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Inbreeding Depression in the Introduced Spider Latrodectus geometricus

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Inbreeding Depression in the Introduced Spider *Latrodectus geometricus*

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By
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ABSTRACT

The brown widow spider (*Latrodectus geometricus)* is thought to be native to South America or Southern Africa, but its distribution has expanded to most continents by human introduction. In the continental USA, *L. geometricus* was first documented in south Florida in the 1930's. In the early 2000's a population expansion occurred, and this species is now found in Florida, Georgia, South Carolina, Alabama, Mississippi, Louisiana, Texas, and southern California. Introduced species may face many obstacles when establishing a new population. One common obstacle might be severe inbreeding following founder events or genetic bottlenecks. The purpose of this study was to quantify inbreeding depression in an introduced population of *L. geometricus*. I predicted that if inbreeding was common for many generations during the introduction, many deleterious alleles should have been purged resulting in lower genetic load and consequently minimal fitness differences between inbred and outbred offspring. To assess the consequences of inbreeding, I compared hatching number between clutches produced by full-sibling crosses and crosses between unrelated individuals. The percentage of unhatched eggs/clutch was roughly 3-fold higher in inbred relative to outbred offspring, indicating a fitness cost to inbreeding. The results suggest that significant inbreeding has been avoided during the introduction of this species in the southeastern US. This may be the result of multiple and continued introductions being common during the recent range expansion leading to a minimization of inbreeding.

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Introduction

 An introduced species is one that has established populations in an environment where they are not native (Beebee & Rowe 2008). An invasive species is an introduced species, which can alter or interrupt an ecosystem by out-competing native species, which has a profound impact on the environment's biodiversity (Beebee & Rowe 2008). A little less than half of the species listed under the Endangered Species Act are considered at risk mainly due to competition with and predation by non-native species (Pimental *et al.* 2004). One study estimated the costs associated with damages to ecosystems by invasive species at $120 billion per year in the United States alone (Pimental *et al.* 2004).

 When species are introduced to a new environment, they can face many obstacles in order to establish a new population (Hedrick & Garcia-Dorado 2016). Some of these obstacles include adjusting to the environment, finding mates, and inbreeding (Beebee & Rowe 2008). In order for an introduced population to become established, individuals must be able to survive and reproduce at sustainable levels in the new environment. Many introduced populations go extinct early in the introduction because they are not able to overcome obstacles such as climate changes, finding food, or competing with native species (Hedrick & Garcia-Dorado 2016). If survival is possible, initial numbers may be a small enough that there is high probability to breed with siblings or other relatives (Facon *et al.* 2011). Inbreeding can ultimately help the population establish itself by increasing numbers, or it may facilitate the eradication of the newly introduced population if the fitness cost is too high.

 Inbreeding can lead to inbreeding depression when deleterious alleles, or alleles that result in reduced fitness relative to other alleles, are common (Hedrick & Garcia-

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Dorado 2016). Inbreeding depression is a decrease in viability and fitness due to increased frequency of homozygosity of deleterious recessive alleles (Neaves *et al.* 2015), and is commonly associated with reproductive traits and other traits that directly relate to fitness (Crnokrak & Roff 1999). Related individuals are genetically more similar to each other than they are to unrelated individuals. Siblings or close relatives are more likely to carry the same deleterious recessive allele than two unrelated individuals in a population. This results in a higher probability that sibling matings will produce offspring that are homozygous for deleterious recessive alleles. For example, if siblings that are heterozygotes carrying deleterious alleles mate, each offspring has a 25% of being homozygous recessive for the deleterious allele. If inbreeding levels are high and there are multiple genes carrying recessive alleles in the genome, a large proportion of offspring are not likely to survive, which causes a decrease in the number of individuals in a population (Beebee & Rowe 2008). From a sibling heterozygote cross, around 50% off the offspring could also be heterozygotes, which means there may be a large amount of individuals carrying the deleterious allele. As these offspring continue to inbreed, there will be a higher amount of deleterious alleles purged out as the homozygous recessive offspring die. After many generations, there is a possibility of only having individuals that are homozygous dominant, and the deleterious allele is not present in the population any longer. At this point, genetic load, the amount of unfavorable genetic traits present in the population, is reduced to a very low number. In any populations, the genetic load can be quantified by the reduction of fitness when inbreeding occurs (Aviles & Bukowski 2005).

