Samantha Wunderlich[1]

Predator Driven Trait Changes in *Anolis smaragdinus*

Abstract

Background: Research on the evolution of traits in regard to predator-prey relationships can illuminate the effects of migrating species and invasive species on native populations, which is increasingly relevant with heightened anthropogenic environmental disturbances shifting territories of native species.

Methods: We used a replicated field experiment to test the effects that predators have on the phenotype of *Anolis smaragdinus* in the Bahamas. It was predicted that *A. smaragdinus* would rapidly evolve over six generations to have shorter fore and hind limb lengths due to the presence of the predator, *Leiocephalus carinatus*, driving *A. sagrei* and *A. smaragdinus* into higher habitats, which selects for shorter limb lengths.

Results: ANCOVAs were performed for male and female *A. smaragdinus*, where only two of the thirteen measured traits, ulna length and third toe length in females, were found to have a significant year by treatment interaction between 2011 and 2017. We observed a general reduction in mean trait size when predators are present. Therefore, predators may be driving *A. smaragdinus* to higher perching sites which selects for smaller limb length. Also, it was found that populations of *A. smaragdinus* may be able to evolve in as little as six generations when predators and competitors are present.

Limitations: A longer study is needed to see if other trait changes would become significant over time, since the residual means are trending in one direction. Another suggestion is to do a study with fewer experimental islands and larger founder populations. This would violate the meaning of a true founder population; however, it may allow *A. smaragdinus* to become fully integrated within the new environment and lead to a smaller chance of extinction.

Conclusion: The study showed that the introduction of predators may cause significant phenotypic changes in female *A. smaragdinus'* ulna and third toe length, while also showing a general reduction in mean trait size when predators are present. Therefore, predators may be driving *A. smaragdinus* to higher perching sites which selects for smaller limb length. However, the study did not show a significant change in either the fore limb length or the hind limb length between 2011 and 2017 which was predicted originally.

Introduction

With a rapidly changing climate, species are being driven into predator-occupied territories to remain in optimal environmental conditions (1). It is essential to study how species interact with each other when they have previously been ecologically naive, since the interactions between invasive and native species can affect each group's behavior, morphology, and survival rates (2). Research on trait evolution resulting from predator-prey relationships can illuminate the effects of migrating species and invasive species on native populations, which is increasingly relevant with heightened anthropogenic environmental disturbances.

There are many examples of directional trait changes in prey species, such as the directional evolution of the peppered moth towards more darkly-colored wings in the presence of high smog levels (3-4) and the evolution of Mullerian mimicry in butterflies (5-6). These classic studies exemplify a directional evolutionary response caused by increased predation on organisms that are not adapted to a novel or changing environment. These are just some of the possible mechanisms that prey species have evolved to escape predation; another mechanism is behavioral change. It has previously been shown that when predators are introduced to prey, the latter change their use of habitat (7-8). This mechanism was illustrated by studies showing crayfish moving to more protective habitats in response to the presence of predators (9-10). Therefore, prey are able to adjust their use of habitat as a strategy to avoid predation.

Anolis is a genus that has been studied extensively with regard to mor-

phology and behavior in the Bahamas (11). *Anolis* species have repeatedly radiated into different ecomorphs, which are adapted to different microhabitats (15-17). The species *Anolis smaragdinus* and *Anolis sagrei* are two different ecomorphs which have developed said adaptations (18). *A. sagrei* is a competitive species that is able to reach high population densities, is highly invasive, and can outcompete many native species of lizards (19-21). It is part of the trunk-ground ecomorph, signifying that it perches on the lower trunks of trees and on objects on the ground. *A. smaragdinus* is part of the trunk-crown ecomorph, as it has a tendency to occupy the uppermost trunks of tall trees and the lower canopy. Consequently, it is better adapted to perching and running on smaller branches (22) and it is larger than *A. sagrei* (23).

