

Comparative Analysis of the Dispersal Behaviors of Californian and Hawaiian *Tetragnatha* Spiders

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ABSTRACT

Dispersal behaviors and biogeographical patterns are inextricably linked as organisms evolve and adapt to their environments to ensure the best chance of survival. Long distance dispersal has allowed organisms to colonize populations in remote locations. However, once established, dispersal reduction becomes necessary to maintain the population. While some organisms have evolved to lose phenological traits to prevent long distance dispersal, spiders are unable to lose the physical structures used for dispersal, since silk is used not only for dispersal, but also mating, feeding, and habitat establishment. Therefore, dispersal reduction in spiders must occur in behavioral changes rather than phenological changes. In order to understand how dispersal behavior changes for spiders living on islands, *Tetragnatha stellarobusta* spiders from Hawaii and *Tetragnatha versicolor* spiders from California were observed in dispersal trials and tiptoe, rappelling, and bridging behaviors were recorded. For all three behaviors, *T. stellarobusta* exhibited more conservative dispersal behavior than *T. versicolor*, demonstrating dispersal reduction in the island species.

KEYWORDS

Dispersal reduction, ballooning, evolution, ecology, biogeography

INTRODUCTION

Understanding biogeographical history is crucial to understanding the evolution of organisms and their traits. Dispersal is an integral part of this biogeographical history, specifically for studying the speciation and diversification of organisms. While long-distance dispersal has largely been regarded as seemingly random and largely unsuccessful occurrences, these events have led to the establishment of entire ecosystems in remote locations and can explain how similar organisms can exist in geographically distant areas. Dispersal, specifically long-distance dispersal, comes at a cost. Therefore, reduction in dispersal has evolved throughout many lineages, more commonly for organisms in island systems. Individuals that are able to reach an isolated island and continue to disperse widely will likely be unsuccessful, and those that do not disperse widely will populate the island and create offspring with similar dispersal patterns (Gillespie et al. 2012). In these scenarios, selection against individuals that disperse will occur.

Charles Darwin was the first of many to make observations about the reduction in dispersal behavior after he found that island bird and beetles had insufficient wings for flight (Waters et al. 2020). Wings largely have the sole purpose of dispersal, and when reduced dispersal becomes favorable, sufficient and large wings become selected against and flightlessness becomes more prominent. Reduced dispersal is not so simple when the primary method of dispersal serves more than one purpose. The primary method of dispersal for spiders is to use silk. While silk is necessary in order for spiders to disperse, silk is not exclusively used for dispersal. In addition to dispersal, spiders use silk for catching prey, creating webs, burrowing, and building egg sacs (Lubin and Suter 2013). Because of the versatility and necessity of silk for multiple functions, dispersal reduction in spiders is more prevalent in behavioral changes rather than morphological ones. While many studies have shown loss of wings in organisms with reduced dispersal ability, few studies have investigated reduced dispersal in organisms that have retained their capacity for dispersal movement (Jessop et al. 2018).

For spiders, the most common methods of dispersal are ballooning and rappelling, both of which utilize silk. Both behaviors consists of pre-dispersal behavior in which the spider determines if air and environmental conditions are suitable. Ballooning is long-distance aerial dispersal in which spiders appear to be flying. The necessary drag for ballooning is provided by the silk and the spider's body, and spiders are able to control drag with their posture and length of silk (Suter

1991). Most ballooning occurrences travel a few hundred meters, but maximum recorded dispersal distances have reached hundreds of kilometers (Reynolds et al. 2007). While the behavior of ballooning is common, the conditions required for spiders to balloon are specific and unique to each species and individual. Suggested stimuli that affect a spider's ballooning propensity include temperature and humidity changes, light intensity, stress, air movement, age, and size (Weyman 1993). Comparative studies of ballooning behavior in spiders have been conducted, for example variation in dispersal behavior has been found wasp spider *Argiope bruennichi*, which has undergone rapid range expansion, with spiders closer to the edges of the range demonstrating reduced ballooning behavior as those spiders are in close proximity to the boundaries of the suitable habitat for the population (Wolz et al. 2020). However, island and continental differences have not been directly investigated in spider dispersal behaviors. In addition to ballooning, rappelling is a common dispersal behavior for spiders. Rappelling behavior occurs when a spider remains tethered to their original surface using a safety thread while it moves around its environment. While rappelling is a method for spiders to navigate their immediate environment itself, rappelling can also serve as a precursor to other dispersal behaviors, including ballooning and bridging (Lubin and Suter 2013).