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The consequences associated with population bottlenecks can involve two different courses that may either result in a population becoming extinct or the population ultimately thriving (Facon *et al.* 2011). Deleterious mutations could be lost (termed "purging of genetic load"), during bottlenecks, leading to an increase in mean population fitness in introduced areas relative to native areas (Facon *et al.* 2011).

When related individuals inbreed, there is an increased frequency of offspring that are homozygous for the deleterious alleles. If a high number of these alleles have lethal effects the homozygous individuals will not survive and the number of individuals in the population will decrease to a very low number or possibly to a level of extinction. If some of the individuals survive, the population may increase in average fitness (Pekkala *et al.* 2012). Of the individuals that survive, they should have a lower frequency of deleterious alleles because individuals carrying these alleles had high mortality levels. If the frequency of deleterious alleles decreases, subsequent inbreeding events should show lower levels of inbreeding depression (Facon *et al.* 2006). The process of eliminating these deleterious alleles by sacrificing some individuals is known as purging of deleterious alleles, which usually occurs over many generations, and can lead to a mean increase in population fitness (Pekkala *et al.* 2012, Tayeh et al. 2013). Once these alleles are completely purged, the population has increased fitness relative the original population, which may facilitate an increase in population size (Beebee & Rowe 2008). If there are a large number of individuals in an introduced population, inbreeding may not occur. The resulting population is constantly growing and there may be an increase in the number of deleterious alleles, or genetic load, over generations since no alleles are being

purged out from inbreeding. This scenario of population growth is common in larger or expanding populations.

  Latrodectus geometricus, also known as the brown widow spider, is a common introduced species on most continents. Thought to be originally native to the South America or Southern Africa, populations of brown widow spiders have quickly expanded across the globe by human introduction (Brown *et al.* 2008). In 1935, the first brown widows spiders were found present in the United States and confined to southern Florida (Vetter *et al.* 2012). These spiders can now be found in all of Florida, many parts of Georgia, South Carolina, Mississippi, Louisiana, Texas, and even some areas of southern California (Brown *et al.* 2008). Brown widows were first seen in Georgia in the 1990's, South Carolina in 2001, Mississippi in 2005, and other dates shown in Figure 1. It is possible that these spiders experienced a bottleneck event in southern Florida, which helped them to establish themselves and drastically expand their range; however, it is also possible that these spiders are so widespread due to multiple introductions to different locations.
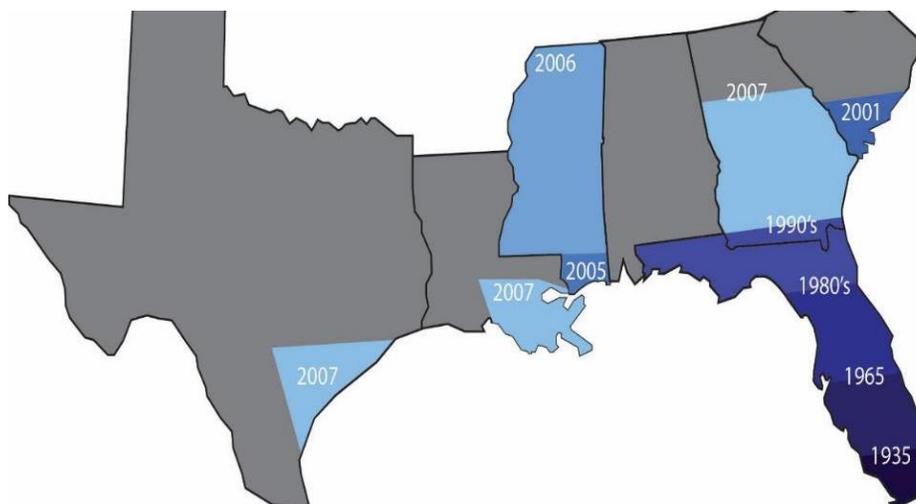

Figure 1. Dates of first observance of *L. geometricus* in the US.(Modified from Brown *et al.* 2008)

In this experiment, I will be determining the severity of inbreeding depression in introduced populations of *Latrodectus geometricus*. I will produce outbred and inbred crosses to determine if there are differences in fitness between them. If introduced populations have a long history of inbreeding due to bottlenecks in small populations in south Florida, they may be expanding because the population has been through purging of deleterious alleles and is recovering with more viable individuals. In this scenario a large decrease in fitness would not be expected when the spiders are inbred in the lab. The populations of these spiders could also be expanding due to continuous introductions, which would entail large genetic variation that has allowed the population to adapt to the environment (Roman & Darling 2007). If this is the case, the spiders will likely have a large genetic load and may be expected suffer severe inbreeding depression when they are inbred in the lab.

