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POPULATION ECOLOGY – ORIGINAL RESEARCH

Spatial aggregation of aquatic habitats affects oviposition patterns in *Aedes* mosquitoes

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Abstract

Colonization, including oviposition, is an important driver of population and community dynamics both within and across habitat patches. Most research has focused on the roles of habitat availability or quality on colonization and its outcomes. However, the spatial distribution of habitats also likely affects these processes. We conducted field experiments in Georgia, USA, using clustered and dispersed arrays of equal numbers of oviposition patches to investigate how patch aggregation influenced oviposition by *Aedes* mosquitoes. We tested the effects of aggregation on: (1) the total number of eggs an array received, (2) the proportion of patches within an array that received eggs, and (3) the number of eggs per colonized patch. We compared results to predictions from three models (Field of Dreams, Propagule Redirection, and Excess Attraction), which vary in the degree to which arrays attract colonists and apportion those colonists among patches. Clustered arrays received 22% more eggs than dispersed arrays, with clustered patches significantly more likely to receive eggs. At the species level, *A. albopictus* responded more to clustering than did *A. triseriatus.* These results are inconsistent with Propagule Redirection, but support the Excess Attraction and Field of Dreams models. Although clustered arrays occupied a relatively small area, they attracted at least as many ovipositing mosquitoes as did dispersed arrays. However, the number of eggs per colonized patch did not differ between clustered and dispersed arrays. Therefore, density dependence among larvae, and hence the production of adult mosquitoes on a per-patch basis, should be similar in dispersed and clustered landscapes.

Keywords Colonization · Density dependence · Habitat availability · Meta-populations

Introduction

Colonization of habitat patches is a critical driver of population dynamics and community structure within individual patches (Binckley and Resetarits 2005; Vonesh et al. 2009) and across collections of patches (Hanski 1998; Loreau and Mouquet 1999; Mouquet and Loreau 2003). Colonization is influenced by a variety of factors intrinsic to the patch, such as resource availability (Fader and Juliano 2014), predator density (Rieger et al. 2004; Dussault et al. 2005), and competitor density (Keen 1982), all of which likely contribute to patch quality. However, external conditions, such as the composition of the inter-patch matrix (Baum et al. 2004; Bender and Fahrig 2005) and the quality of neighboring patches (Resetarits and Silberbush 2016), also can influence the colonization of a focal patch.

Habitat availability (i.e., the number or density of patches) is another extrinsic factor that can drive patch-level colonization, although its effects appear to vary among systems. For example, Stier and Osenberg (2010) found that larval fish arriving in a reef environment after a pelagic larval stage had lower settlement rates on corals that were surrounded by other corals (high patch density) compared to isolated corals (low density). Their results indicated that the high-density corals competed with one another for a relatively fixed number of larvae, which distributed themselves across the available coral patches. In other words, when more corals were available, larvae were divided among them, resulting in fewer larvae per individual coral.

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However, although Stier and Osenberg (2010) observed lower densities of fish per coral in the experimental arrays with multiple corals, these arrays of coral habitat received slightly greater total settlement than single isolated corals, suggesting that the local pool of available larvae increased with the addition of habitat, although much less than expected given the amount of added habitat. A more extreme example of this latter pattern occurred in studies of the colonization of small ponds by aquatic beetles. As in the fish-coral study, these ponds were previously unoccupied, so potential colonists were coming from outside the experimental system. When habitat quality was similar among experimental ponds, beetles colonized individual ponds at a constant rate, regardless of whether there were one or four ponds in the local neighborhood (Resetarits and Binckley 2009; Resetarits and Binckley 2014). Thus, in contrast to the results for reef fish and corals, increasing the number of ponds in a local area yielded a proportional increase in the number of beetles colonizing that area. In yet another study in an oyster reef system, Keller et al. (2017) found results that depended on the species being considered and its life stage; some results fit the reef fish pattern and other results resembled the beetle pattern. Together, these studies illustrate that different colonization patterns can occur in different systems, with the drivers of these patterns likely related to the strength of signals produced by patches (which would likely increase with an increase in the number of patches in a local area), signal detection capabilities of potential colonists, and mobility of those colonists (e.g., Hamman et al. 2018).

These results can be viewed with respect to two competing processes: (1) how a region containing habitat patches attracts potential colonists; and (2) how those potential colonists get divided up amongst the available patches in that region. The Propagule Redirection Hypothesis (Osenberg et al. 2002; Hamman et al. 2018) asserts that a collection of patches in a local area attracts no more total colonists than an isolated patch, and as a result, a constant number of colonists get distributed across the patches, resulting in proportionately fewer colonists per patch. In contrast, if an area with more patches attracts proportionately more colonists, and those individuals then get evenly distributed among patches, the number of colonists per patch will be the same for a single isolated patch or a cluster of many patches. This scenario has been called the Field of Dreams Hypothesis (Palmer et al. 1997; see also Stier and Osenberg 2010; Keller et al. 2017; Hamman et al. 2018 for further elaborations), and results in a proportionate increase in colonists in areas with more patches. A more extreme version of Field of Dreams could arise if areas with multiple patches attracted a disproportionately large number of colonists compared to a single patch, and thus the number of colonists per patch would increase with patch number. We refer to this scenario as the Excess Attraction Hypothesis.