The objective of this study is to compare dispersal behaviors between species of Tetragnatha spiders. This study aims to determine the factors that cause some spiders to have a higher dispersal propensity than others, and will investigate the correlation between dispersal behavior and geographical distribution of these spiders. Factors observed include age and region. I hypothesize that dispersal reduction will be observed in Hawaiian spiders in comparison to Californian spiders and dispersal behavior will be highest in younger spiders as smaller spiders are better able to disperse aurally.

METHODS

Study species

The spider genus Tetragnatha, commonly known as long-jawed orb-weavers, has undergone adaptive radiation, which is the rapid diversification of an ancestral species into several ecologically different species. Because of the isolation of oceanic islands, the Hawaiian islands

now contain around 60 species of Tetragnatha that are incredibly diverse in color, shapes, sizes, and behaviors (Gillespie). Within Hawaii, some species have a greater distribution than others. Most are endemic to only one island or area, while some are distributed across the majority of islands. Tetragnatha can also be found in California, and those found in California have origins tied to those in Hawaii. Greater genetic diversity can be found between the continental Tetragnatha of California and the island Tetragnatha of Hawaii. Two species of Tetragnatha spiders were tested in this study: *T. stellarobusta* from Hawaii and *T. versicolor* from California. The *versicolor* are brown web-builders from California and the *stellarobusta* are brown web-builders from Hawaii.

Spider collection and care

Adult spiders were collected by hand. Hawaiian *T. stellarobusta* were collected from Waikamoi Preserve in Maui in June 2019. Californian *T. versicolor* were collected from Lake Anza in Tilden Regional Park in September 2019. Adult spiders were fed *Drosophila melanogaster* weekly and were kept in plastic containers 6x6x15 centimeters in size with a mesh lid for ventilation and a stick to provide substrate. Californian adult spiders were kept at room temperature with natural sunlight. Hawaiian adult spiders were kept inside of an incubator that maintained a temperature of cycle of 19 – 21°C and a light cycle of 12 hours light and dark, mimicking the natural environment of the spiders.

Female spiders were checked weekly and any eggs found were removed from their adult container to be incubated. To incubate the eggs, the silk coating the eggs were carefully removed with tweezers and the eggs were then placed directly into a deli cup lined with a cupcake liner paper and perforated with ventilation holes. The deli cups were kept in a larger container with a wet paper towel at the bottom to maintain adequate humidity. All incubation containers were kept inside of the incubator. Incubating eggs were checked weekly to see if the spiders had hatched. Once hatched, the clutches of spiderlings were moved from the deli cups to small plastic containers 3x3x6 centimeters in size with small mesh placed inside to provide substrate and a mesh lid for ventilation. The age of the clutches of spiderlings were determined as days since they first hatched. Spiderling containers were also kept in the incubator at the same light and temperature cycle as the eggs.

Dispersal behavior trials

To test for dispersal behavior with the spiderlings, a modified 20-gallon tank was used to reduce the effects of air drafts in the room. To create a constant draft, a fan tilted upward at an angle of 70 degrees was placed at one end of the tank. Using a paint brush, individual spiderlings were carefully placed on the tip of a vertical wooden skewer placed 20 centimeters away from the fan. The skewer was placed in a small cup with water to prevent the spiders from rappelling down onto the base of the tank. From the location of the stick, the wind speed from the fan was consistently between 1.1 meters per second and 1.3 meters per second. We used red light headlamps in a dark room to run the dispersal trials as the study spiders are nocturnal.

