4 weeks from June 2003 to October 2003, and from May 2004 to August 2004 (five sampling periods year⁻¹). The contents from each container were placed in sterile plastic containers and taken to the laboratory for analysis. In the laboratory, all second-, third- and fourth-instar larvae and pupae were counted, and the larvae were identified to genus. First-instar larvae cannot easily be detected with the naked eye, and extraction procedures to quantify them accurately require passing samples through sieves and rinsing them with water (Lounibos 1983). Analyses were restricted to later instars to reduce the potential mortality due to these disturbances. Samples were returned to their respective containers within 24 h. On the last sampling date in 2004, all third- and fourth-instar larvae were collected, preserved in ethanol, and identified to species to distinguish *O. triseriatus* from *Ochlerotatus japonicus* (Theobald), a recent invader at low abundance during this experiment (Darsie & Ward 2005).

Data were analysed using repeated measures ANOVA in PROC GLM in SAS ver. 9.1 to test for the effects of region, habitat, tree within habitat, litter, and habitat × litter interaction on mosquito density. Density of larvae (number per container) was ln(X + 1)-transformed to meet assumptions of homoscedasticity and normality, and data were examined separately for each year to compare colonization dynamics with the dynamics of diapausing individuals the following year.

**PERFORMANCE**

This study was a factorial field experiment to test the effects of density-dependent feedback and heterogeneity on performance. Specifically, it tested the influence of habitat type (deciduous forest, evergreen forest), litter type (maple leaves, pine needles), and larval density (low, medium, high) on larval survival, larval development time and adult fecundity in midsummer 2004. Density treatments (low, 40; medium, 140; high, 320 larvae per container) were based on densities observed in 2004 in the study described above, where containers were colonized by the natural population. The low-density treatment was selected to reflect the lower densities of larvae observed, but to be high enough that possible mortality due to the transportation of first-instar larvae would not remove the entire cohort from containers. The medium-density treatment was selected to reflect the mean number of larvae observed in naturally colonized containers. The high-density treatment was selected to reflect the highest densities of larvae observed (although, because of low hatching rates, this level was not the absolute highest density observed).

Four trees along a transect within each habitat were selected with adjacent trees 50–70 m apart. Six containers were placed on each tree, one for each litter × density treatment. Litter × density treatments were blocked by tree within each habitat to control for the effects of individual trees on larval development. All tree holes receive rainwater inputs either directly from the canopy (leaves) or via stem flow (water travelling down the trunk of the tree). Studies have demonstrated that this water can bring a variety of different nutrients into the tree hole and can influence larval development (Carpenter 1982; Walker *et al*. 1991; Kaufman *et al*. 1999). Because the nutrient input may vary from tree to tree, litter × density treatments were blocked within each habitat by tree, resulting in six containers (one for each litter × density treatment) per tree. Containers were constructed as described above, covered with a 1-mm mesh netting to prevent oviposition from adult mosquitoes in the field, hung on selected trees in a random compass orientation, and filled with water (1 l) and leaves (11 g l⁻¹) 2 weeks prior to addition of larvae.

First-instar *O. triseriatus* larvae were hatched from eggs collected from laboratory colonies and added to the containers in densities defined by the treatment on 1 August 2004. Laboratory colonies were established in April 2004 from *O. triseriatus* larvae collected from Trescott Hill and Boston Lot, and raised in standard laboratory conditions (22 °C, 80% humidity ± 10%; 16 : 8 day : night light regime; Munstermann & Wasmuth 1985). Females obtained blood meals from a guinea pig housed according to standard Institutional Animal Care and Use Committee protocol. Because there were not
enough larvae to establish treatments, additional larvae were obtained from a laboratory colony at Michigan State University (MSU; donated by W. Morgan): \(1%\) of the individuals used in this experiment came from the MSU colonies. Mosquitoes from the MSU colonies were raised in similar laboratory conditions and were genetically related to the species in our study area (Munstermann 1985). Just prior to the experiment, eggs were simultaneously hatched by immersing them in a protozoa medium made by dissolving one ‘protozoan pellet’ (Carolina Biological Supply Co., Burlington, NC) in 1·5 l water. First-instar larvae were transported immediately to the field containers.

