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Vertical Habitat Stratification in Sympatric and Allopatric Populations of *Aedes hendersoni* and *Aedes triseriatus* (Diptera: Culicidae)

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Abstract

Vertical habitat stratification in populations of *Aedes hendersoni* Cockerell (Diptera: Culicidae) and *Aedes triseriatus* (Say) (Diptera: Culicidae) has been observed to varying degrees throughout the species' sympatric range, and potential causes of the phenomenon, including species competition and interaction, have been debated extensively. Stratification patterns in oviposition in allopatric, sympatric, and marginally sympatric populations of both species were investigated and compared in this research to detect any pattern differences related to species composition. Expected patterns were observed in sympatric populations, with *Ae. hendersoni* preferentially ovipositing in canopy habitats, whereas *Ae. triseriatus* preferred basal habitats. Allopatric populations presented a strong shift toward basal preference in the former and a slighter but significant shift toward canopy in the latter. Marginal populations of *Ae. hendersoni* showed intermediate height preferences, whereas preferences of marginal and sympatric *Ae. triseriatus* did not differ. The convergence of habitat selection in allopatric populations and corresponding divergence in sympatric populations support interspecific competition-based hypotheses regarding the origin of the stratification phenomenon, although plausible alternative or contributing explanations are numerous and warrant further study.

Key words: Aedes triseriatus, Aedes hendersoni, niche diversification, resource partitioning, competition

Oviposition site selection by gravid *Aedes hendersoni* Cockrell (Diptera: Culicidae) and *Aedes triseriatus* (Say) (Diptera: Culicidae) females is vital to the success of their offspring, since larvae and pupae are necessarily confined within container habitats during important developmental stages, and since the quality of a larval habitat greatly influences the health and survival of the individuals within it. Larval habitat quality is subject to temporal and spatial variation in resource levels, chemical composition, risk of desiccation, and community structure (Fish and Carpenter 1982, Walker and Merritt 1988, Teng and Apperson 2000, Kaufman et al. 2001), and depends heavily on the location of the habitat (Williams et al. 2007). Basal habitats, e.g., receive and maintain more water and nutrients via leaf drop and stem flow than do similarly structured canopy habitats, and therefore support more larval development success (Carpenter 1982, Walker et al. 1991, Kaufman et al. 2007). Williams et al. 2007).

Species Interactions

Among treehole mosquitoes, oviposition site selection by gravid females appears to be based on chemical (Copeland and Craig 1992a,

Trexler et al. 1998, Yee and Yee 2007) and other environmental cues (Debboun and Hall 1992, Yee and Yee 2007, Ellis 2008). The presence of mosquito larvae within a habitat has been observed to both deter and attract oviposition by gravid females, and therefore appears to be a highly context-specific cue (Edgerly et al. 1998). A thriving group of larvae within a container may indicate a relatively permanent habitat and entice oviposition, yet interspecific and intraspecific competition and cannibalism can impede larval success (Livdahl 1982, Edgerly and Livdahl 1992, Teng and Apperson 2000). Larvae also may serve as a deterrent. Ae. triseriatus regularly outperforms Ae. hendersoni in interspecific competition experiments, whereas each performs oppositely in intraspecific competition experiments: Ae. triseriatus perform relatively worse against Ae. triseriatus, but Ae. hendersoni perform relatively better against Ae. hendersoni (Copeland and Craig 1992b). If competition coefficients can be inferred from these asymmetric responses to con- and heterospecific densities, the potential for competitive exclusion of Ae. hendersoni by Ae. triseriatus is indicated where the two species occupy the same containers. Further, Ae. hendersoni is susceptible to decreased competitive ability as well as increased mortality relative to *Ae. triseriatus* when infected with *Ascogregarina barretti* (Vavra) (Eugregarinida: Lecudinidae), a common protozoan parasite of *Ae. triseriatus* (Copeland and Craig 1992b). There are ample reasons to expect that competition with *Ae. triseriatus* has been a significant historical problem for *Ae. hendersoni* in the sympatric parts of the two species' ranges.