A total of 156 dispersal trials were run. Once the spiderlings were positioned on the skewers, the fan was turned on signaling the beginning of the trial. Throughout the trial, three dispersal behaviors were recorded: “tip-toe”, “rappelling”, and “bridging”. Tip-toe behavior occurs when spiders stand on the tips of their legs and raise their abdomen upward in the air. Rappelling behavior entails the spiderling allowing itself to fall from the skewer and ride the air while remaining attached to the skewer with a safety thread. Bridging occurs when spiders release silk that attaches to a new surface while remaining tethered to their original surface, and the spider uses its silk as a bridge between the two surfaces. Trials were run for four minutes or until the spider bridged to the end of the tank. During that time, the time of the first tiptoe, number of tiptoe behaviors, number of rappels, and duration of rappels were recorded. Temperature, humidity, spider age, and species were recorded for every trial. The tank was wiped with a brush in between trials in order to remove any silk left by the previous spider.

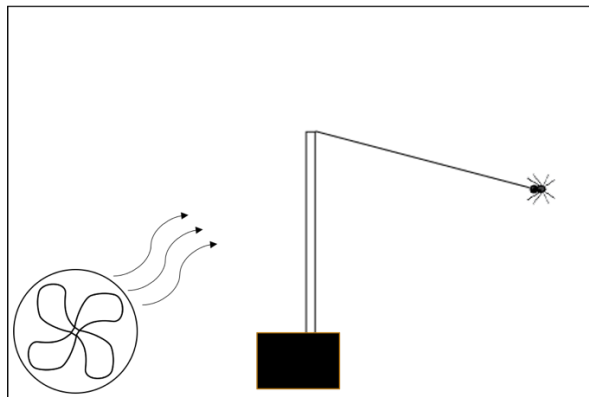


Figure 1. Diagram of experimental set up

Statistical analysis

Differences in the dispersal behavior between *T. versicolor* and *T. stellarubusta* spiders and age patterns were analyzed using R-studio Version 3.6.1 (2019). To determine if differences in ballooning propensity exist between spiders from continental and island locations exist, I used T-tests to examine the number of tip-toes observed within trials and the time of the first tiptoe in a trial. To determine if differences in dispersal propensity exist between species, I used a T-tests to compare number of rappels and duration of rappels within trials. To determine the significance of bridging behavior between the two regions, I used a Chi-Squared Test. To examine the effect of age in dispersal behavior across the two species, I categorized the ages that I had recorded in days into categories of weeks and used the Kruskal-Wallace test to compare spider age to the number of times spiders tiptoed and rappelled during trials. Lastly, I used a T-test to compare the ages of the spiders that had bridged to the ages of the spiders that had not.

RESULTS

Dispersal behavior across species

I conducted 156 dispersal trials, 61 of those observations were *T. versicolor* from California and 95 were *T. stellarobusta* from Hawaii. Though the time at which the two species performed the first tiptoe behavior were not significantly different (T-test, $df = 13.087$, $p = 0.2161$), the average tiptoe counts for *T. versicolor* spiders (2.820 ± 3.304) were significantly higher than *T. stellarobusta* spiders (0.505 ± 1.147) (T-test, $df = 69.386$, $p = 1.459e-6$). Similarly, while the average duration that the spiders rappelled for were not significant across the two species (T-test, $df = 295.45$, $p = 0.9488$), *T. versicolor* spiders rappelled more on average (2.525 ± 2.481) than *T. stellarobusta* spiders (1.737 ± 2.179) (T-test, $df = 116.02$, $p = 0.04484$). Lastly, *T. versicolor* spiders successfully bridged more often than *T. stellarobusta* spiders (Chi-Squared = 8, $df = 1$, $p = 0.004687$).