Methods

All spider rearing was carried out in a 27 $℃$ incubator with 50-60% humidity and a 12 hour light-dark cycle.

Rearing spiders for breeding

The spiders used for mating were raised in the lab so mating and relationship history were known. Spiders and egg sacs were collected from different areas around Statesboro, GA. The egg sacs were opened and offspring were put in individual cages and then fed 3-4 wingless *Drosophila* twice a week. Some of the spiders collected

produced clutches in the lab after they were caught, and these egg sacs were treated in the same manner. Once the spiders were mature enough for their sex to be determined visually (Mahmoudi *et al.* 2008) females were fed small mealworms while the males were still fed *Drosophila*. Outbred and inbred crosses were then created within the collection of spiders by grouping siblings or non-siblings.

Breeding spiders

Taking into account where the wild caught spiders were found, five outbred and five inbred crosses were established. The inbred crosses were planned between full siblings and the outbred crosses were between spiders whose mothers or egg sacs had been collected from different locations. When each of the females appeared large enough to breed, they were fed a mealworm and the pre-determined male was placed in the same cage with the female the next day. By feeding the female a day before the male was placed in the cage, the male had a higher chance of surviving mating because the female was distracted by food or full from the meal. Once the male was put in the cage, the pair was observed to see if there was any interaction. The male and female were left in the cage together for two days or until the male had been killed. After two days, the male and female were observed under a microscope to determine if they had mated. If the male's reproductive organs were in tact, there was a chance the two had not mated, so the female was observed for one week to detect the production of an egg sac. If the female did not produce a clutch after two weeks, other spiders were selected from the collection and another cross was set up.

Colleting egg and hatching numbers

When the female spiders began producing egg sacs, the day when the clutch was first seen was recorded. After two days, the sac was removed from the cage containing the female and placed in a petri dish. The egg sac was opened by using forceps and the number of eggs was recorded. The petri dishes containing the eggs were checked daily and the date of the first hatched egg was recorded. The eggs were then observed and the number of unhatched eggs was counted each day until the number did not change. Each time each female laid an egg sac, the clutch number, egg number, hatching date number, and the unhatched eggs numbers were all collected. Difference between inbred and outbred treatments in hatching numbers was determined using MANOA.

Development time

Development time was defined as the length of time from the date each clutch was produced to the date of the first egg hatching. This data set was recorded for each of the first three egg sacs produced by each female. Differences between inbred and outbred treatments were assessed by MANOVA tests.

Successive Clutches

To test for differences in successive clutches from individual females, the processes from the hatching number and development time were repeated for the first three egg sacs a female produced. This test was to determine if there was different maternal investment into any of the successive clutches.

Inbreeding Coefficient

The coefficient of inbreeding depression was calculated by using the mean inbred (X_1) and outbred (X_O) percent hatched numbers. This value was also calculated in a way

to standardize by the level of inbreeding for full sibling mating (F=0.25) by dividing the inbreeding coefficient by 0.25 (Crnokrak & Roff 1999). These two values can be compared to other experiments regardless of the measure of fitness used (Crnokrak & Roff 1999).

$$\delta = 1 - (\frac{X_1}{X_0})$$

Results

      Six inbred (full sibling) and outbred crosses were created from lab-reared spiders. The first three clutches were used for determining hatching number and development time. The clutches from each spider, inbred and outbred crosses, contained from 100-120 eggs (Table 1).

Table 1: Average egg numbers for Clutches 1, 2, and 3, of inbred and outbred crosses

	Clutch 1	Clutch 2	Clutch 3	Total number of eggs
Inbred	121 ($\pm$ 10.2)	125 ($\pm$ 11.3)	106 ($\pm$ 18.6)	1990
Outbred	118 ($\pm$ 8.1)	121 ($\pm$ 9.9)	109 ($\pm$ 6.0)	2084