Containers were sampled every other day until all larvae pupated (a total of 53 days). On each sampling date, the contents of each container were emptied into a white plastic tray, the pupae present in the container were removed, and the larvae and other contents were replaced. Pupae were taken to the laboratory and incubated at 22 °C with a 16 : 8 day : night light regime until adults emerged. After adults emerged, they were dried at 80 °C for 7 days, sexed and weighed (Cahn C-35 microbalance, Thermo Fisher Scientific, Waltham, MA).

Three measures of performance were recorded. Survivorship in each container was calculated as the proportion of the original cohort that survived to the pupal stage (includes males + females). Development time was defined as the time from the start of the experiment until pupation for each surviving individual. Female mass was used as a surrogate of fecundity, as mass is linearly related to fecundity (Livdahl 1982).

Proportion surviving, female development time and adult female mass were also combined into an overall per capita population growth rate for each container (Livdahl 1982). Per capita growth rate was calculated as:

\[
r' = \frac{\ln \left( \frac{1}{N_0} \sum x \hat{w}_i A_i f(\hat{w}_i) \right)}{D + \left( \sum x \hat{w}_i A_i f(\hat{w}_i) \right) \left( \sum x \hat{w}_i A_i f(\hat{w}_i) \right)}
\]

where \(x\) is a given day, \(\hat{w}_i\) is the average mass of females emerging on that day, \(A_i\) is the number of females emerging on day \(x\), and \(N_0\) is the initial number of female larvae in the cohort (assuming a 1 : 1 male-to-female ratio) (Livdahl 1982). \(D\) is a constant determined by the delay between adult emergence and oviposition, estimated as 7 days (Livdahl 1982). \(f(\hat{w}_i)\) is a function converting female biomass into the expected per capita number of female offspring, and is calculated using the formula (Livdahl 1982):

\[
f(\hat{w}_i) = 7.13 + 45.85\hat{w}_i.
\]

Although several studies have used equation 1 to examine population growth in mosquitoes, it may not always be appropriate because it assumes completely overlapping generations. Many mosquito populations, including that examined here, are primarily univoltine, with largely non-overlapping cohorts present in the population at any one time. Thus accounting for development time in a measure of population growth rate or fitness gives a false impression. Similarly, the equation assumes that populations are at a stable age distribution; but because of the seasonal dynamics of some mosquito populations this assumption is often violated (Strand et al. 1999). For this reason, reproductive potential (female eggs in generation \(t + 1\) per female egg in generation \(t\)) for each container was also calculated as the product of survival probability and fecundity given survival (according to Strand et al. 1999). As above, fecundity (the expected per capita number of female offspring) was calculated using equation 2, where \(\hat{w}_i\) is the average mass of females per container (Livdahl 1982).

This formula for reproductive potential also assumes a 1 : 1 male-to-female ratio.

Multivariate anova in PROC GLM in SAS ver. 9·1 was used to test for the effects of habitat (deciduous, evergreen); litter (maple, pine); density (40, 140, 320 larvae per container); and all two- and three-way interactions on average female development time, average female mass, and proportion surviving. Female development time and mass were analysed here because female demographics determine population dynamics in this system (Livdahl 1982). Development time and mass were in-transformed to meet assumptions of homoscedasticity and normality. To dissect the overall responses, I performed univariate anovas on each of these components of fitness in SAS ver. 9·1 to test the effects of habitat, tree within habitat, litter, density, and all two- and three-way interactions among habitat, litter, and density. Univariate anovas were similarly performed for per capita growth rate and reproductive potential. For all anovas, tree within habitat was considered a random effect, while the remaining variables were considered fixed effects.

Results

Oviposition

Females laid approximately twice as many eggs in containers in the deciduous habitat than in the evergreen habitat (Fig. 2, \(F_{1,9} = 81·33, P < 0·0001\)). Oviposition was highest in late July and lowest at the beginning and end of the season (Fig. 2). Litter type and the habitat \(\times\) litter interaction had no statistically significant effect (\(P > 0·8\) for both).