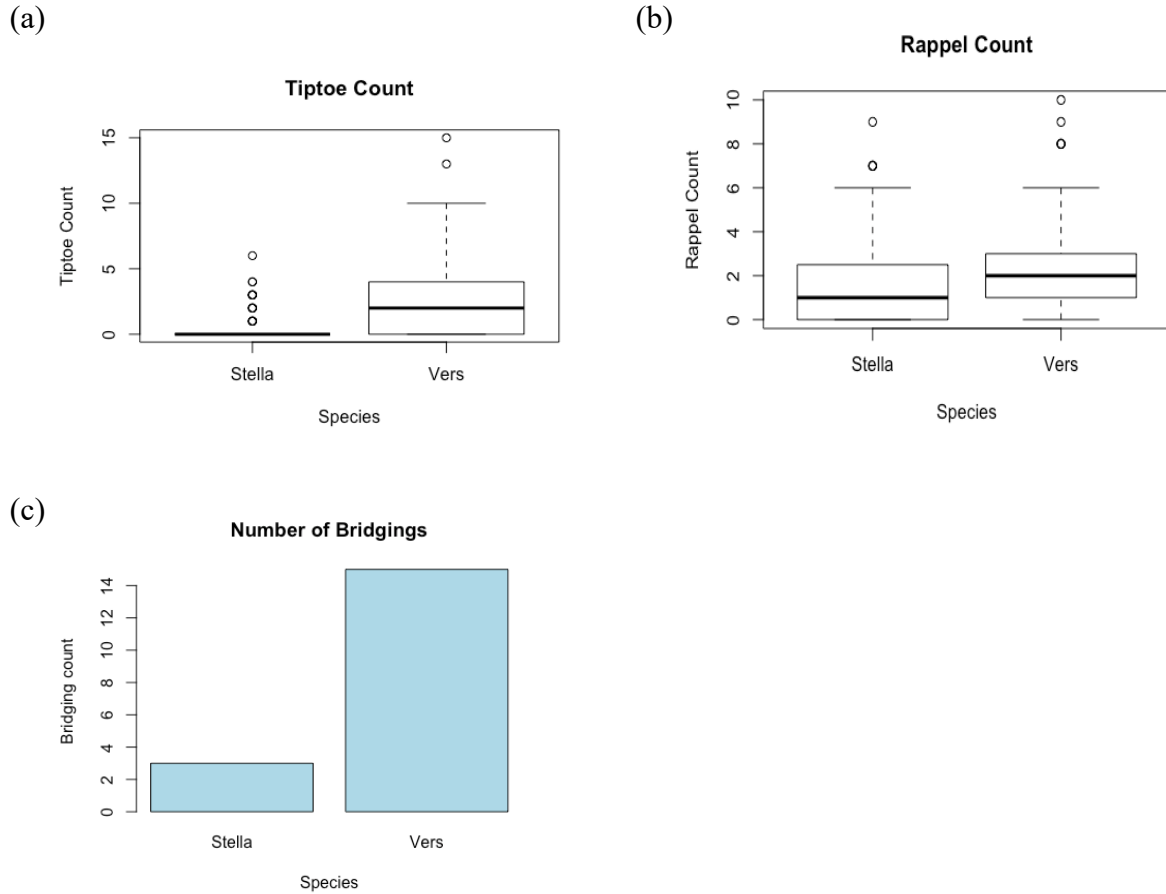
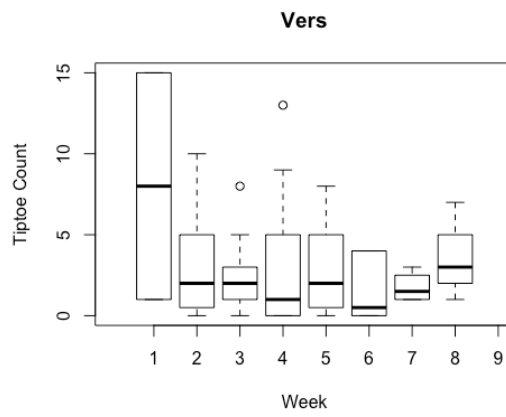
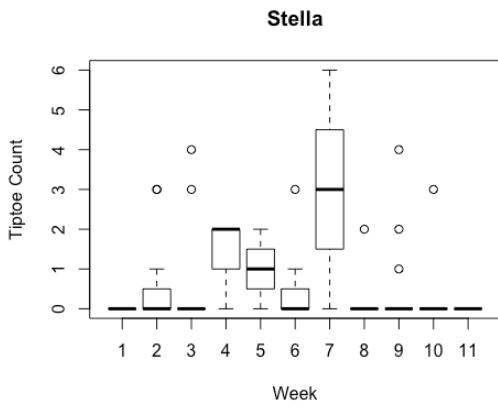


Figure 2. Species comparisons for dispersal behaviors. All plots show *T. stellarobusta* behavioral counts on the left and *T. versicolor* behavioral counts on the right. (a) Box-and-whisker plot comparing tiptoe counts. (b) Box-and-whisker plot comparing rappel counts. (c) Bar chart comparing bridging counts.