The numbers of eggs that did not hatch were used to calculate the percent unhatched of each group of successive clutches. The mean percent unhatched eggs of successive clutches did not differ for either inbred (C1=0.293 $\pm$ 0.062, C2=0.192 $\pm$ 0.053, C3=0.273 $\pm$ 0.083) or outbred crosses (C1=0.084 $\pm$ 0.018, C2=0.049 $\pm$ 0.029, C3=0.076 $\pm$ 0.029). MANOVA F=1.704, DFnum=2, DFden=7, p=0.249 for inbred and F=1.064, DFnum=2, DFden=7, p=0.374 for outbred (Figure 2).
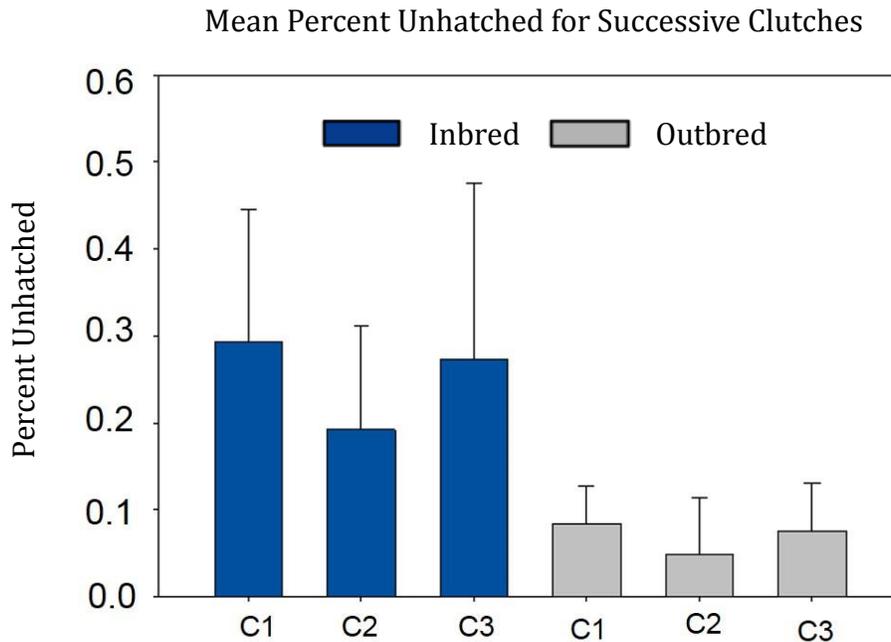
Figure 2. Mean % unhatched eggs (± s.e.) for each of the first three clutches of eggs for inbred and outbred crosses. Hatching numbers did not differ between successive clutches for inbred (F=1.704, DFnum=2, DFden=7, p=0.249) or outbred (F=1.064, DFnum=2, DFden=7, p=0.374).

Egg clutches 1-3 did not differ allowing all eggs sacs to be combined to compare a total number of eggs that did not develop to hatching between inbred and outbred corsses. The average percentage of unhatched eggs was 20% higher in inbred crosses (0.269 ± 0.062) compared to the outbred crosses (0.071 ± 0.015) (Figure 3). This difference was found to be significant (F=7.495, DFnum=1, DFden=8, p=0.0255. The coefficient of inbreeding depression for percent hatched was 0.2137. When this number was standardized for full sib mating (F=0.25), the standardized coefficient of inbreeding was 0.855.
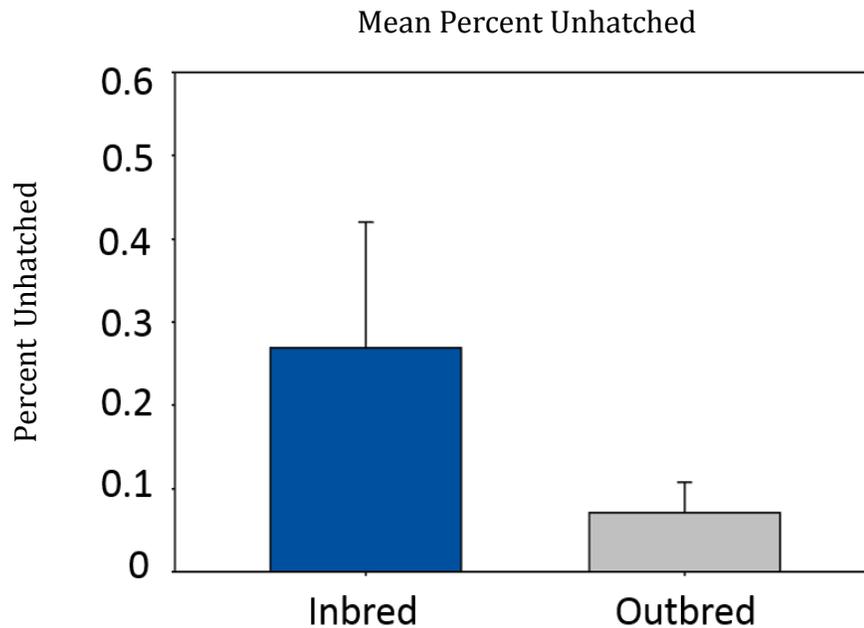
Figure 3. Total mean % unhatched eggs ($\pm$ s.e.) of six inbred and and six outbred crosses.   The number of unhatched eggs was 20% higher in inbred relative to outbred crosses (F=7.495, DFnum=1, DFden=8, p=0.0255).