Species Geographic Distributions

Both *Ae. triseriatus* and *Ae. hendersoni* occupy vast geographical ranges, and much of North America is inhabited by one or both species. *Ae. triseriatus* occurs abundantly in the eastern United States and small portions of southern Canada, with its range extending westward, generally to the Mississippi River valley. *Ae. hendersoni* shares much of the same range, although it is absent in most of Maine, peninsular Florida, and extreme southern Texas. The range of *Ae. hendersoni* also extends much further west than that of Ae. *triseriatus*, reaching the southeastern region of British Columbia and eastern Washington and Oregon in the north as well as the northern regions of Nevada and Utah, eastern Colorado and New Mexico, and mid-northern Texas to the south (Fig. 1, Darsie and Ward 2004).

Vertical Distributions

Field collections in several previous studies have indicated a vertical stratification in the oviposition site selection between these species, with *Ae. triseriatus* more prevalent in basal habitats and *Ae. hendersoni* more common in canopy habitats (Scholl and DeFoliart 1977, Sinsko and Grimstad 1977, Clark and Craig 1985, Walker et al. 1987, Copeland and Craig 1990, Debboun and Hall 1992), although the pattern has been observed in varying degrees throughout the species' sympatric range. Because basal larval habitats appear to be more suitable oviposition sites, and because *Ae. triseriatus* is the superior larval competitor, researchers have suggested that oviposition by *Ae. hendersoni* in canopy habitats may provide an example of resource partitioning driven by interspecific competition (Schreiber et al. 1988, Copeland and Craig 1992b). Supporters of this theory argue that the

competitively inferior *Ae. hendersoni* has been driven to suboptimal canopy habitats to avoid competition with *Ae. triseriatus*. Evidence for this ecological concept, however, is difficult to identify with confidence and has been debated with some vigor (e.g., Menge and Sutherland 1976, Connell 1980, Bradshaw and Holzapfel 1983).

Competition and Resource Partitioning

One main difficulty in settling this debate is that while the supposed driver of resource partitioning is interspecific competition, competition itself is difficult to observe and measure in nature, as it is rarely a direct and apparent phenomenon. In addition, if the theory is correct, the presumed causative factor of resource partitioning should become less important as a result, rendering the interaction a hypothetical historical factor. Hence, the 'ghost of competition past' (sensu Connell 1980) has been invoked to explain both the coexistence of species which have similar resource needs, as well as the differences in resource use by coexisting, ecologically similar and related species. Although it is difficult to identify a direct and singular cause of the current state of habitat segregation, Copeland and Craig (1990, 1992b) argue that regardless of the origin of such a phenomenon, interspecific competition is likely to help maintain that segregation.

A major body of literature has been built around this supposition, including many studies of apparent niche separation and community structure with the interpretation that interspecific competition is the means by which community structure is shaped (e.g., Schoener 1974, Diamond 1978, Connell 1980, James and Boecklen 1984). While many additional factors are indeed influential on community structure, it has been proposed that convincing evidence for competition among species exists in cases where a single limiting resource is identified and manipulated (Connell 1961, Dayton 1971), and that spatial divergence between competing species may be understood as a result. Further, it is argued that competition can be demonstrated in systems where the behavioral mechanisms of competition are identifiable, such as in cases of interference competition (Hallacher 1977, Larson 1980 referenced in Hines 1982). However, even when evidence of interspecific competition is present, it remains difficult to



Fig. 1. Range maps for Aedes triseriatus (horizontal lines) and Ae. hendersoni (vertical lines), redrawn from Darsie and Ward (2004).

build a complete set of evidence in support of competition as a direct cause of resource partitioning.