Influence of age on dispersal behavior

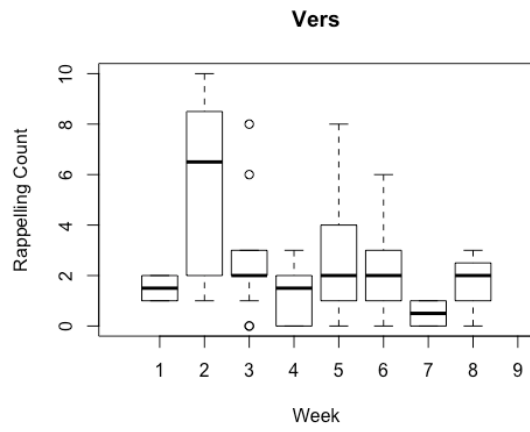
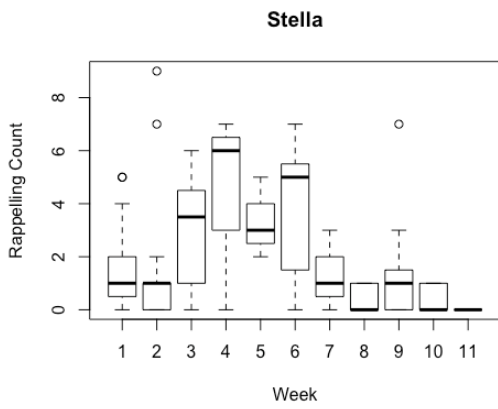
For both study species, the distribution of tiptoe counts by age in weeks did not vary significantly (KW Test, *T. stellarobusta* $p = 0.081$, *T. versicolor* $p = 0.881$). Rappelling counts on the other hand also did not show significant differences by age in weeks for *T. versicolor* (KW test, $p = 0.075$), but did show that *T. stellarobusta* had a significant peak in tiptoe counts at 4 weeks (KW test, $p = 0.018$). The ages of the spiders that did bridge were not significantly different than the ages of the spiders that did not bridge (T-test, $df = 25.58$, $p = 0.7519$).

(a) (b)



(c)

(d)



(e)

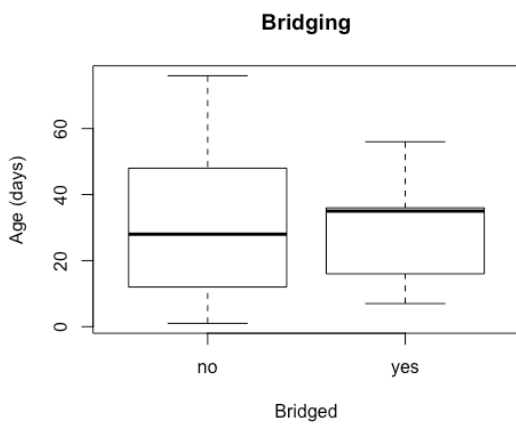


Figure 3. Age result data for dispersal behaviors.

DISCUSSION

The probability of surviving a dispersal event determines dispersal propensity, and variation in spatial habitat alone reduces dispersal rates (Weyman 1993). For all three behaviors observed (tiptoe, rappelling, bridging), Hawaiian spiders performed the behaviors less often than the Californian spiders, suggesting a relationship between dispersal behavior and whether the spiders were from island or continental regions. Age had almost no statistically significant results for the behaviors tested for both of the spider species.

Biogeography and dispersal patterns

This study found significant differences between *T. versicolor* spiders from California and *T. stellarobusta* spiders from Hawaii for all dispersal behaviors tested. Tiptoeing is the behavior which is most directly linked to ballooning, or long-distance aerial dispersal, and this behavior was much more common in the species from California. This finding supports our initial hypothesis that island spiders have a reduction in dispersal behavior in comparison to continental spiders, specifically in ballooning behavior in which the spider has less control over the dispersal. Previous studies on Lycosid wolf spiders have found that tiptoe behavior is only performed for ballooning (Bonte et al. 2007). Other studies with Linyphiid spiders have noted that while tiptoe behavior can precede both long-distance ballooning and short-distance rappelling, spiders perform the tiptoe behavior in order to test the suitability of air conditions for ballooning, and can ultimately take off from this position (Bonte et al. 2009). Because tiptoe behavior was less frequent in the Hawaiian spiders than Californian spiders in this study, dispersal propensity is suggested to be reduced in island species.