The first date the clutch was seen and the date the first egg from that clutch hatched were used to calculat the development time. The development time of successive clutches did not differ for either inbred (C1=8.33 $\pm$ 0.56, C2=9.40 $\pm$ 0.75, C3=9.40 $\pm$ 0.51 days) or outbred crosses (C1=8.83 $\pm$ 0.55, C2=8.00 $\pm$ 0.45, C3=8.00 $\pm$ 0.52 days) with inbred F= 0.1987, DFnum=2, DFden=7, p=0.8243 and outbred F=0.787, DFnum=2, DFden=7, p=0.763 (Figure 4).
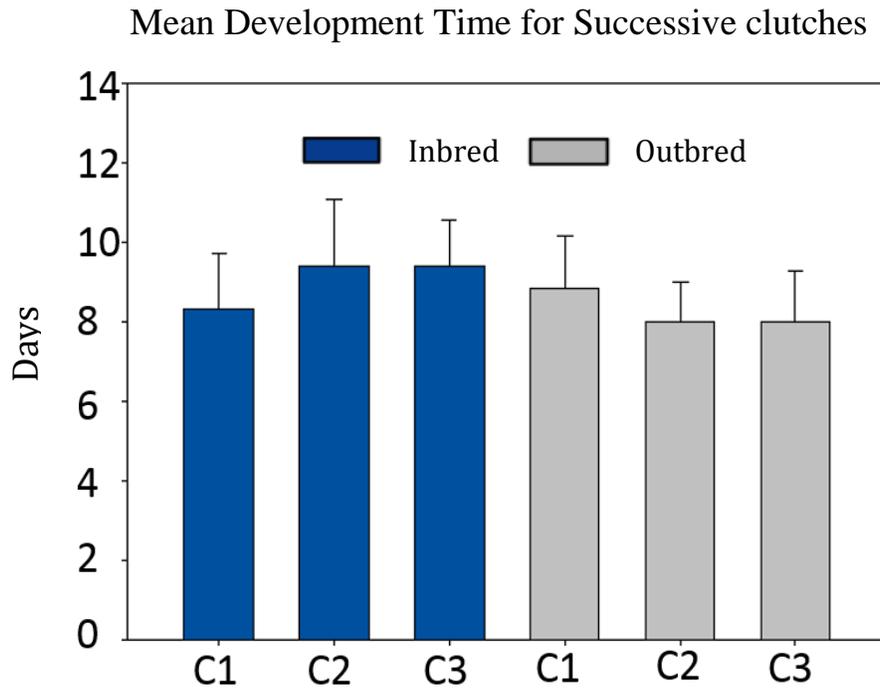
Figure 4. Mean development time ($\pm$ s.e.) in days for each of the first three clutches of eggs for inbred and outbred crosses. Development time did not differ between successive clutches for inbred (F=0.1987, DFnum=2, DFden=7, p=0.8243) or outbred (F=0.787, DFnum=2, DFden=7, p=0.763) crosses.