Spatiotemporal pattern of larval density

The most abundant tree-hole mosquito species in both regions was \(O.\) triseriatus. In 2003, \(O.\) japonicus, \(Culex\) pipiens L. and Anopheles barbieri Coquillett were also found in containers at very low abundances. \(Culex\) pipiens and \(A.\) barbieri were each found in one container in 2003 and not at all in 2004. On the last sampling date in 2004, 1540 larvae were collected and identified to species. Of these, 99·7% were \(O.\) triseriatus and 0·3% \(O.\) japonicus (five in a container with \(O.\) triseriatus larvae and maple leaves located in the evergreen habitat at Trescott Hill).
Colonization was slow in 2003, and larvae did not appear in containers until July (Fig. 3a). In 2004, much higher densities than in 2003 were observed in all containers due to overwintering eggs (Fig. 3b). In both years, densities were higher in deciduous than in evergreen forests (habitats in 2003, \( F_{2,18} = 4.74, P < 0.05 \); habitat in 2004, \( F_{1,15} = 7.81, P < 0.01 \)). There was no effect of tree, litter type, or habitat × litter interaction in either year (\( P > 0.12 \) for all effects), although densities were slightly higher in containers with maple leaves than in containers with pine needles.

**PERFORMANCE**

In the larval density experiment, MANOVA showed strong effects of a habitat × litter interaction (Wilks’ \( \lambda \) approximation: \( F_{2,34} = 7.88, P = 0.002 \)) and a strong main effect of larval density (\( F_{2,18} = 118.31, P < 0.0001 \)) on survivorship, female mass and larval development time.

Univariate ANOVAs showed a significant litter × density interaction (\( F_{2,10} = 9.62, P = 0.0006 \)) and a significant habitat × density interaction (\( F_{2,10} = 3.71, P = 0.04 \)) for female development time (Fig. 4a). In general, development time increased with density. At low densities, female development times were similar across all habitat and litter treatments, while at medium and high densities, female development time was shortest in containers with maple leaves that were located in deciduous forests. The mean female-to-male ratio of adults did not differ among treatments (\( P > 0.8 \) for all effects) and was not significantly different from 1:1 across all containers (\( P > 0.4 \)).

Female mass displayed a significant main effect of density (\( F_{2,16} = 764.88, P < 0.0001 \)) and a significant habitat × litter interaction (\( F_{1,16} = 15.42, P = 0.0005 \)) (Fig. 4b). The habitat × density interaction was marginally significant (\( F_{2,16} = 2.72, P = 0.08 \)). In general, mass decreased with increasing density. At low densities, mass was similar across all habitat and litter treatment levels. At medium and high densities, mass was highest in containers with maple leaves that were located in deciduous habitats.

The proportion of larvae surviving to the pupal stage decreased with increasing larval density (\( F_{1,29} = 19.65, P < 0.0001 \)), but was unaffected by the habitat (\( F_{1,29} = 0.08, P = 0.75 \)) or litter treatments (\( F_{1,29} = 0.04, P = 0.80 \)) (Fig. 4c). *Per capita* growth rate, a measure synthesizing larval survival, larval development time and adult mass, declined with increasing larval density (\( F_{2,29} = 46.16, P < 0.0001 \)), but did not differ among habitats (\( F_{1,29} = 2.70, P = 0.11 \)) or litter types (\( F_{2,29} = 2.41, P = 0.13 \)) (Fig. 4d). Reproductive potential, calculated as the product of probability of survival and average fecundity given survival, also declined with increasing larval density (\( F_{2,29} = 80.44, P < 0.0001 \)) (Fig. 4e), but did not differ among habitats (\( F_{1,29} = 0.04, P = 0.84 \)) or litter types (\( F_{1,29} = 0.07, P = 0.79 \)).

**Discussion**

The preference–performance hypothesis predicts that females should distribute their offspring among habitats to maximize fitness (Rausher 1983; Schriber 1983; Thompson 1988; Valladares et al. 1991; Nuñio et al. 2004). Here, *O. triseriatus* mosquitoes laid more eggs in deciduous forests than in adjacent evergreen forests. Although the movement of adult mosquitoes was not examined in the current study, results from a mark-release-recapture (MRR) study conducted in New Hampshire suggest that this pattern may be due to weak habitat preferences (Ellis 2008). In the MRR study, dispersal was not limiting at the scale at which differences in oviposition and larval densities were observed here (0.02 km² in 2 weeks), and females exhibited a weak preference for ovipositing in habitats with more understorey vegetation over habitats with little understorey vegetation. Other studies have also suggested that woodland mosquito species such as *O. triseriatus* are often found in dense ground vegetation, possibly because of the resources and resting sites it provides (Service 1971; Beier et al. 1982; Clements 1999). Because the deciduous forests in this study had more understorey vegetation than the evergreen forest habitats, it is possible that the higher oviposition activity and larval densities observed here were due to weak selective behaviours in response to understorey vegetation.