Although rappelling is very common in all spiders for movement within their immediate environment, such as for descending from branches or making webs, rappelling also has strong links to being a precursor to longer distance dispersal behavior (Lubin and Suter 2013). Studies have categorized rappelling as a pre-ballooning behavior directly, specifically when spiders continued to release silk while rappelling in order to create a sail for ballooning, at which point the spiders will cut the safety silk thread that kept them anchored to the original surface (Tolbert 1977;

Wolz et al. 2020). In our study, the Californian spiders had higher rappel counts than Hawaiian spiders, further supporting the hypothesis that island organisms have reduced dispersal behavior.

This variation of behavior of the two study species remains consistent for the last dispersal behavior observed, which is bridging. In practice, the behaviors of bridging and ballooning via rappelling are very similar. In both behaviors, the spider remains tethered to its original surface while releasing additional silk. The difference being what happens to the extra released silk. The distal end of the silk attaches to a new surface resulting in bridging, while the released silk creates a sail and the safety thread is cut resulting in ballooning (Lubin and Suter 2013). Due to the pre-dispersal behaviors being very similar, it can be difficult to determine the intention of the spider when releasing that extra silk. In this study, bridging was the most definitive dispersal behavior observed, as it was the only one in which the spider was actually able to completely leave its original surface. The difference between the bridging counts for Hawaiian and Californian spiders was the most significant of the behaviors observed, with Hawaiian spiders again exhibiting reduced dispersal behavior.

Age and dispersal behavior

This study found that age had a relatively low influence on the dispersal behavior of spiders across the two species tested. Significant results relating age and dispersal behavior were only found in *T. stellarobusta* for rappelling behavior, but no significant difference was found in *T. versicolor* or for tiptoe and bridge behaviors. The average age of the spiders that did complete a tiptoe and bridge were not significantly different than the average age of the spiders that did not complete that behavior. Similarly, no difference was found between weeks for tiptoe behavior, implying that dispersal behavior is not dependent on age. A relationship was found between age for *T. stellarobusta* spiders and rappelling count, at around 4 to 6 weeks.

These findings may be the result of measuring age rather than instar. My hypothesis for higher dispersal activity in younger spiders was based on the finding that adult spiders are often too large to disperse long distances (Weyman 1993). However, all subjects were slow growing and were within the first instar. While the subject species included a wide age range of 0 to 60 days, the lack of instar range can explain the homogenous behavioral pattern. Arthropods experience developmental stages, known as instars, which more directly correlate to differences in appearance

and behavior (Aiken and Coyle 2000). Expected differences in dispersal behavior would be more visible if subjects were compared based on instar, rather than age. Other studies have intentionally limited their scope to test only spiders within a certain instar, further supporting the expectation that spiders within the same instar will behave similarly (Walter et al. 2005). In order to understand the changes in dispersal behavior throughout the lifespan of spiders, future studies could include trails of spiders in different instars. Studies have found conflicting results on the instar at which dispersal behavior is the highest, for example, peak ballooning activity was found during the third and fourth instar for *Erigone arctica* spiders (Weyman 1993), while the peak for Lycosids was found during the second instar (Walter et al. 2005). These conflicting reports suggest that instar-based dispersal behavior depend on the species of spider. While this study was limited in not being able to determine a specific peak for ballooning or dispersal behavior for *Tetragnatha* spiders, this study can confirm that dispersal and pre-dispersal behaviors are common in *Tetragnatha* spiders within the first instar.

Future research

To further inspect the relationship between biogeography and dispersal behavior, species with more diverse population distributions could be tested, such as Hawaiian *Tetragnatha* spiders found on multiple islands. Lastly, while some species in the *Tetragnatha* genus build webs, other species have lost ability to build webs and have more spines on their legs which serve as their primary prey capture method. Possible links could exist between dispersal behavior and web-building attributes as a sign of habitat investment.