Egg clutches 1-3 did not differ in development time allowing all eggs sacs to be combined to compare a total development time between inbred and outbred corsses. The average development times for inbred crosses (9.04 $\pm$ 0.34 days) and outbred crosses (8.28 $\pm$ 0.29 days) were not a significantly different (F= 1.4596, DFnum=1, DFden=8, p=0.2952) (Figure 5).
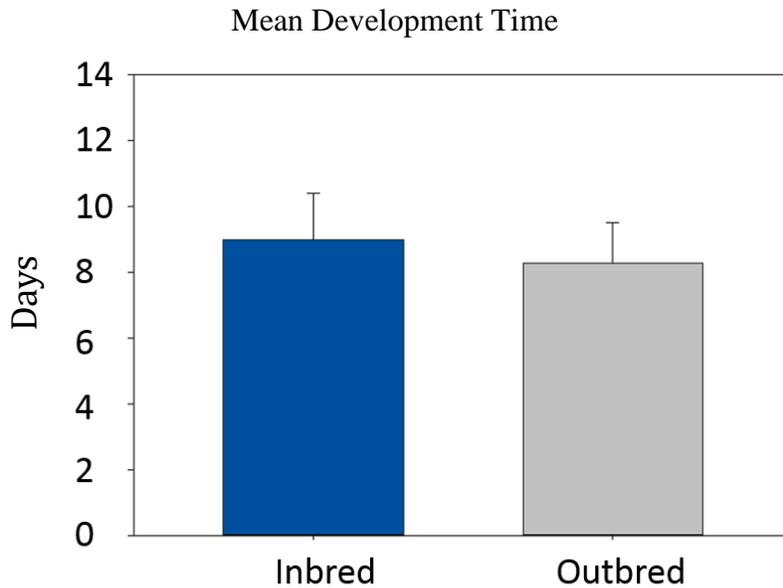
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Figure 5. Total mean development time ($\pm$ s.e.) for inbred and outbred crosses. Development time did not differ between cross type (F=1.4596, DFnum=1, DFden=8, p=0.2952).

Discussion

Brown widow spiders have expanded their introduced range to most continents. This species must have traits amenable to successfully establish viable populations during introductions. From an initial introduction in southern Florida in 1935, the range has increased drastically over the last 20 years to cover the entire southeastern US (Brown *et al.* 2008). Introduction of a small number of individuals into a new environment can increase the probability the population will face inbreeding, which may be necessary in overcoming propagule pressure (Facon *et al.* 2011) .

The objective of this study was to determine if reduced fitness occurs in inbred crosses from an introduced population of *L. geometricus*. I can use this information to make conclusions about genetic load and inferences about levels of inbreeding during the introduction. The hatching numbers and the development time of inbred and outbred

clutches were used to show the extent of inbreeding depression in this population. The inbreeding coefficient was used to compare the level of inbreeding found in this study compared to other studies.

The results of this experiment showed a significantly higher number of eggs that did not hatch in clutches produced from inbreeding relative to outbred clutches. The significant difference in hatching number shows that there is a significant decrease in fitness when these spiders are inbred. Because hatching number is affected, this suggests that deleterious alleles in the population have a measurable effect on fitness and there are a significant number of lethal alleles individually or in combination. On the other hand, there was not a significant difference in development time in terms of time to hatching. This suggests that there are few deleterious alleles in this population that affect the development time or they are masked by lethal alleles early in development. The results also showed no significant different between clutches, which is consistent with equal maternal investment into each clutch.

Hatching number proved to be a good measurement of fitness. One way to interpret the results of development time is that there is no significant difference because there are no deleterious alleles that affect development time to hatching. Another way to interpret the data is that development time to hatching may not have been a good measure of fitness and there may be a better factor to measure, like time to reach sexual maturity or the time interval for all of the eggs to hatch. The final way to interpret this data is that individuals with a high load of deleterious alleles for early development were lethal, so early development time was not observable.

Since there was a reduction in fitness in inbred crosses, pertaining to hatching numbers, I am able to conclude that this introduced population possesses significant genetic load (inbreeding load). Had there been a history of significant and prolonged inbreeding in the southeastern US during the introduction, minimal fitness differences between inbred and outbred clutches would have been expected (Hedrick & Garcia-Dorado 2016). Although the data of when these spiders came into the US suggests that a bottleneck was likely (Facon *et al.* 2011), one possible explanation is multiple introductions of this species into different areas in order for the population to spread.