Originally, these hypotheses were formulated with respect to the effects of habitat abundance (e.g., areas with high vs. low numbers of available habitat patches). However, they can be extended to consider the effects of the spatial distribution of patches (e.g., dispersed vs. clustered), keeping the regional abundance of patches constant, but changing the local density. Thus, an array of highly dispersed patches could be viewed as having low local density, with each patch behaving like a single isolated patch in the original formulations of the hypotheses.

Although disentangling all the factors driving colonization patterns in a system is difficult, understanding which colonization scenario (e.g., Field of Dreams or Propagule Redirection) dominates is a necessary step towards a more complete understanding of the dynamics of that system. Colonization patterns set the stage upon which post-colonization effects (e.g., density-dependent growth, survival, or reproduction) act to determine the production of new colonists (Palmer et al. 1997; Stier and Osenberg 2010; Hamman et al. 2018). Thus, considering how different species respond to the density and spatial aggregation of habitat patches can have important implications: e.g., for management and restoration of threatened populations or the control of disease vectors that depend on patchily distributed habitat types.

Terrestrial organisms with aquatic larval stages, such as many insects and amphibians, are prime examples of species whose population dynamics may be influenced by the spatial distribution of habitat patches. For these organisms, females frequently search for suitable habitat patches in which to oviposit. For example, container-breeding mosquitoes in the genus *Aedes* lay eggs in small, often ephemeral, bodies of water such as tree holes or artificial containers in human-altered landscapes. These mosquitoes also have the behaviorally plastic capacity for skip oviposition, which allows them to distribute eggs from a single batch into multiple larval habitats. Females tend to spread eggs out more when multiple habitats patches are available in small (~ 1 m^2) areas (de Abreu et al. 2015; Davis et al. 2015), although they do this less if some of the patches are of lower quality (Oliva et al. 2013; Davis et al. 2015). This ability potentially allows them to reduce competition between their offspring by depositing fewer eggs per patch, and/or could serve as a bet-hedging strategy, enabling them to spread out their eggs in case one patch becomes uninhabitable.

Density-dependent competition during the larval stage affects *Aedes* mosquito population dynamics (Briegel 1990; Armbruster and Hutchinson 2002; Walsh et al. 2011; Gilles et al. 2011; Yoshioka et al. 2012), and influences their competence as disease vectors (Alto et al. 2008, 2005). Thus, how eggs get distributed among habitat patches could affect adult mosquito production as well as spatial patterns of disease transmission, which is a major public health concern, as these mosquitoes are important vectors for many human diseases such as Zika, dengue, and chikungunya. Research investigating factors that drive patterns of oviposition by female mosquitoes has primarily focused on patch characteristics that indicate habitat quality, such as resource quantity (Yoshioka et al. 2012; Fader and Juliano 2014) or type (Dieng et al. 2003; Ponnusamy et al. 2008; Reiskind et al. 2009), the presence of conspecifics or heterospecific competitors (Zahiri et al. 1997; Allan and Kline 1998; Edgerly et al. 1998; Yoshioka et al. 2012; Fader and Juliano 2014; Wasserberg et al. 2014; Fonseca et al. 2015), or the presence of predators (Pamplona et al. 2009) and parasites (Lowenberger and Rau 1994). However, little is known about how the spatial distribution of suitable habitat patches affects mosquito oviposition behavior.

To address this question, we conducted a field experiment on *Aedes* mosquitoes in NE Georgia, USA. Specifically, we sought to determine whether Propagule Redirection, Field of Dreams, or Excess Attraction Hypotheses capture the spatial patterns of oviposition in this system, extending these colonization paradigms to consider the spatial aggregation of patches, and not simply patch availability: i.e., we compared oviposition in arrays of habitat patches containing an equal number of spatially clustered or dispersed patches. We compared the three hypotheses with respect to three measures of egg deposition: (1) the total number of eggs per array of habitat patches; (2) the number of patches in an array that received eggs; and (3) the average density of eggs per patch in patches that received eggs. We also considered how skip oviposition would influence these patterns.

In the absence of skip oviposition, the three hypotheses can be easily distinguished (Table 1, Appendices A–B in Online Resource 1). Propagule Redirection predicts that the total number of eggs per array and the number of eggs per colonized patch will be lower in the clustered arrays (Fig. S1 in Online Resource 1). Specifically, a cluster of patches will receive the same number of eggs as a single dispersed patch. Therefore, due to the greater areal spread of the dispersed patches, we would expect the entire array (and the cues produced by its patches) to be encountered by a greater number of females, and receive more total eggs than a cluster of patches. This is essentially a sampling effect, where dispersed habitats can be thought of as sampling gravid mosquitoes over a larger area, whereas patches in a clustered array have overlapping sampling areas, and thus compete for the same set of females, resulting in fewer eggs per colonized patch compared to dispersed patches.