Others claim that although interspecific resource competition may occur, most populations do not persist at high enough equilibrium densities to make this a strong enough influence to drive the evolution of niche segregation (Wiens 1977), and that resources shared between species occur frequently in nature without competition (Birch 1979, referenced in Strong 1984). In addition, many researchers point out that competition cannot be identified confidently as the driving force behind niche differentiation because there are simply too many additional potential drivers in the environment, including predation and disturbance (Chesson and Huntly 1989, Arlettaz et al. 1997). Members of this camp further maintain that the intensity of interspecific competition cannot be measured accurately in nature, and that the exhibition of niche segregation alone cannot provide an explanation of the origin of that segregation (Strong 1984, Arlettaz et al. 1997).

The hypothesis that competition has been a driving factor in promoting and maintaining habitat segregation between congeneric and coexisting species is potentially falsifiable. If similar habitat use occurs in areas where potential competitors are absent relative to areas of sympatry, the competition hypothesis can be rejected. Otherwise, differences in habitat use between sympatric and allopatric populations would be consistent with and supportive of the competition hypothesis.

This hypothesis was tested in this study by comparing vertical habitat preferences for *Ae. triseriatus* and *Ae. hendersoni* in sympatric and allopatric parts of each species' range. If avoidance of competition has driven vertical stratification of oviposition site selection, then in the absence of *Ae. triseriatus* and the associated threat of interspecific larval competition, gravid allopatric *Ae. hendersoni* females should select the more favorable basal habitats for oviposition. In addition, if competition with *Ae. hendersoni* has been a factor in oviposition choices by *Ae. triseriatus*, an upward change to canopy habitat should be expected. This phenomenon was investigated in allopatric populations of *Ae. triseriatus* located in North and South Dakota, allopatric *Ae. triseriatus* located in Maine, and in sympatric Massachusetts populations.

Materials and Methods

Sampling

Locations potentially supporting populations of *Ae. triseriatus* and *Ae. hendersoni* were identified based on published range maps for

each species (Darsie and Ward 2004). Sympatric populations were sampled from several sites across Massachusetts. Potential sampling locations were selected based on several criteria. Locations had to be accessible and sampling efforts feasible, to contain deciduous forest or mixed forest that was composed mainly of deciduous growth, sufficiently mature or dense enough (based on satellite imagery) to support both basal and canopy larval habitats, and to be sufficiently tall to permit clear separation between forest floor and canopy (suitable habitat was confirmed in the latter two cases only upon visitation). Sites were selected in Maine, Massachusetts, North and South Dakota (Table 1, Fig. 2). Collections took place in Maine during the summer of 2011, in Maine, North Dakota, and South Dakota during the summer of 2012, and in Massachusetts during the summers of 2010–2014.

At each sampling site, five basal and five canopy ovitraps were set along a rough transect such that each location had a low and a high trap. Transect placement and shape were dictated by the presence of large trees suitable for ovitrap use, thus transect lengths, shapes, and orientation were irregular among sampling sites. Distances between ovitrap locations along each transect were similarly irregular. Ovitraps were 500 ml black drinking cups lined with cardboard and baited with approximately 30 g of leaf litter. They were filled with 400 ml of water, and secured with string either basally or in the canopy. Basal ovitraps were placed directly on the forest floor and attached adjacent to the north side of mature trees (hardwood trees at least 20 cm in diameter), while canopy ovitraps were suspended at least 3.5 meters above the forest floor, most often between 4.5 and 6.0 meters. Canopy ovitraps were suspended on the north sides of trees, and located immediately adjacent to the base of a branch such that they were somewhat braced and sheltered. This was done in an effort to mimic naturally occurring and desirable larval habitat, which was deemed more important than strict adherence to a specific height requirement. Some variability in the height of suspended canopy ovitraps was inevitable, as trap location was dictated by the availability of suitable branches. Variability also occurred in the length of time traps remained in the field before retrieval, due to logistic constraints of travel.