Previous research has found that *A. sagrei* moves to higher vegetation to escape the predator, *Leiocephalus carinatus*, (24). Similarly, *A. carolinensis* will partition its habitat when *A. sagrei* is introduced as a competitor species (25). *A. smaragdinus* is subgroup of *A. carolinensis* (26); therefore, they are in the same ecomorph and are highly related. Shorter limbs of *A. carolinensis* and *A. smaragdinus* help increase manoeuvrability on branches; previous research has found that in *A. sagrei* natural selection favours shorter hind limbs when they occupy higher perches (27). However, that study did not look at the changes in limb length over multiple generations. Another study has found that the toe pads of *A. carolinensis* evolve rapidly when *A. sagrei* are introduced (28). Literature regarding predator introduction and the consequent habitat shift of *A. sagrei* on other species of lizard, such as *A. smaragdinus*, is lacking. It has been suggested that two closely co-occurring related species could diverge when interactions

are negatively occurring (29). Therefore, we predict that *A. smaragdinus* will rapidly evolve over six generations to have shorter fore and hind limb lengths due to the presence of the predator, *L. carinatus*, driving *A. sagrei* and *A. smaragdinus* into higher habitats, which selects for shorter limb lengths due to competition. Additionally, by using a portable x-ray machine to measure changes in *A. smaragdinus'* traits between 2011 and 2017, we will be able to obtain more precise measurements compared to using a tape measure or a caliper since gravity can affect growth (30).

Methods

Field Site

16 small islands located near Staniel Cay, in Exuma Bay of Grand Bahama Bank (26.17°, −76.44°) were chosen for our experiment. These islands have been used for experimentation since 1977 (31). Islands were included in the study if they met the following criteria: presence of robust *Anolis sagrei* (brown anole) population; absence of any other detectable lizard or top-predator species; and presence of trees more than two meters tall which are deemed necessary to support *Anolis smaragdinus* (green-anole) populations (32). *A. sagrei* was censused on the 16 islands in May 2011, during which time no other lizard species were detected.

Islands were given one of four treatments: control islands had only *A. sagrei* on them as the native lizard species, and the remaining experimental islands had *A. sagrei* with *L. carinatus* and *A. smaragdinus*; *A. sagrei* with only *A. smaragdinus*; or *A. sagrei* with only *L. carinatus*. Island treatments were assigned by a random coin flip. Islands were first stratified by size and measured in terms of vegetated area, such that two replications of each treatment would be assigned to the eight smallest and the eight largest islands. *A. smaragdinus* were collected from Staniel Cay by noose since *L. carinatus* were absent on this island; thereby both *Anolis* were ecologically naive to the predator, *L. carinatus*. On other islands these lizards co-exist, therefore they were not evolutionarily naive.

Field Work

In 2011, experimental islands were surveyed for initial lizard species and populations that were present. *A. smaragdinus* was caught opportunistically from Staniel Cay with a noose and pole or by hand and kept captive for a couple of days. We used Isoflurane to anesthetize the lizards (33) and x-rayed them using a portable x-ray machine. After x-rays were taken, the lizards were weighed, and a small piece of tail tissue was obtained for DNA sequencing. *A. smaragdinus* was assigned randomly in groups of ten or eleven to islands with five to seven *L. carinatus* of unknown sex. These numbers were chosen to simulate colonization by small founder populations (34).

In 2017, we surveyed the lizard populations on the experimental islands and caught *A. smaragdinus* to measure phenotype traits with a portable x-ray scanner, holding them captive for a maximum of 24 hours. After x-rays were taken, the lizards were weighed and a small piece of tail tissue was extracted for DNA sequencing. In order to prevent the immediate recapture of individuals, each lizard was marked with nail polish that would be removed with the lizards next molting. When catching lizards, bodies were checked for nail polish and tail integrity to ensure no recapture of individuals. *A. smaragdinus* were found on only two of the four experimental islands at the time of measurement.

Analysis

One researcher measured skeletal traits related to locomotion and niche using the ObjectJ plugin for ImageJ (35; Fig. 1). Measurements were taken on both sides of the *A. smaragdinus* sagittal plane in order to average the measurements. If a lizard had an abnormality, such as a missing claw, then only the data from the undamaged side of the lizard was used for that specific measurement.