Based on the preference–performance hypothesis (Rausher 1983; Schriber 1983; Thompson 1988; Valladares et al. 1991; Nuñio et al. 2004), this weak oviposition preference should be an evolutionary consequence of fitness differences among habitats; a preference for ovipositing in deciduous forest habitats would evolve if offspring performance is higher in those habitats for a given density. Weak preferences, such as...
those observed here, would result if performance is not consistently higher in the preferred habitat over time, or if opposing selection pressures prevent fixation of the genes governing a particular habitat preference (Rausher 1984).

In accordance with these expectations, results were not consistent with large, distinct differences in performance between deciduous and evergreen habitats that would generate strong habitat preferences. At low densities, offspring performance did not differ among habitats or litter types, suggesting that in years when overall population size is small and larval densities are low, no fitness difference exists between deciduous and evergreen habitats. According to expectations of the IFD (Fretwell et al. 1970), females should distribute their offspring equally among deciduous and evergreen forests when population densities are low, to equalize expected fitness. As larval densities increase, however, development time was shortest in containers with maple leaves that were located in deciduous habitats for a given density (Fig. 1c). Although female mass was also highest in deciduous forests for a given density, differences were less pronounced. Nevertheless, these results suggest that as density increases, performance is increasingly higher in containers with maple leaves that are located in deciduous habitats. Because maple leaves decompose more quickly than pine needles (Delaney et al. 1996; Dieng et al. 2002), and faster-decaying litter enhances mosquito growth (Dieng et al. 2002), high-quality maple leaves may lessen the effects of competition and support higher larval densities than pine needles. Similarly, precipitation from deciduous canopies tends to be less acidic and higher in base cations than evergreen canopies (Pryor & Barthelmie 2005), which could stimulate microbial growth and generate higher larval densities (Kaufman et al. 1999). The effects of habitat and litter are, however, only evident when larval densities are high enough to draw resources down to limiting levels.

Although performance differed among treatments at high density for some measures of fitness, these effects were not as strong when individual measures of performance were combined into one overall measure of per capita population growth (Livdahl 1982). The strong density-dependent mortality in the larval stage appeared to mask the relatively smaller effects of habitat type and litter type on time to emergence and adult fecundity. Livdahl (1982) similarly found that the effects of environmental heterogeneity on independent measures of fitness (survivorship, development time, adult mass) were not detected by his composite measure of population growth. However, the effects of environmental heterogeneity on development time and fecundity at high density could have substantial impacts on fitness that are not
evident in this simple metric of population growth. For example, if there is a threshold size below which adults are not able to reproduce, then the effects of habitat and resource type on adult mass could be extremely important in determining fitness. Haramis (1985) found that smaller adult *O. triseriatus* have a lower probability of completing a single gonotrophic cycle in the field, possibly because lower mass reduces flight capability and the ability to find hosts.

Similarly, studies have demonstrated that voltinism (univoltine vs. bivoltine) can be determined primarily by differences in development time (Burke *et al*. 2005). Minor differences in development time generated by environmental differences among habitats may therefore have substantial effects on annual population growth by determining the number of generations that can be completed in a given period of time. Although voltinism may be important in determining population dynamics in some populations of tree-hole mosquito, *O. triseriatus* populations in the north-east, where this study was conducted, are primarily univoltine. Development time has less influence on overall population growth in these populations and, for this reason, measures of reproductive potential (calculated as the product of the survival probability and fecundity given survival) may be more appropriate (Strand *et al*. 1999), and any effects of habitat on population growth probably operate primarily through its effect on female mass and fecundity. Results also demonstrated that reproductive potential did not differ among habitats. This suggests that the effects of habitat variables on performance and population growth may be less pronounced in more northern populations of *O. triseriatus*.