Identification

Eggs were removed from ovitrap liners, counted under magnification, and then identified based on egg surface morphology; namely the structure and arrangement of outer chorionic cells and

Table 1. Sample site locations, collection dates and species occurrence categories

State	City	Species occurence	Collection dates		
Maine	Bangor 1	Allopatric (Ae. triseriatus)	July 2011; July 2012		
	Bangor 2	Allopatric (Ae. triseriatus)	July 2011; July 2012		
	Orono	Allopatric (Ae. triseriatus)	July 2011; July 2012		
Massachusetts	Easthampton	Sympatric	July–Oct. 2011; April 2012		
	Hadley	Sympatric	July-Oct. 2011; April 2012		
	Uxbridge 1	Sympatric	Aug. 2010; July-Aug. 2011		
	Uxbridge 2	Sympatric	Aug. 2010; July-Aug. 2011		
	Worcester	Sympatric	June-Aug. 2013; June-Aug. 2014		
North Dakota	Bismark	Allopatric (Ae. hendersoni)	July–Aug. 2012		
	Fort Ransom 1	Marginal	July–Aug. 2012		
	Fort Ransom 2	Marginal	July–Aug. 2012		
	Homen 1	Allopatric (Ae. hendersoni)	July–Aug. 2012		
	Homen 2	Allopatric (Ae. hendersoni)	July–Aug. 2012		
	Mandan	Marginal	July–Aug. 2012		
	Valley City	Marginal	July–Aug. 2012		
South Dakota	Spearfish	Allopatric (Ae. hendersoni)	July–Aug. 2012		



Fig. 2. Locations of sampling sites in Massachusetts (sympatric populations), Maine (allopatric *Ae. triseriatus* populations), and North and South Dakota (allopatric *Ae. hendersoni* populations, and marginal sympatric populations).

chorionic reticulum, and the amount and arrangement of tubercles within the cells located on the ventral and lateral surfaces of the eggs (Zaim et al. 1977, Linley 1989, Linley and Craig 1993, Haddow 2009). Morphological species identification was confirmed molecularly in pooled samples of unhatched eggs. Pooled samples consisted of 20 eggs of each species, sampled randomly from at least four separate ovitrap liners at each collection site. Molecular identification was achieved via PCR amplification using a conserved reverse primer (5' CGCGCCTGACTATCTTCAAT 3'), and species-specific forward primers for both *Ae. hendersoni* (5' CACCGAAGAGAGAGAGAGAAAA 3') and *Ae. triseriatus* (5' CATCAAGAGGTTAACGAG 3') (Wilson et al. 2014). Amplified molecular product was visualized through agarose gel electrophoresis and either methylene blue or ethidium bromide staining.

Analysis

Categories of species occurrence were defined according to the presence or absence of a congener (sympatric, allopatric), and a third category (marginal) was created based on the unexpected discovery of *Ae. triseriatus* at low frequencies at four North Dakota sampling sites, which had not been previously reported. Data were aggregated within each category and analyzed based on total eggs identified for each species in all ovitraps. Because these species lay eggs in clusters, individual eggs cannot be considered independent events. Therefore to increase the likelihood of independence, oviposition events were estimated for each species within each ovitrap based on predicted egg batch sizes of 22.7 for *Ae. hendersoni* and 30.3 for *Ae. triseriatus* (Walker et al. 1987), and data were analyzed using transformed values. χ^2 goodness of fit one sample tests were used to test for significant preferences for oviposition height (H_o: No oviposition height preference, even distribution of eggs; H_a: Oviposition height preference for low or high ovitraps; df = 1; $\alpha = 0.05$).