R (v. 1.1.456) (36) was used to prepare the data for analysis. Sagittal plane measurements that were averaged were converted into millimeters. *A.*

smaragdinus were checked for recapture by looking at the Euclidean distance between measurements of each island by sex, and if this value was zero then the x-rays and field notebook data was analyzed. Additionally, only adult lizards (male snout-vent length (SVL) > 45.0 mm; 37) were used in subsequent analyses. Due to unclear literature regarding size cut-offs for adult females, a threshold was obtained using graphical analysis which determined a cut off of SVL > 37.5 mm. After data cleaning, there were a total of 39 lizards removed based on SVL measurements and field data. Analysis of Covariance (ANCOVA) was used to evaluate changes in phenotype measurements over time with respect to experimental treatment. Since *A. smaragdinus* are sexually dimorphic (38), analysis was separated by sex. We pooled lizards by treatment, whether a *L. carinatus* was present or absent, and used linear models of the form trait ~ SVL + year*treatment with the uncorrected size measurements. The year by treatment interaction was used to test whether predation caused phenotypic evolution.

In order to examine the phenotypic trait changes more generally, we looked at the changes in the relative size-corrected traits through time by treatment. Size corrections were performed using SVL as a covariate for body size where the relative trait measurements were the residuals from sex-specific linear regressions of trait by SVL. These measurements were compared to the residual lengths means of each trait to determine the direction of trait change found in the ANCOVA.
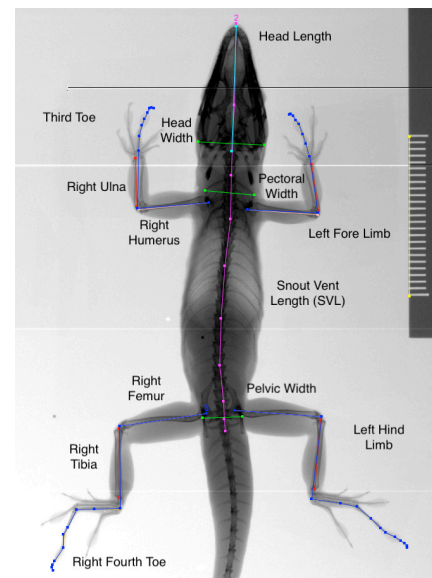

Fig. 1. Object J Screenshot of Vector Measurements of Skeletal Traits. Measured traits include snout-vent-length (SVL), head width, head length, pectoral width, right and left humerus, right and left ulna, right and left third toe, right and left forelimb, pelvic width, right and left femur, right and left tibia, right and left fourth toe, right and left hind limb, and ruler.

Results

In 2011, we analyzed 78 lizards and in 2017, we analyzed 83 lizards; 84 female and 77 male (N=161) x-rays of *A. smaragdinus* were used for this study. For both males and females, measured traits tended to become smaller between 2011 and 2017 on islands with predators and larger on islands without predators. When predators were present on the islands, the means of the residual lengths of the traits decreased from 2011 to 2017 in all traits for the males except their pelvic width, humerus, and SVL (which all increased). When the predators were absent, the means of the residual lengths increased in all traits except for head width, pelvic width, and femur length, where they decreased. In females the means of the residual lengths of traits decreased from 2011 to 2017 for all traits except for pelvic width and pectoral width when predators where present. When predators were absent, all the means of the trait residual lengths increased in females.

We performed ANCOVAs for male *A. smaragdinus*, where none of the thirteen measurable traits were found to have a significant year by treat

ment interaction between 2011 and 2017 (p > 0.05) when using the uncorrected traits. ANCOVAs were conducted for female *A. smaragdinus* to determine if there was variation between the trait measurements on a year by treatment interaction between 2011 and 2017. Only two of thirteen measured traits were found to have a significant year by treatment interaction: third toe length ($F_{1, 79}$ = 4.292, p = 0.0415; Fig. 2) and ulna length ($F_{1, 79}$ = 4.231, p = 0.043; Fig. 2). The rest of traits were not found to be significant on a year by treatment interaction basis (p > 0.05).
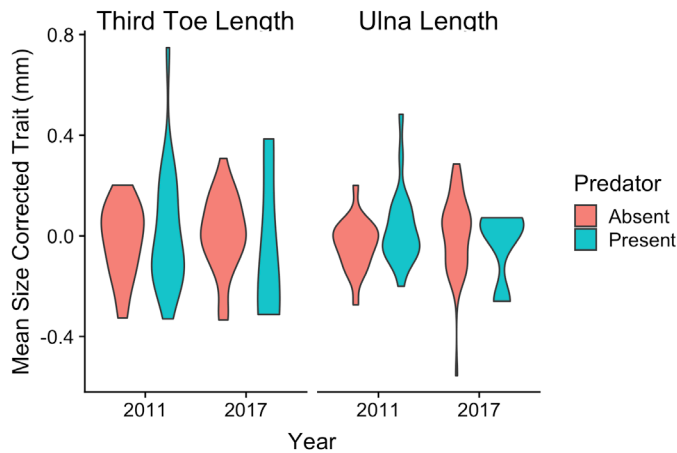