In contrast, the Field of Dreams Hypothesis predicts that the number of eggs per array and the eggs per colonized patch will be equal in the clustered and dispersed arrays (Fig. S2 in Online Resource 1). These predictions arise from the assumption that the number of female mosquitoes that visit and oviposit in a clustered array increase in proportion to the number of patches in the cluster. Because the clustered patches occupy a much smaller area of the landscape than the dispersed patches, this means that the cluster of patches must draw in mosquitoes from a larger area than a single dispersed patch to achieve the same per-patch oviposition rate. Specifically, the total area sampled by a clustered array must be equal to the area sampled by a dispersed array. However, at the patch

Table 1 Summary of different colonization and skip oviposition scenarios and their predicted oviposition outcomes for patch- and array-level responses in dispersed (D) vs. clustered (C) treatments (assuming that arrays are not saturated with mosquitoes)

Colonization scenarios			Predicted oviposition patterns		
Hypothesis	Description	Skip oviposition	Array response (total eggs per array)	Binomial response (% patches with eggs)	Patch response (eggs per colonized patch)
Propagule Redirection (dilution)	A cluster of habitat patches receives the same no. of colonists as a single dispersed patch	No	C < D	C < D	C < D
		More in C	C < D	C ? D	C ≪ D
		More in D	C < D	C ≪ D	C ? D
Field of Dreams (proportionate increase)	Each patch receives an equal no. of colonists, irrespective of clustering	No	C = D	C = D	C = D
		More in C	C = D	C > D	C < D
		More in D	C = D	C < D	C > D
Excess Attraction (greater than proportionate increase)	Each patch receives disproportionately more colonists than a single patch when it is near other patches	No	C > D	C > D	C > D
		More in C	C > D	C ≫ D	C ? D
		More in D	C > D	C ? D	C ≫ D

Separate predictions are given for scenarios where skip oviposition occurs more in either clustered or dispersed patches. “C ? D” indicates that the pattern is ambiguous, and could either be C > D, C = D, or C < D, depending on the degree of skip oviposition. If skip oviposition occurs similarly across both treatment types, predictions remain the same as the case in which skip oviposition does not occur level, the increased number of females attracted to the clustered array gets allocated among patches within the cluster, resulting in the same number of ovipositing females (and thus eggs) per patch: i.e., the number of eggs deposited into a patch is independent of the proximity of its neighbors.

Finally, the Excess Attraction Hypothesis predicts that the total number of eggs per array will increase when patches are clustered: i.e., the attractive effect of clustering more than compensates for the redistribution of females among patches in the cluster. As result, the number of eggs per colonized patch is also greater (Fig. S3 in Online Resource 1).

In addition, if the landscape is not saturated with mosquitoes, such that some patches do not receive eggs, then the three hypotheses can be further distinguished from one another based on the frequency of patches that receive eggs within each array (i.e., the presence/absence of eggs). Under Propagule Redirection, the dispersed array is expected to "sample" more females on the landscape, and therefore receive more eggs per patch. Therefore, the probability (Pr) that a patch in the dispersed array is found and oviposited in by at least one female would be greater than in the clustered array (i.e., Pr(0) in a Poisson process declines as the expected number of events—in our case, the expected number of ovipositing females visiting a patch—increases). Thus, predicted treatment effects on the proportion of patches that receive eggs should mirror the prediction with respect to the total number of eggs per array (Table 1, Figs. S1–S3 in Online Resource 1).

If not all patches receive eggs, and if skip oviposition occurs to different degrees in the two treatments, then the predictions regarding the proportion of patches receiving eggs and the number of eggs per colonized patch, but not the total number of eggs per array, will change (Table 1; Figs. S2, S4, S5 in Online Resource 1). In the treatment in which skip oviposition is more common, the frequency of patches with eggs will increase relative to the other treatment, because the eggs will be distributed more evenly across the available patches (Table 1). This effect will reduce the number of eggs per colonized patch in the treatment with more skip oviposition, since the same total number of eggs would be spread out among more patches. As a result of these effects, skip oviposition slightly blurs the distinctions among the three hypotheses with respect to the proportion of patches receiving eggs and eggs per colonized patch, although the three hypotheses remain distinguishable (see Appendix C in Online Resource 1 for more details).

Methods

Field sites

Five forested sites with populations of the native Eastern tree-hole mosquito, *Aedes triseriatus* (also known as *Ochlerotatus triseriatus*: Reinert 2000), and the invasive Asian tiger mosquito, *Aedes albopictus*, were selected within or near the city of Athens, Georgia, USA—three in Whitehall Forest, and two in Oconee Forest Park (GPS coordinates in Table S1, Online Resource 1). These forests are separated by approximately 4 km, and experimental sites within a forest were separated by 0.25–1 km. All sites contained a mixture of tree species, primarily deciduous species dominated by maple and oak, as well as a few individual pines. Sites were set up in the forest interior, approximately 50 m from its edge, in areas with relatively low underbrush density. As it was a drought year, most sites had few natural larval habitats such as water-filled tree holes. Those that did generally had only one or two natural habitats within 20 m of any experimental patch. Preliminary analyses did not provide any demonstrable effect of this small variation in natural habitats, so we did not explore their effects further.