To test for differences in height preferences in different parts of the ranges of these species, contingency tables were constructed using the estimated oviposition events, and tests for independence between rows and columns were performed with χ^2 tests. In each of these tests, rows were the position of traps (high, low), where low traps were always basal and high traps were always at least 3.5 m above ground. Contingency tests included: a test for differences in species preferences within sympatric sites, a test for differences among preferences of each species within three species occurrence categories (sympatric, allopatric and marginal), and a test for differences in the proportions of oviposition events occurring in the high traps for the six combinations of species and occurrence category. Results from the latter 2×6 contingency table were further analyzed for pairwise differences in the fraction of high trap oviposition events using the Marascuilo procedure for comparing all possible pairs of proportions (Marascuilo and McSweeney 1967). Confidence intervals for these proportions were calculated by the Agresti-Coull method as recommended by Whitlock and Schluter (2015). For illustrative purposes, ordinary least squares regression lines for plots of oviposition events versus height were constructed for each species at each combination of species and occurrence category.

Results

Stratification Patterns

Relationships between eggs laid and height of traps are summarized in Fig. 3 for each combination of species occurrence category and species.

Sympatric Ae. hendersoni and Ae. triseriatus

At all sympatric sites sampled, these species showed consistent and marked tendencies toward high and low traps, respectively. Data for each sympatric site appear in Table 2; significant differences of proportions from 0.5 occurred for each species at each site. Aggregate data are shown in Fig. 4.

Areas Marginal for Ae. triseriatus

Estimated oviposition events for each species appear in Table 2, with an aggregated summary in Fig. 5. Results of tests for stratification varied among sites, particularly for *Ae. triseriatus*, although the number of oviposition events were quite low for that species.

Allopatric Ae. triseriatus Sites

Data for the three sites in Maine are provided in Table 2. No *Ae. hendersoni* were found in these locations, and at these sites, *Ae. triseriatus* oviposition in high traps occurred with a significantly higher frequency, relative to sympatric sites (Fig. 5).

Allopatric Ae. hendersoni Sites

Traps at four sites in the Dakotas did not receive any *Ae. triseriatus* eggs. Site-specific data appear in Table 2. *Ae. hendersoni* in these localities displayed a distinct preference for low traps, with frequencies of high trap oviposition events occurring significantly below 0.5 in each case.

Comparisons Among Occurrence Categories and Between Species

Ae. triseriatus

Tests for differences among the three occurrence categories sampled for *Ae. triseriatus* revealed no significant difference between



Fig. 3. Scatterplots of eggs collected per trap per sampling interval transformed by y' = ln(y + 1) against trap height for each combination of occurrence category (allopatric, marginal, sympatric) and species. Robust regressions and confidence intervals for predicted values are overlaid for each combination.

	Sampling location	Ν	Ae. triseriatus			Ae. hendersoni	
			EOE	Fraction high	N	EOE	Fraction high
Sympatric populations	Worcester, MA	7,130	235	0.09	2,835	125	0.88
	Uxbridge 1, MA	2,896	96	0.22	835	37	0.94
	Uxbridge 2, MA	4,483	148	0.05	942	42	0.84
	Hadley, MA	2,190	72	0.05	1,207	53	0.93
	Easthampton, MA	7,728	255	0.08	2,975	131	0.95
Marginal populations	Fort Ransom 1, ND	60	2	0.08	1,141	50	0.02
	Fort Ransom 2, ND	420	14	0.07	1,899	84	0.64
	Mandan, ND	16	1	1.00	334	15	0.63
	Valley City, ND	110	4	0.00	997	44	0.18
Allopatric populations	Homen 1, ND				2,324	102	0.34
	Homen 2, ND				807	36	0
	Bismarck, ND				487	21	0.22
	Spearfish, SD				210	9	0.40
	Bangor 1, ME	461	15	0.13			
	Bangor 2, ME	708	23	0.25			
	Orono, ME	477	16	0.14			

Table 2. Total egg counts (*N*) and estimated oviposition events (EOE) at all sites sampled, for each species, with the fraction of those events that occurred within high ovitraps

Significant deviations from 0.50 (χ^2 , 1 df, α = 0.05) based on EOE are indicated in bold; all sites deviated significantly from 0.50 when based on total egg counts.