Fig. 2. Violin Plot of the Mean Size Corrected Traits, Third Toe Length and Ulna Length. This plot looks at the change in the mean size corrected trait in millimeters between years 2011 and 2017 for females on islands with or without predators.

Discussion

We used a replicated field experiment to test the effects that predators have on *Anolis smaragdinus* phenotype in the Bahamas. It was predicted that *A. smaragdinus* would rapidly evolve over six generations to have shorter fore and hind limb lengths due to the presence of the predator, *L. carinatus*, driving *A. sagrei* and *A. smaragdinus* into higher habitats, which selects for shorter limb lengths. ANCOVAs were performed for male and female *A. smaragdinus*. Only two of the thirteen measured traits were found to have a significant year by treatment interaction between 2011 and 2017: ulna length and third toe length in females.

In general, mean residual lengths of the traits decreased about one millimeter from 2011 to 2017 when predators were present. Notable exceptions are SVL in males and pelvic width and pectoral width in females, where the mean residual lengths increased. When predators were absent the traits tended to increase in their mean residual lengths. However, in males none of these differences were significant, and in females only the changes in the third toe length and ulna length were significant on p < 0.05 in the ANCOVA; we did not find a significant change in the whole fore limb or the full hind limb in the ANCOVA. A possible explanation for the reduction in limb length trends is that when a predator is introduced into *A. smaragdinus*' habitat, the lizards change the way they use their habitats. This would coincide with previous research in that lizards will begin to perch higher to avoid the predator (39). Another possible reason for the reduction in the mean residual trait lengths between 2011 and 2017 is that islands are generally populated by a small population of colonizers that are especially susceptible to genetic drift. Over a period of six generations, it is possible for extremely small founder populations to experience genetic drift due to the founder effect. However, previous research has found that *Anolis* maintains genetic diversity over seven generations in small populations on Dominica (40). Also, the mean residual lengths of the traits tended to increase in predator absent islands. This may be explained by the lack of predators preying on *A. sagrei* and *A. smaragdinus*, which usually forces *A. smaragdinus* into higher vegetation and selects for smaller phenotypes (41). Furthermore, *A. smaragdinus* were transplanted from

very large islands with very tall vegetation to smaller islands. Therefore, the changes we see may be due to adapting to new, smaller islands rather than the predator's effects. Previous research has found that natural selection can adjust morphology of lizards after exposure to habitats of different structures (42).

We may not have found significance in many traits in the ANCOVA due to the low population size of *A. smaragdinus* and high population densities of *A. sagrei* in our experiment. In order to simulate a founder population, we only transplanted 10-11 *A. smaragdinus* on to each island, simulating a colonization event. This gives a low statistical power for our ANCOVA. Additionally, only two out of four experimental islands had *A. smaragdinus* remaining on them. Therefore, our results are conditional that the lizards survive to establish a colony from 2011 and 2017. Furthermore, all experimental islands had high density populations of *A. sagrei*. Even though they are less adapted to arboreal habitats, their sheer density may have led to interspecific competition between them and *A. smaragdinus* for habitat space, leading to them outcompete *A. smaragdinus*.