The coefficient of inbreeding depression is used as a way to standardize inbreeding depression from the experiment presented here with studies of other species in the wild (Crnokrak & Roff 1999). A study by Crnokrak and Roff (1999) shows the difference of the coefficient of inbreeding depression in wild and captive populations. The coefficient of inbreeding depression usually ranges from 0 to 1, but the number may be outside of that range if the inbred offspring are more successful than outbred offspring (Crnokrak & Roff 1999). The coefficient of inbreeding depression found in our experiment (0.2137) is similar to those found for wild populations that did not have a history of inbreeding. Crnokrak & Roff (1999) found that the coefficient of inbreeding was on average 0.268 in Homeotherms, 0.197 in Poikilotherms, and 0.264 in plants. When corrected for levels of inbreeding, these values were 0.818, 0.661, and o.552 respectively. The inbreeding coefficient corrected for full sib mating in this study was 0.855 suggesting a high cost of inbreeding.

In nature, it may take many generations of inbreeding to completely purge deleterious alleles from inbreeding population. A study conducted on small population

size of *Drosophila* involved 19 generations of inbred offspring to completely purge the genetic load (Swindell & Bouzat 2006). In order for this population of spiders to purge all of their genetic load, there would likely have to be many generations of inbreeding to unmask all deleterious recessive alleles (Swindell & Bouzat 2006). The individuals that are homozygous recessive for the deleterious allele would perish; therefore, the remaining individuals would have a higher frequency of non-deleterious alleles and there would be a lower frequency of the deleterious alleles in the population. If the population survives this purging of the deleterious alleles, the average overall fitness of the population should improve (Swindell & Bouzat 2006). This improvement in population fitness could be a catalyst for population expansion in introduced species.

When there are multiple introductions of populations into an area, the individuals may never encounter inbreeding situations due to the large population numbers. Multiple introductions also allow for more genetic variation in the population, which could also mean a higher genetic load (Roman & Darling 2007). The widespread distribution of these spiders across the southeastern US and the reduction of fitness when inbred indicates that multiple introductions into different areas are likely. The expanding population of this species is similar to the pattern of expansion of human populations around the globe. When there is nothing to keep the amount of deleterious alleles in check, the genetic load increases gradually and the fitness of the species may be compromised if a bottleneck situation were to occur (Facon *et al.* 2011).

Although the data collected suggests that these spiders do not commonly inbreed in nature, there are plenty of opportunities for these populations to be exposed to potential inbreeding situations. When searching for these spiders around the Statesboro

area, many buildings had twenty or more spiders around the exterior with most of them having multiple egg sacs in their web. These buildings supply a great sanctuary for siblings to live in close contact with each other, which may expose the population to inbreeding. If these spiders around the same building are not relatives, there must be a way for them to effectively disperse to different locations in order to avoid inbreeding, or they may have other tactics to avoid mating with siblings. Since I have found that these spiders experience significant inbreeding depression, the explanation may be the latter. From a study of the subsocial spider *Stegodyphus lineatus* it was suggested that spiders may carry few deleterious alleles because of a high incidence of sibling matings in nature (Bilde *et al.* 2005). However, a study of the sexually cannibalistic spider *Argiope bruennichi* that had a recent range expansion showed a decrease in hatching rate of 29% in inbred relative to outbred crosses (Zimmer *et al.* 2014). This level of inbreeding depression is similar to values reported in the brown widow in this study suggesting inbreeding is not common.

There are many mechanisms that can be used in order for species to avoid inbreeding in nature. Some of these mechanisms that pertain to spiders include different timing of maturation to reproductive age between genders, chemical signaling, kin recognition, or dispersal (Bilde *et al.* 2006). In one study, it was found that certain spiders can favor more compatible (non-sibling) sperm when involved in polyandry and get rid of sibling sperm they may also have stored (Welke & Schneider 2009). When siblings were paired together in our experiment, they would usually mate; however, the result may have been different if the spiders had been given a choice between a sibling and

unrelated mate. With all of these mechanisms, organisms can usually avoid inbreeding in nature, which decreases the chances of inbreeding depression (Bilde *et al.* 2006).

Purging of alleles can be shown in lab settings, but this process has not commonly been observed in wild populations (Facon *et. al* 2011). *Latrodectus geometricus* have been very successful in establishing and expanding introduced populations on most continents. In summary, the results found in this study suggest that these successes are most likely not due to purging out any deleterious alleles in early introduction. While inbreeding can play a major role in helping the population to become established, in the southeastern US any levels of inbreeding were not sufficient enough to remove a large portion of deleterious alleles (Hedrick & Garcia-Dorado 2016). *Latrodectus geometricus* may have mechanisms to avoid inbreeding in nature even in small populations. It is likely that the recent range expansion in the southeastern US has involved introduction events maintaining genetic diversity.

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