Fig. 4. Mosaic plot of trap data for *Aedes triseriatus* and *Ae. hendersoni* in sympatric sites, pooled across five locations. Numbers are based on estimated oviposition events. Width of each vertical column indicates relative abundance of each species, and height of each rectangle indicates relative frequency of occurrence within high and low traps.

the height selections of sympatric and marginal populations, while allopatric populations differed significantly from each, showing a higher frequency of oviposition in high traps (Fig. 5).

Ae. hendersoni

Tests for differences among the three occurrence categories sampled for *Ae. hendersoni* revealed significant differences for all comparisons, with allopatric populations showing lower habitat preferences, populations from areas marginal to the range of *Ae. triseriatus* showing intermediate height preferences, and populations

Fig. 5. Proportions of oviposition events occurring in high ovitraps, for all species occurrence category and species combinations. The overall proportion, pooled across sites is shown for each group, and 95% confidence intervals are shown for each proportion. Groups that are not significantly different (as indicated by Marascuilo pairwise contrasts) are indicated by common letters below each column.

sympatric with Ae. triseriatus showing distinctly higher habitat preference.

Data for the six combinations of species occurrence category and species appear in Fig. 5. Comparing the allopatric populations of both species, the frequency of oviposition events in high traps is similar and not significantly different. Results in Fig. 5 show that a clear difference between these species is apparent in sympatry, and convergent habitat preference has occurred for each species in the absence of the other.

Discussion

Oviposition Events and Total Egg Numbers

Our statistical tests relied mainly on estimates of the number of oviposition events, based upon previously published estimates of batch sizes for both species (Walker et al. 1987). While this deliberately conservative approach made independence of observations more likely for χ^2 tests, numerous egg counts for both species fell well below those batch size estimates, suggesting that batch size can vary. Many factors are likely to affect realized egg batch sizes in nature, including bloodmeal success and efficiency, physiology and behavior of gravid females, and highly varied spatial and temporal environmental pressures. Multiple batches laid within a gonotrophic cycle (skip oviposition) is a possible factor for both species, as has been found in a number of other aedine species including Aedes aegypti (Linnaeus) (Diptera: Culicidae) (Apostol et al. 1994, Reiter et al. 1995, Colton et al. 2003, Reiter 2007, Rey and O'Connell 2014), Aedes albopictus (Skuse) (Diptera: Culicidae) (Gaugler et al. 2011, Yoshioka et al. 2012, Rey and O'Connell 2014, Davis et al. 2015), and Aedes japonicus (Diptera: Culicidae) (Theobold) (Oliver and Howard 2005), and has been suggested as a likely oviposition strategy in Ae. triseriatus (Kitron et al. 1989).

Sympatric Stratification

The preferences of Ae. hendersoni and Ae. triseriatus for high and low habitats, respectively, are clearly evident in this study. This observation is consistent with expectations of competition-based resource partitioning, and thus supports the viability of competition as one possible explanation, or as a complimentary driving factor. Because the degree of vertical stratification observed in sympatric populations is varied and typically incomplete, it is likely that additional factors are at play, and that additional research in a variety of areas will prove illuminating. For example, a longer growing season in southern climates may permit more temporal separation of the larval stages of the two species, while varied geography and complex landscape features may alter spatial distributions throughout the sympatric range. The presence or absence of additional species are likely to affect both larval habitat selection and the degree of interspecific competition between Ae. hendersoni and Ae. triseriatus. This includes additional competitive container breeding species as well as differentially harmful infectious agents such as A. barretti (Copeland and Craig 1992b) or potentially competition-mediating predators such as Toxorhynchites rutilis (Coquillett) (Diptera: Culicidae) (Bradshaw and Holzapfel 1983). While it does not overwinter successfully in the sympatric areas sampled in this study (Livdahl, personal observation), T. rutilus does occur in at least one Massachusetts sampling location, although occurrences are only recent (Dennehy and Livdahl 1999) and only late in the season after relatively mild winters.