Experimental setup and oviposition assays

Previous field studies on *Aedes* mosquitoes used ground-deployed oviposition patches (Trexler et al. 1998), and in our observations of natural larval habitats near these sites, the majority of tree holes that we found containing water and mosquito larvae were at the base of trees. Therefore, at each site, 12 black plastic cups (10 cm diameter) simulating natural oviposition patches were deployed on the forest floor, 6 in a clustered and 6 in a dispersed habitat array. Cups were staked to the ground, and had two holes drilled in the sides to prevent water from overflowing or submersing mosquito eggs during rain events. In the clustered array, five cups were positioned in a circle 1 m from a central cup. As mosquitoes can detect olfactory cues from larval habitats on a scale of meters, and visual cues on similar or slightly larger scales (Day 2016), we presumed that if a mosquito encountered one patch in a clustered array, it also would be able to perceive other patches in the array. In the dispersed arrays, cups were arranged in three staggered rows of two cups each, separated from the clustered array and from each other by ~ 20 m (Fig. 1). This distance was used, because it is likely beyond the olfactory and visual detection ranges of *Aedes* mosquitoes (Bidlingmayer and Hem 1980; Day 2016), although mosquitoes can move more than 20 m over the experimental time frame (Niebylski and Craig 1994; Honório et al. 2003; Marini et al. 2010; Medeiros et al. 2017).

At the start of each oviposition assay, the top half of each cup was lined with seed germination paper (Anchor Paper Co., St. Paul, Minnesota), which was held in place with three paperclips. Cups were filled with 430 mL of a solution of deionized (DI) water infused with the dried leaves of the most common species of oak in the region, the white oak (*Quercus alba*), and a 1:1 mixture of yeast and lactalbumin. This solution was fermented at room temperature for 3 days

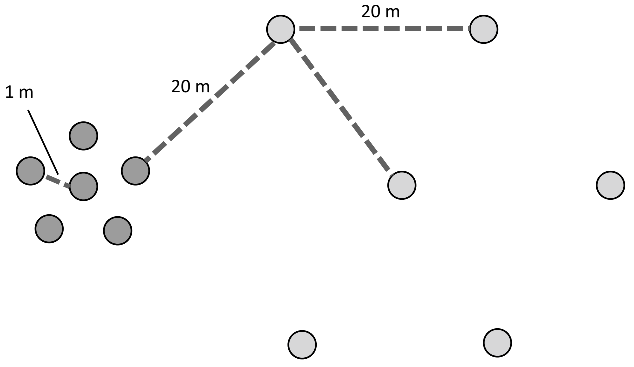

Fig. 1 Experimental setup at each site. Experimental oviposition patches (circles) were deployed in clustered (dark gray) and dispersed (light gray) arrays. Clustered arrays had five patches distributed 1 m from a central patch, while dispersed arrays had six patches arranged in three staggered rows, with ≥ 20 m between patches. The two arrays were positioned ≥ 20 m from one another

before the oak leaves were removed and the solution was homogenized and apportioned into the experimental cups. *Aedes* mosquitoes prefer to oviposit in leaf-infused water compared to DI or tap water, and in the eastern US, oak leaves are an effective oviposition attractant (Trexler et al. 1998). Cups were deployed for 7 days, after which the egg papers and cups were collected and taken back to the lab. In the lab, cup water was checked for mosquito hatchlings (which only occurred in a few cups), and eggs laid on the oviposition paper were counted under a dissecting microscope, and then induced to hatch. Larvae in the cup water were assumed to come from eggs still attached to the paper (as some eggs were observed missing their shell caps, indicating that larvae had hatched). After hatching, the larvae (from both cups and from egg paper) were reared to adulthood, so they could be identified to species (details are provided in the larval rearing section, below).

The oviposition assay was conducted three times to increase replication, with the three temporal blocks beginning on 25 July, 23 August, and 7 September 2016. After the first round of the experiment, each cup was surrounded by a cylinder of hardware cloth fencing (1 cm mesh and 18 cm in diameter) that reached 4 cm higher than the rim of the cup, which reduced interference by wildlife, but did not appear to affect mosquito oviposition rates.

Larval rearing

After the egg papers were collected from the field, they were incubated in the lab for 7 days at room temperature (23–24 °C), and then submersed in a solution of DI water and 0.15 g/L of a 1:1 mixture of lactalbumin and yeast. Hatchlings were counted and transferred to experimental rearing containers (Mosquito Breeder, BioQuip Products) with 300 mL of DI water and 0.4 g of pellet fish food (Cichlid Gold, Hikari), where they matured to adulthood. Containers were checked for new hatchlings approximately every other day for a week after the eggs were first submersed, although most eggs hatched in the first 24 h. Larvae were limited to < 50 individuals per rearing container to reduce competitive effects on adult emergence. Rearing containers were kept at 27 °C in a 14:10 light–dark cycle (fluorescent lighting) in an incubator (Percival). After adults eclosed from the pupae, they were collected from the rearing containers and frozen until they were counted and identified to species under a dissecting microscope.