Conclusion

In this study, island spiders demonstrated reduced dispersal propensity in comparison to continental spiders. This finding supports Charles Darwin's theory that long-distance dispersal traits are less common in island species after his observation that the majority of beetles on the island of Madeira were unable to fly (Waters et al. 2020) by investigating dispersal methods other than flightlessness. Long-distance dispersal carries a higher risk on islands and areas of smaller land mass than areas of larger land mass. Organisms on continents are more likely to survive long

distance dispersal, especially when methods offer less control to the organism. Prior to this study, dispersal differences between continental and island organisms have primarily been limited to research on flightlessness due to morphological changes. The results of this study demonstrate that behavioral changes, even while organisms still have the phenological capacity to disperse, have evolved over time to reduce dispersal in remote regions.

ACKNOWLEDGEMENTS

This study would not have been possible without the mentorship, guidance, and support of Seira Ashley Adams of Berkeley Evolab. Other members of Berkeley Evolab that I would like to thank are Professor Rosemary Gillespie and the graduate students for their feedback and advice, and the undergraduate students for helping run dispersal trials. Thank you to the ESPM 175 teaching team, specifically Patina Mendez and Leslie McGinnis, for their constant support over the past year. I would also like to thank my ESPM 175 cohort, especially Dara Sengchanthavong, Nhu Nguyen, Tiffany Lwin, and my peer review group, for allowing me to share ideas and learn from you all. Last but not least, I would like to thank my family, partner, and friends for encouraging me throughout my undergraduate career and always being willing to listen to me ramble on and on (and on) about spiders.

REFERENCES

- Aiken, M., and F. A. Coyle. 2000. Habitat distribution, life history and behavior of Tetragnatha spider species in the Great Smoky Mountains National Park 28:97–106.
- Bell, J. R., D. A. Bohan, E. M. Shaw, and G. S. Weyman. 2005. Ballooning dispersal using silk: world fauna, phylogenies, genetics and models 95:69–114.
- Bonte, D., B. Bossuyt, and L. Lens. 2007. Aerial dispersal plasticity under different wind velocities in a salt marsh wolf spider 18:438–443.
- Bonte, D., N. D. Clercq, I. Zwertvaegher, and L. Lens. 2009. Repeatability of dispersal behaviour in a common dwarf spider: evidence for different mechanisms behind short- and long-distance dispersal 34:271–276.

- Gillespie, R. G., B. G. Baldwin, J. M. Waters, C. I. Fraser, R. Nikula, and G. K. Roderick. 2012. Long-distance dispersal: a framework for hypothesis testing. Elsevier, Kidlington.
- Jessop, T. S., A. Ariefiandy, D. Purwandana, J. Imansyah, C. Ciofi, Y. J. Benu, D. A. Fordham, D. M. Forsyth, R. A. Mulder, and B. L. Phillips. 2018. Exploring mechanisms and origins of reduced dispersal in island Komodo dragons.
- Lubin, Y., and R. B. Suter. 2013. What is the function of “pre-dispersal” behavior in juvenile social spiders (*Stegodyphus dumicola*: Eresidae)? American Arachnological Society.
- Reynolds Andy, M., A. Bohan David, and R. Bell James. 2007. Ballooning dispersal in arthropod taxa: conditions at take-off 3:237–240.
- Suter, R. B. 1991. Ballooning in spiders: results of wind tunnel experiments 3:13–25.
- Tolbert, W. W. 1977. Aerial dispersal behavior of two orb weaving spiders 84:13–27.
- Vandergast, A. G., R. G. Gillespie, and G. K. Roderick. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian Tetragnatha spiders: fragmentation, rapid population growth and the potential for accelerated evolution 13:1729–1743.
- Walter, A., P. Bliss, and R. F. Moritz. 2005. The wasp spider *Argiope bruennichi* (Arachnida, Araneidae): ballooning is not an obligate life history phase 33:516–522.
- Waters, J. M., B. C. Emerson, P. Arribas, and G. A. McCulloch. 2020. Dispersal Reduction: Causes, Genomic Mechanisms, and Evolutionary Consequences. Elsevier Ltd.
- Weyman, G. S. 1993. A review of the possible causative factors and significance of ballooning in spiders 5:279–291.
- Wolz, M., M. Klockmann, T. Schmitz, S. Pekár, D. Bonte, and G. Uhl. 2020. Dispersal and life-history traits in a spider with rapid range expansion 8:1–11.