Our research has shown that phenotypic traits can change significantly in as little as six generations when strong enough selection is present. This coincides with previous research showing that the interaction of species can cause phenotypic changes granted that the selection is strong enough on the trait (43). However, another study found that when birds and snakes were introduced to *A. sagrei* populations, selection did not alter phenotypic traits (44). Therefore, different types of predators may have different effects on prey and competitive species. Additionally, a longer study is needed to see if other trait changes would become significant over time, since the residual means are trending in one direction. Another suggestion is to do a study with fewer experimental islands and larger founder populations. This would violate the idea of a true founder population being a small group of members; however, it may be relevant as populations of species are migrating or invasive species are introduced into new habitats. Furthermore, it would ensure success of the *A. smaragdinus* and it would limit confounding variables thereby increasing colonization success (45). One technical advantage of our study is that we used x-ray measurements to compare the differences in limb lengths between 2011 and 2017. This gave us more precise measurements than measuring the outer limbs with a measuring tape or caliper since the bones themselves were measured and not the bones and tissue. Lastly, this allowed us to measure traits not frequently looked at like pectoral width and pelvic width.

Conclusion

Our study shows that the introduction of predators may cause significant phenotypic changes in female *A. smaragdinus*' ulna and third toe length. It also shows a general reduction in mean trait size when predators are present. Therefore, predators may be driving *A. smaragdinus* to higher perching sites which selects for smaller limb length. Our study did not show a significant change in either the fore limb length or the hind limb length between 2011 and 2017. Therefore, we show that populations of *A. smaragdinus* may be able to evolve in as little as six generations when predators and competitors are present. This is relevant since anthropogenic environmental disturbances may push invasive species and predators into new territories, therefore affecting the morphology of other species.

Acknowledgements

References

1. Rahel FJ, Olden JD. Assessing the effects of climate change on aquatic invasive species. Conservation biology. 2008;22(3):521-33.
2. Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE. The spread of invasive species and infectious disease as drivers of ecosystem change. Frontiers in Ecology and the Environment. 2008;6(5):238-46.