Overall, rearing success (i.e., the proportion of adults that successfully eclosed out of all the eggs that were oviposited in each experimental cup) was variable and averaged 42%. We evaluated if this variable rearing success could lead to biases in our estimates of species composition by examining the relationship between the proportion of adults that were a given species and the total rearing success associated with that cup (Appendix E, Fig. S6 in Online Resource 1). There was no correlation, so we assumed that the proportional representation of eggs was equal to the relative abundances of adults, and we estimated the number of eggs laid by a species in each cup as the proportion of adults of that species that emerged from the cup times the total number of eggs deposited in it. 26 of the 180 cups received eggs, but had zero adults successfully emerge. These cups usually had very low numbers of eggs (1–10 eggs), and were omitted from the species-level analyses, but were included for analyses based on the raw, combined species data set (using all eggs), i.e., the "pooled species" response. Based on these proportions, *Aedes* mosquitoes comprised 97% of the eggs oviposited in our experimental cups over the course of all three rounds of the experiment. 61% were *Aedes albopictus,* 36% were *Aedes triseriatus*, and 3% were *Orthopodomyia signifera*. Therefore, we conducted analyses on three sets of the data: (1) the pooled species (using all eggs), and species-level data based on: (2) *A. albopictus*, or (3) *A. triseriatus*. The pooled species analyses were conducted because of the known effects of larval competition (with both hetero- and conspecifics) on mosquito survival, size, and fecundity at adulthood (Livdahl and Willey 1991; Edgerly et al. 1993; Walsh et al. 2011; Gilles et al. 2011; Yoshioka et al. 2012).

Statistical analyses

To determine whether the oviposition patterns were better predicted by the Propagule Redirection, Field of Dreams, or Excess Attraction hypotheses, we analyzed three responses for each set of data: (1) the total number of eggs per array; (2) the proportion of cups that received eggs; and (3) the average number of eggs for cups that received eggs. The results were compared to the expectations in Table 1.

Oviposition patterns across habitat arrays

To evaluate if the total number of eggs oviposited in the six cups varied between the clustered vs. dispersed arrays, we used generalized linear mixed effects models with habitat treatment (dispersed vs. clustered) and temporal block (i.e., experimental round) as fixed effects, and site as a random effect. Temporal block and site were included to account for potential differences in experimental conditions. We treated temporal block as a fixed effect, because it had only three levels (one for each round of the experiment), which is insufficient to accurately estimate the among-level variance (Crawley 2002). We did not include interactions with treatment, because we had limited observations with which to fit models with interaction terms and no a priori reason to expect the effect of treatment on oviposition patterns to change with experimental round or site. Egg counts were overdispersed relative to a Poisson distribution, as would be expected because females lay many eggs. Therefore, we used a negative binomial distribution, which fit the data better.

Oviposition patterns at individual larval patches

To understand drivers of oviposition patterns, we considered oviposition at a cup to be a two-part process: (1) a binomial process describing the presence or absence of eggs in a cup, and (2) a count process describing the number of eggs oviposited in a cup, given that it received eggs. For the binomial process model, we fit a logistic regression (logit link function and binomial family) to the presence/absence of eggs in the cups within each array, with the same random and fixed effects as in the array-level oviposition model. For the count process, first, we calculated the mean number of eggs per cup in each array using only the cups that received eggs (i.e., "colonized" cups). Then, we fit a zero-truncated negative binomial model to the mean egg counts per colonized cup, again with habitat treatment and temporal block as fixed effects and site as a random factor.

Model testing

For both *Aedes* species, as well as for the pooled species response data, full models containing effects for treatment, temporal block, and site were fit to each of the three response variables (egg counts per array, the proportion of cups in an array that received eggs, and the egg counts per colonized cup). Full models were then compared to nested models, which dropped habitat treatment as a predictor variable, using $\mathrm{AIC_c}$ (AIC for small sample sizes). This approach allowed us to determine if habitat treatment was an important predictor. Model comparison results are summarized in Table S3 in Online Resource 1. We then used Wald χ^2 tests to calculate approximate P-values for the fixed factors of the models that included treatment and block (Bolker et al. 2009).

Occasionally, an array received zero eggs from one of the *Aedes* species, which reduced the sample size for that treatment. Specifically, *A. albopictus* had $n = 13$ (rather than 15) in both treatments for the egg counts per colonized cup, as there were two instances for each treatment in which no cups in an array received eggs; the sites and rounds in which this occurred differed between the treatments. Zeroes were more common for *A. triseriatus*, which did not oviposit any eggs in either treatment in three sites in the first temporal block. To deal with this problem, we excluded the first temporal block from the array-level egg count analyses ($n = 10$) for this species. Similarly, analyses of egg counts per colonized cup had $n = 10$ in the clustered treatment, and $n = 12$ in the dispersed treatment.

Models were fit using the *lme4* package (Bates et al. 2015) in R 3.3.2 (R Core Team 2016), with the Wald χ^2 tests performed using the *car* package (Fox and Weisberg 2011). In all cases, model predictions were graphically compared to the observed data to ensure that they provided a reasonable fit. $\mathrm{AIC_c}$ values were compared using the *bbmle* package (Bolker and R Development Core Team 2017).