Allopatric Diversification and Convergence

In areas sampled beyond the range of *Ae. triseriatus, Ae. hendersoni* exhibited a reduced fraction of oviposition in high traps, suggesting that the vertical position axis of their niche has shifted downward in allopatry, made possible perhaps by the absence of the strong competitor *Ae. triseriatus* at basal sites. In allopatric areas sampled in Maine, *Ae. triseriatus* shows a higher fraction ovipositing in higher traps, indicating an upward shift in habitat preference relative to sympatric populations. Both of these shifts are consistent with the competition hypothesis for the evolution of resource partitioning. Moreover, the two species show similar vertical habitat preferences

in allopatric locations (Fig. 5), a result that is also consistent with a competition-based hypothesis.

Marginal Situations

Preference for basal habitats by Ae. hendersoni was maintained at two sites (Fort Ransom 1 and Valley City sites, ND) which were designated as marginal, and which had two of the lowest proportional values for Ae. triseriatus eggs (<5% and <10%, respectively). Meanwhile, the marginal site with the highest proportional value of Ae. triseriatus eggs (>18%) was the only site in North and South Dakota where a shift to a significant preference for canopy habitats was observed. This supports speculation that Ae. hendersoni alters its oviposition behavior in response to Ae. triseriatus presence (either evolutionarily or by behavioral plasticity), and suggests a possible proportional threshold for eliciting such a behavior. It is also possible that populations of Ae. triseriatus must first become sufficiently established, both quantitatively and temporally, before a shift in Ae. hendersoni oviposition behavior occurs. As these localities are beyond the published range of Ae. triseriatus, it is possible that Ae. triseriatus occurs only sporadically or has only recently occupied those areas. In either case, adaptive responses to competition with that species should be less marked, consistent with the intermediate vertical habitat preference of Ae. hendersoni. Whether these responses are the result of natural selection or behavioral plasticity remains to be addressed. The latter would require a mechanism of species recognition, while the former would require a genetic basis for flight elevation tendencies.

Future Research and Inquiry

The consistency of these results with theoretical expectations does not constitute proof that competition has caused the evolution of different height preferences of these two species in sympatry, because alternative explanations can be posed, and not all of them can be refuted. In particular, parasitism by *A. barretti* within the range of its native host *Ae. triseriatus*, and the differential adverse influence that species has on *Ae. hendersoni*, may have been a contributing factor. Additional factors include, but are not limited to, behavioral plasticity, possible differences in habitat availability as well as climatic differences between and within allopatric and sympatric ranges. Further work in allopatric regions might be revealing, e.g., as Maine, southern Texas and peninsular Florida all have contrasting climates and are all areas with allopatric *Ae. triseriatus* populations. Similarities or differences among the three regions could inform us about the importance of climate in driving vertical habitat preferences.

An apparent association exists between habitat type and height, and the species could be using elevation as a cue to lead them toward a favored habitat type. Two major categories of tree holes, pans, and rot holes, can each be found at many levels, but pans tend to be more common basally and rot holes seem more common at higher levels. As such, habitat type may be another aspect of the species' niches that has diverged in sympatric situations, in association with height. If so, we expect that allopatric populations would be more opportunistic and likely to be distributed more evenly across both habitat types.

Differential hatch times and critical diapause photoperiods of the two species represent a temporal dimension by which divergence could reduce competition in sympatry. To our knowledge, no extensive study of the hatch times of these two species has been performed.

Prior to and during this project, the North and South Dakota region was experiencing a severe drought, and although many naturally occurring larval habitats were located, almost all of those observed in the region (with one exception) were dry. Since suitable larval habitats were so rare, it is possible that gravid females were less selective about oviposition location. Conversely, it is possible that the drought conditions could actually strengthen selection for basal habitats based on their typical resistance to desiccation. In either case, as each specific ovitrap location offered both a basal and a corresponding canopy trap, the opportunity to select one or the other was present to every gravid female, thus it can safely be concluded that basal traps were preferred. An expanded study to cover multiple years, including drought and non-drought years, would be necessary to clarify the role of drought.

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