Strong density dependence and the divergence of fitness among habitats with increasing density (Fig. 1c), such as that observed here, may have profound evolutionary consequences for the P–P relationship and the evolution of oviposition preferences. According to expectations of the IFD (Fretwell *et al*. 1970), if there is intense competition among larvae, and organisms behave ideally, ovipositing females should exhibit some degree of conspecific avoidance until all available oviposition sites are occupied. When oviposition sites become limiting, however, females should distribute offspring based on density and resources.

Here there was no difference in performance when densities were low, but as densities increased, performance was increasingly higher in deciduous forests (Fig. 1c). In this case, females should alter their search strategies as densities change. Edgerly *et al*. (1998) have demonstrated that *O. triseriatus* females may exhibit conspecific avoidance early in the season (which would match expectations based on intense density dependence), but conspecific attraction later in the season. While the authors attribute conspecific attraction behaviours to cues indicating habitat suitability for overwintering habitat, females could also be responding to interactive effects between high densities and environmental factors. Flexible oviposition strategies have been observed in several other organisms (e.g. galling sawfly, Craig, Itami & Price 1989; butterfly, Gibbs *et al*. 2005; frogs, Marsh & Borrell 2001; golden egg bug, Reguera & Gomendio 2002), but very few, if any, have examined changes in oviposition behaviour in response to density × environment interactions.

Results also suggest that if fitness differences are themselves density-dependent (Fig. 1c), and the regional abundance of organisms vary temporally, then the strength of selection for oviposition or habitat preference may also vary. Based on results of the current study, there would be relatively weak selection for an oviposition preference in tree-hole mosquitoes when population densities (and thus larval densities) were low, while at high population densities (and thus high larval densities), differences in performance would generate stronger selection for oviposition preferences. Temporal variability in the strength of selection for preferences due to, for example, environmentally generated differences in population density with time, could then generate consistent but weak preferences for deciduous habitats. If weak preferences were exhibited across all densities, the preference–performance relationship would be negative when population densities are relatively low, but positive when densities are high.

Nonlinear density dependence, and differences in fitness among habitats that are themselves density-dependent, may thus explain, in part, how different P–P relationships may be observed in the same species (e.g. butterflies, Bossart 2003). More broadly, differences in the strength and nature of density dependence experienced by different organisms could explain the conflicting results that sometimes support (e.g. butterflies, galling and leaf-mining insects, reviewed by Price 1994) and sometimes refute the P–P hypothesis (e.g. sawfly, Mayhew 2001; Digweed 2006; moths and butterflies, reviewed by Price 1994).

**Conclusions**

Originally the oviposition–offspring hypothesis assumed that density dependence was not operating (or at least was not important) (Jaenike 1990), and that fitness differences among habitats were consistent across densities (e.g. the IFD, Fretwell *et al*. 1970). The theory then predicted that selection pressures would result in oviposition specificity for the host or habitat that resulted in the highest performance for offspring across all densities. Results presented here demonstrate that these assumptions may be violated in natural systems, and that complex interactions between competition and environmental heterogeneity can generate a divergence in fitness among habitats with increasing offspring densities. This has profound implications for the nature of the P–P relationship and the evolution of oviposition preferences. Failure to recognize the interaction between offspring density and environmental heterogeneity may therefore limit our understanding of evolutionary processes influencing life-history traits and behaviour.

**Acknowledgements**

This research was supported by NSF Grant DEB-0209736 to Mark A. McPeek and an endowed award by The R. Melville Cramer 1877 Foundation. Mark McPeek, Kathy Cottingham, Matt Ayres, Doug Bolger, Marcel Holyoak and an endowment awarded by The R. Melville Cramer 1877 Foundation. Mark McPeek, Kathy Cottingham, Matt Ayres, Doug Bolger, Marcel Holyoak and two anonymous reviewers made helpful comments on the manuscript, and Bill Morgan donated several hundred eggs. Thanks to Cayelan Carey for her field and lab work, and to Thomas Morrison for his help in the field.
References


Barrera, R. (1988) Multiple Factors and their Interactions on Structuring the Community of Aquatic Insects of Treeholes. Pennsylvania State University, University Park, PA, USA.


and species. *Journal of the American Mosquito Control Association, 16*, 175–188.


Received 17 August 2007, accepted 6 November 2007

Handling Editor: Rolf Ims