Results

Egg counts per habitat array

On average, the clustered habitat arrays received 22% more eggs than did the dispersed arrays, although this pattern was not significant ($p = 0.22$; Fig. 2, Table 2). *Aedes albopictus*, which comprised the majority of eggs, showed a similar trend, ovipositing 38% more eggs in the clustered arrays compared to the dispersed arrays; however, again, this pattern was not significant ($p = 0.27$; Fig. 2, Table 2). Model comparisons for the pooled species response and for *A. albopictus* found equivocal support ($\mathrm{dAIC_c} \leq 2$) for the model with habitat treatment as a predictor, compared to the model without treatment (Table S3 in Online Resource 1). In contrast, *A. triseriatus* egg counts were similar in the clustered and dispersed arrays ($p = 0.23$; Fig. 2, Table 2), and the model without habitat treatment was better supported by the data (Table S3 in Online Resource 1). Oviposition varied among temporal blocks for *A. triseriatus* ($p < 0.001$), with higher oviposition rates in later rounds of the experiment; oviposition by *A. albopictus* or the pooled species response did not vary among the three temporal blocks ($p = 0.49$ and 0.08, respectively).

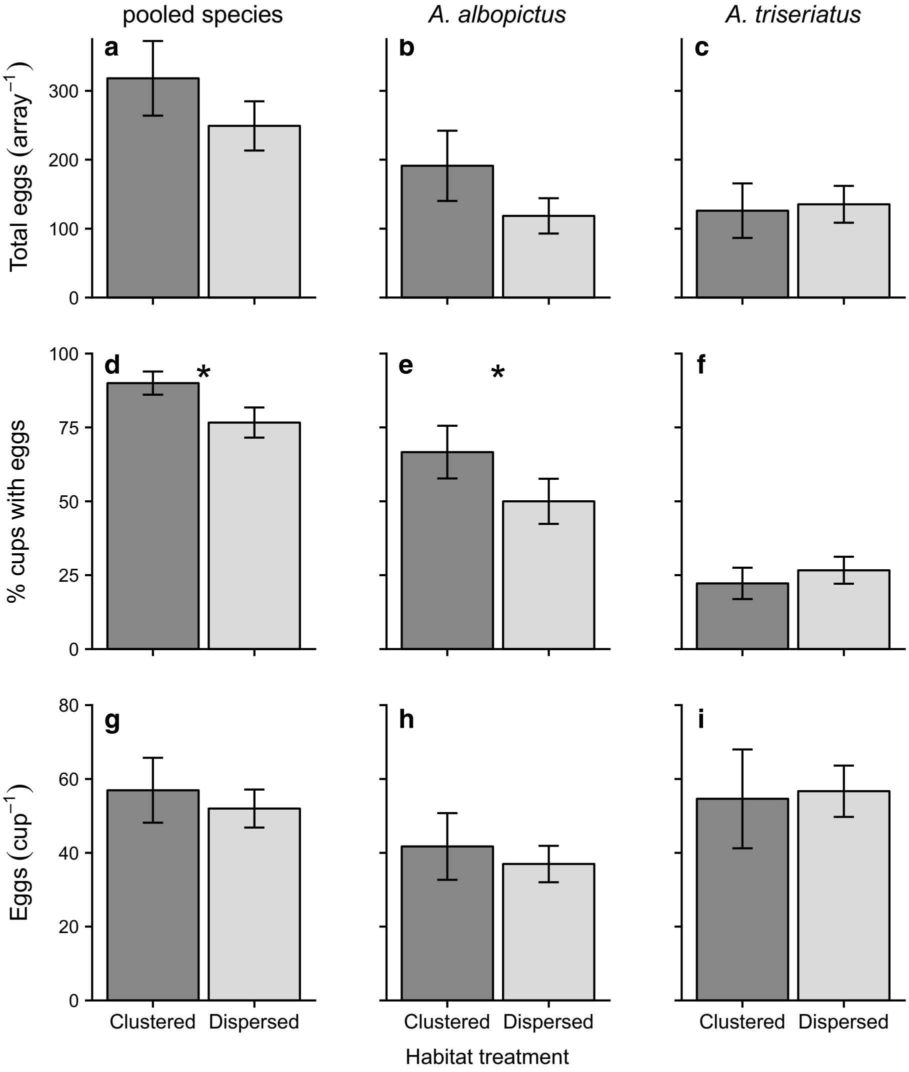

Fig. 2 The effects of spatial aggregation of habitat patches (habitat treatment) on container-breeding mosquito oviposition patterns. The top row (**a**–**c**) shows the total egg counts for clustered (dark gray) vs. dispersed (light gray) habitat arrays: i.e., the sum of the eggs oviposited in the six cups within each array. The middle row (**d**–**f**) shows the percentage of cups that received at least 1 egg within an array (i.e., the non-zero cups), and the bottom row (**g**–**i**) shows the mean number of eggs per cup in cups that received eggs in each array. Columns indicate mosquito species, with the pooled species column representing the raw egg counts for all species (including *Aedes albopictus*, *Aedes triseriatus*, and *Orthopodomyia signifera*). Bars represent group means ± SE ($n = 15$, based on five sites and three temporal blocks, with the following exceptions: **c**, $n = 10$; **h**, $n = 13$; **i**, $n = 10$ in the clustered treatment, and $n = 12$ in the dispersed treatment). An asterisk denotes a significant difference between habitat treatments (see Table 2)

Proportion of cups receiving eggs

Habitat treatment (clustered vs. dispersed) was a significant predictor of whether an experimental cup received eggs from any species of mosquito ($p = 0.017$), with on average 15% more clustered cups receiving eggs than dispersed cups (5.4 vs. 4.9: Fig. 2). This result was primarily driven by *A. albopictus*, which oviposited in 68% of the experimental cups (compared to 29% used by *A. triseriatus*) and deposited eggs in 25% more cups in the clustered arrays relative to the dispersed arrays ($p = 0.016$). However, dispersed and clustered cups were equally likely to receive eggs from *A. triseriatus* ($p = 0.46$). These results were supported by the model comparisons (Table S3 in Online Resource 1). Temporal block was a significant factor in all models (Table 2). In general, more cups received eggs in the later experimental rounds, except for *A. albopictus*, which had the most cups with eggs in the second round, and similar numbers of cups with eggs in the first and last rounds.