3. Clarke CA, Mani G, Wynne G. Evolution in reverse: clean air and the peppered moth. Biological Journal of the Linnean Society. 1985;26(2):189-99.
4. Grant BS. Fine tuning the peppered moth paradigm. Evolution. 1999;53(3):980-4.
5. Turner JR. Studies of Müllerian mimicry and its evolution in burnet moths and heliconid butterflies. Ecological genetics and evolution: Springer; 1971. p. 224-60.
6. Kapan DD. Three-butterfly system provides a field test of Müllerian mimicry. Nature. 2001;409(6818):338.
7. Schoener TW, Spiller DA, Losos JB. Predators increase the risk of catastrophic extinction of prey populations. Nature. 2001;412(6843):183.
8. Schoener TW, Spiller DA, Losos JB. Predation on a common Anolis lizard: can the food-web effects of a devastating predator be reversed? Ecological Monographs. 2002;72(3):383-407.
9. Stein RA, Magnuson JJ. Behavioral response of crayfish to a fish predator. Ecology. 1976;57(4):751-61.
10. Mittelbach G. Predator-mediated habitat use: some consequences for species interactions. Environmental Biology of Fishes. 1986;16(1-3):159-69.
11. Johnson MA, Revell LJ, Losos JB. Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in Anolis lizards. Evolution: International Journal of Organic Evolution. 2010;64(4):1151-9.
12. Andrews RM. Growth rate in island and mainland anoline lizards. Copeia. 1976:477-82.
13. Martin AP, Palumbi SR. Body size, metabolic rate, generation time, and the molecular clock. Proceedings of the National Academy of Sciences. 1993;90(9):4087-91.
14. Reznick DN, Shaw FH, Rodd FH, Shaw RG. Evaluation of the rate of evolution in natural populations of guppies (Poecilia reticulata). Science. 1997;275(5308):1934-7.
15. Losos JB, Jackman TR, Larson A, de Queiroz K, Rodrıguez-Schettino L. Contingency and determinism in replicated adaptive radiations of island lizards. Science. 1998;279(5359):2115-8.
16. Schluter D. The ecology of adaptive radiation: OUP Oxford; 2000.
17. Butler MA, Sawyer SA, Losos JB. Sexual dimorphism and adaptive radiation in Anolis lizards. Nature. 2007;447(7141):202.
18. Losos JB. Ecomorphology, performance capability, and scaling of West Indian Anolis lizards: an evolutionary analysis. Ecological Monographs. 1990;60(3):369-88.
19. Losos JB, Marks JC, Schoener TW. Habitat use and ecological interactions of an introduced and a native species of Anolis lizard on Grand Cayman, with a review of the outcomes of anole introductions. Oecologia. 1993;95(4):525-32.
20. Campbell TS. Analyses of the effects of an exotic lizard (Anolis sagrei) on a native lizard (Anolis carolinensis) in Florida, using islands as experimental units. 2000.
21. Gerber GP, Echternacht AC. Evidence for asymmetrical intraguild predation between native and introduced Anolis lizards. Oecologia. 2000;124(4):599-607.
22. Losos JB, Irschick DJ. The effect of perch diameter on escape behaviour of Anolis lizards: laboratory predictions and field tests. Animal Behaviour. 1996;51(3):593-602.
23. Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S. Morphological characteristics of the lizard Anolis carolinensis from South Carolina. Herpetologica. 1995:401-11.
24. Schoener TW, Spiller DA, Losos JB. Predation on a common Anolis lizard: can the food-web effects of a devastating predator be reversed? Ecological Monographs. 2002;72(3):383-407.
25. Kamath A, Stuart YE, Campbell TS. Behavioral Partitioning by the Native Lizard Anolis carolinensis in the Presence and Absence of the Invasive Anolis sagrei in Florida. Breviora. 2013:1-10.
26. Hedges SB, Burnell KL. The Jamaican radiation of Anolis (Sauria: Iguanidae): an analysis of relationships and biogeography using sequential electrophoresis. Caribbean Journal of Science. 1990;26(1-2):31-44.
27. Losos JB, Schoener TW, Langerhans RB, Spiller DA. Rapid temporal reversal in predator-driven natural selection. Science. 2006;314(5802):1111-.
28. Stuart YE, Campbell T, Hohenlohe P, Reynolds RG, Revell L, Losos J. Rapid evolution of a native species following invasion by a congener. Science. 2014;346(6208):463-6.
29. Brown WL, Wilson EO. Character displacement. Systematic zoology. 1956;5(2):49-64.
30. Cox RM, Calsbeek R. Severe costs of reproduction persist in Anolis lizards despite the evolution of a single-egg clutch. Evolution. 2010;64(5):1321-30.
31. Schoener TW, Schoener A. Distribution of vertebrates on some very small islands. I. Occurrence sequences of individual species. The Journal of Animal Ecology. 1983:209-35.
32. Losos JB, Spiller DA. Differential colonization success and asymmetrical interactions between two lizard species. Ecology. 1999;80(1):252-8.
33. Bennett RA, editor Reptile anesthesia. Seminars in avian and exotic pet medicine; 1998: Elsevier.
34. Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB. Founder effects persist despite adaptive differentiation: a field experiment with lizards. Science.
2012;335(6072):1086-9.
35. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature methods. 2012;9(7):676.
36. Team RC. R: A Language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018.
37. Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S. Morphological characteristics of the lizard Anolis carolinensis from South Carolina. Herpetologica. 1995:401-11.
38. Butler MA, Sawyer SA, Losos JB. Sexual dimorphism and adaptive radiation in Anolis lizards. Nature. 2007;447(7141):202.
39. Losos JB, Schoener TW, Langerhans RB, Spiller DA. Rapid temporal reversal in predator-driven natural selection. Science. 2006;314(5802):1111-.
40. Eales J, Thorpe R, Malhotra A. Weak founder effect signal in a recent introduction of Caribbean Anolis. Molecular Ecology. 2008;17(6):1416-26.
41. Losos JB, Schoener TW, Langerhans RB, Spiller DA. Rapid temporal reversal in predator-driven natural selection. Science. 2006;314(5802):1111-.
42. Vitt LJ, Caldwell JP, Zani PA, Titus TA. The role of habitat shift in the evolution of lizard morphology: evidence from tropical Tropidurus. Proceedings of the National Academy of Sciences. 1997;94(8):3828-32.
43. Brown WL, Wilson EO. Character displacement. Systematic zoology. 1956;5(2):49-64.
44. Calsbeek R, Cox RM. Experimentally assessing the relative importance of predation and competition as agents of selection. Nature. 2010;465(7298):613.
45. Ahlroth P, Alatalo RV, Holopainen A, Kumpulainen T, Suhonen J. Founder population size and number of source populations enhance colonization success in waterstriders. Oecologia. 2003;137(4):617-20.