Egg counts per colonized cup

Oviposition in cups that received eggs was approximately the same in the clustered vs. dispersed treatments for both *Aedes* species, and for the pooled data set (Fig. 2), and the models containing an effect of habitat treatment were less supported by the data (Table S3 in Online Resource 1). Thus, habitat treatment was not a significant predictor in any of the egg count models (pooled species: $p = 0.60$; *A. albopictus*: $p = 0.24$; *A. triseriatus*: $p = 0.79$). Egg counts for all three species of mosquito ranged from 1 to 287 eggs per cup; counts for *A. albopictus* and *A. triseriatus* ranged from 1 to 183 and 1 to 186 eggs per cup, respectively. The average number of eggs per cup was 44 ($n = 105$) for *A. albopictus*, and 62 ($n = 44$) for *A. triseriatus*. Temporal block explained significant variation in the *A. triseriatus* egg count model, but not in the pooled species or the *A. albopictus* models (Table 2). In general, the mean number of eggs per colonized cup increased in later experimental rounds for the pooled

Table 2 Summary of *Wald Chi-squared* statistics for full models containing habitat treatment and experimental round (i.e., temporal block) as fixed factors, and site as a random factor

Predictor	*df*	χ^2	*p* value
Array count response—eggs per array			
Pooled species			
Habitat treatment	1	1.478	0.224
Round	2	4.965	0.084
A. albopictus			
Habitat treatment	1	1.205	0.272
Round	2	1.422	0.491
*A. triseriatus**			
Habitat treatment	1	0.050	0.824
Round	1	1.798	0.180
Binomial response—proportion of patches with eggs			
Pooled species			
Habitat treatment	1	5.680	**0.017**
Round	2	6.569	0.037
A. albopictus			
Habitat treatment	1	5.7865	**0.016**
Round	2	14.323	0.001
A. triseriatus			
Habitat treatment	1	0.533	0.465
Round	2	12.034	0.002
Patch count response—eggs per colonized patch			
Pooled species			
Habitat treatment	1	0.200	0.655
Round	2	2.499	0.287
A. albopictus			
Habitat treatment	1	0.272	0.602
Round	2	0.649	0.723
A. triseriatus			
Habitat treatment	1	0.513	0.474
Round	2	15.354	< 0.001

Results are separated by binomial and count process models, which were run separately for pooled data and individual mosquito species. For the array response of *A. triseriatus*, the first round of the experiment was removed due to the high proportion of arrays with zero eggs (indicated by an asterisk). Habitat treatment (the fixed effect of interest) is highlighted in bold when it is significant

species and for *A. triseriatus,* although not for *A. albopictus* (which had similar mean eggs counts across all rounds).

Discussion

Overall, these results led us to reject the Propagule Redirection Hypothesis, which predicted that there would be fewer total eggs, fewer cups with eggs, and fewer eggs per colonized cup in the clustered arrays compared to the dispersed arrays. None of these predictions were supported by the data for either species of mosquito. Although each dispersed array was distributed over an area 400 times greater than a clustered array (i.e., patches were laid out in two pseudo-circles with areas of approximately 3 vs. ~ 1200 m^2), the dispersed arrays did not receive more eggs than the clustered arrays. In addition, both habitat treatments received similar numbers of eggs per colonized cup for both *Aedes* species, and either the same or a higher proportion of cups received eggs (for *A. triseriatus* and *A. albopictus*, respectively). Thus, a clustered array of six cups received at least six times more eggs than did a single, isolated cup in the dispersed arrays (so there was at least a proportionate increase in colonization).

These results are consistent with the Field of Dreams Hypothesis, or potentially the Excess Attraction Hypothesis for *A. albopictus*. Both of these hypotheses are generated by assuming that clustered patches draw in mosquitoes from a larger area than does a single dispersed patch, leading to higher oviposition rates in clustered patches. This effect would arise if the relative magnitude of the signal produced by a clustered array of patches was much more intense at a given distance than the signal produced by a dispersed patch, and extended over a greater distance.

Female mosquitoes are known to use chemosensory and visual cues to search for larval habitats in which to oviposit (Day 2016). Cues of this sort have been modeled as exponential decay functions of distance (Hamman et al. 2018). Therefore, if we assume that an attractive signal diffuses from a cup in a point-source pattern, and that females can detect a cup up to a critical distance at which the signal has decreased to a threshold level, then we can construct a ballpark estimate of the distance from which an isolated cup vs. a cluster of cups attracts females. The area (A_C) from which the clustered cups could attract females would be $\sim \pi r_C^2$, where r_C is the detection distance for a cluster. Assuming that the dispersed cups (i.e., patches) are distant enough from one another that their regions of detection do not overlap (e.g., Fig. S2 in Online Resource 1), then the total area (A_D) from which the six dispersed patches can attract females is $\sim 6\pi r_D^2$ (where r_D is the detection limit for a single isolated patch). Given that the observed ratio of average total oviposition in the clustered vs. the dispersed arrays was 318:249, and assuming that these oviposition rates were proportional to the relative signal areas from which females were attracted (A_C:A_D), then we find that $r_C/r_D = 2.8$: i.e., the clustered patches were detected out to a distance approximately three times greater (or an area almost eight times greater) than a single patch. Thus, the increased attractiveness of the clustered patches more than compensated for any dilution of propagules among clustered patches that would have occurred due to Propagule Redirection-like processes.

Species differences

Skip oviposition behavior occurs when females distribute eggs from a single batch into multiple patches. Previous studies suggest that *A. albopictus*, but not *A. triseriatus*, exhibits skip oviposition (Edgerly et al. 1998; Delatte et al. 2009; Davis et al. 2015). In support of these previous findings, our results indicate that *A. albopictus* females tended to skip oviposit, as 44% of the cups from which *A. albopictus* emerged had fewer than 30 eggs, which is considerably less than its typical egg batch size of 49–74 eggs batch^{-1}, with batch size estimates at the higher end of that range for most temperatures (Delatte et al. 2009; Davis et al. 2015). In contrast, the mean eggs per cup for *A. triseriatus* (62 eggs cup^{-1}) were similar to estimates for the average batch size per female (56–58 eggs; Edgerly et al. 1998). However, 23% of the cups from which *A. triseriatus* emerged had fewer than 30 eggs, suggesting that it also exhibited some skip oviposition behavior, although *A. albopictus* likely exhibited skip oviposition to a much larger degree.

In addition to these differences in skip oviposition behavior, the two species also exhibited some differences in the magnitude of the effect of patch aggregation on their oviposition patterns. *A. albopictus* appeared to be more responsive than *A. triseriatus*, ovipositing on average 38% more eggs in the clustered arrays compared to the dispersed arrays, whereas *A. triseriatus* oviposited 10% fewer eggs in the clustered arrays (although neither difference was significant). Thus, it is more likely that Excess Attraction occurred in *A. albopictus* compared to *A. triseriatus,* although distinguishing between Excess Attraction and Field of Dreams is statistically challenging (particularly given the ambiguity added by skip oviposition).

Landscape-level consequences

One of our motivations in conducting this study was to assess if patch aggregation could establish spatial variation in colonist density, which could shape the effects of density dependence and other post-colonization processes; consequently, influencing the spatial patterning of a dispersing species on a landscape. Our results indicate that, due to a combination of their degree of attraction to clustered patches, as well as their propensity for skip oviposition, both *A. albopictus* and *A. triseriatus* mosquitoes would experience similar larval densities in both aggregation treatments. Therefore, the production of adult mosquitoes on a per-patch basis should be similar in the dispersed versus clustered arrays. Of course, our study only compared two spatial configurations of habitat patches, and patterns could vary under different spatial arrangements, inter-patch spacing patterns, and local densities of patches. However, our results indicate that, in general, regions containing clustered patches would create hotspots of mosquito oviposition and production.

Spatial scales of habitats, their signals, and processes affecting colonization

Previous studies on colonization dynamics have focused on the effects of the addition of habitats. However, our work shows, for mobile organisms, one must also consider the spatial distribution of habitat patches, particularly when distances between patches are on a similar scale as the detection and dispersal abilities of a species of interest. Within this spatial scale, habitat clustering can draw in more colonists on a localized per-area basis than areas with few habitats, following predictions of the Field of Dreams or Excess Attraction hypotheses. This is analogous to patterns found in the pollination literature, where areas with denser plants have higher pollinator visitation rates per plant, and plants with larger floral displays bring in more pollinators (Klinkhamer and de Jong 1990; Grindeland et al. 2005). However, for species with low signal detection sensitivity (and thus a short detection range), or low mobility (e.g., larval reef fish), habitat clustering should be less important for drawing in colonists, but still could influence their colonization patterns once they reach an area with habitat patches; thus, their colonization dynamics should be more reflective of Propagule Redirection (Stier and Osenberg 2010; Hamman et al. 2018). Understanding the ability of potential colonists to perceive and respond to signals given off by habitat patches might help to resolve observed variation in the relationship between habitat availability and colonization in different study systems as well as variation among species in the same system.

Our experiment used patches of similar quality; however, in natural landscapes, patches will vary in quality as well as spatial configuration. In such cases, dispersing organisms might be predicted to be more selective when habitat patches are densely configured, and less selective when there are fewer patches to choose from or there is a greater cost or risk associated with moving between patches. Therefore, interactions between factors operating at different spatial scales could influence how colonization processes play out to affect colonization of individual habitat patches, and thus metapopulation and meta-community dynamics in heterogeneous landscapes. Because of this, future research is needed that considers the scales at which different colonization processes operate, including mechanisms related to signal strength, signal detection, disperser mobility, and colonist behavior.

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