Ontogenetic shift in agonistic behavior of the Southern black widow spider, *Latrodectus mactans* (Araneae: Theridiidae)

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Ontogenetic shift in agonistic behavior of the Southern black widow spider, *Latrodectus mactans* (Araneae: Theridiidae)

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College Scholars Senior Thesis

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Defended: May 1\textsuperscript{st}, 2009
Ontogenetic shift in agonistic behavior of the Southern black widow spider, *Latrodectus mactans* (Araneae: Theridiidae)

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**Summary.**

The behavioral phenotype of an organism may undergo ontogenetic shifts and also change with context. I tested whether aggressiveness exhibited by female black widow spiders (*Latrodectus mactans*) was positively associated with increasing age and with levels of current reproductive investment. Response to a predatory cue (front and rear prods with a probe) was used in the assessment of spider aggressiveness. The prod was applied to test individuals at seven prematuration instars and at three different reproductive stages following maturation: virgin, mated, and egg guarding. In general, aggression levels increased in intensity with developmental stage. Adult females guarding egg cases exhibited the highest levels of aggression in response to the predatory cues. However, within five minutes following the removal of the females’ egg cases, levels of aggressiveness decreased to that recorded for mated females that had yet to produce egg clutches. I conclude from this pattern of test results, that aggressiveness is not merely under ontogenetic control, but is context dependent as well.

Keywords: Antipredator behaviour, ontogeny, aggressiveness, *Latrodectus*, resource value
**Introduction.**

Evolution has equipped animals with different mechanisms to avoid wasting energy when responding to external stimuli. Among these is the ability to shift behavioral characters in response to changing environmental contexts, or behavioral plasticity. Selection should favor behavior that takes into account the expected benefit of an action relative to its cost (Maynard Smith 1982). However, costs and benefits may change based on such contexts as the individual’s age, size, and its reproductive status, as well as the presence of predators and changes in resource value (Glaudas 2006, Mooney and Haloin 2006, Rudolf and Armstrong 2008). Maynard Smith (1982) even suggests behavioral shifts may occur as a result of learning the rules of a game, thus producing a better strategy. In a specific context, the optimal strategy is the one in which the benefits are maximized and the costs are minimized. Individuals should risk injury and invest effort in a manner proportional to the resource they are defending (Maynard Smith 1982, Riechert 1979).

Quite a large number of studies have shown that organisms alter their agonistic behavior when the resource value is manipulated (Enquist and Leimar 1987, Riechert 1979, Riechert 1984). In fact, resource value may be the most important parameter in determining aggressive behavior (Enquist and Leimar 1987, Riechert 1978). Because aggressiveness is costly in terms of energy spent, time lost and possible injury, its exercise must be carefully weighed against resource value. Generally, aggressiveness and resource value vary directly. The cost of a wrong decision is substantially greater as aggressiveness and resource value increase (Tanner and Adler 2009).
Responding to stimuli appropriately is very important to individual fitness (Riechert 1979). If an individual were to respond to a potential mate as it responds to a potential predator, the effects on its fitness would be disastrous. Offspring represent a resource to parents and various studies have concluded independently that the amount of parental effort invested in defense of offspring should be decided by the risk to future reproduction, the value of present offspring compared with expected future generations, and the expected benefit to the offspring resulting from a parental investment (Huang 2006, Goubault et al. 2007, Tanner and Adler 2009). Parents may optimize their fitness by employing this strategy correctly (Mooney and Haloin 2006).

I investigated the effect developmental stage and various reproductive contexts on the aggressiveness of *Latrodectus mactans*. We also looked for behavioral shifts in the aggressiveness of juveniles that may occur over the course of ontogeny. Juveniles gradually develop venom prematuration. They also undergo shifts in predation due to changing size and often changing trophic levels (Rudolf and Armstrong 2008). Shifts in predation often call for shifting anti-predator behavior and aggressiveness.

*L. mactans* makes an interesting test species for maternal defense because of its iterparous (i.e. reproducing multiple times) nature. How often females have an opportunity to oviposit during the breeding season appears to be a crucial factor leading to variation in parental care. A large investment in maternal care is believed to cost the females time and energy that might be applied instead to additional ovipositions (Futami and Akimoto 2005). Using the reproductive investment argument discussed above, the resource value of a particular egg clutch may be low because *L. mactans* has the opportunity to lay additional clutches. Glaudas (2006) also stresses the need to include the high cost of having the venom in resource defense: this can
be an added cost to exhibiting high levels of aggressiveness for clutch defense in the highly venomous *L. mactans*.

I tested females at three different reproductive states to assess the effect of reproductive state on anti-predator and aggressive behavior. I also conducted an egg case removal experiment. Finally, I tested juveniles at each prematuration instar. I hypothesized that, as the adult spider’s reproductive investment increased, her behavioral aggressiveness would increase as well. With the juveniles, I began with the neutral hypothesis that juveniles’ aggressiveness does not correlate positively or negatively with age.

**Methods.**

*Study Organisms, Collection and Lab Maintenance*

*Latrodectus mactans* (Araneae: Theridiidae) were collected as mature adults from various locations within the Southeastern United States. Most of the *L. mactans* used in these trials were collected from a deciduous forest in East Tennessee (36° 6′ 42″ N, 83° 46′ 0″ W) and Central Arkansas (36° 6′ 54″ N, 92° 56′ 7″ W). Adult females were collected between April 2008 and July 2008, and were maintained in the laboratory in clear plastic arenas (1000ml). Females were provided poultry wire as a scaffold for web-building and a silk leaf for cover. All individuals were maintained between 22 and 24° C, and spiders were fed a diet of two-week-old crickets once weekly. Juveniles were maintained in small plastic containers (59ml) and fed primarily termite workers with occasional supplementation of cricket nymphs.

*Assessment of Aggressiveness*
Methods for assessing aggression levels were adapted from the anti-predator test used in Pruitt et al. (2008). Tests were conducted on the third day after spiders were fed to satiation. Spiders remained on their webs during testing. After opening its container, the individual was given time to settle (30 seconds without moving). The individual was lightly prodded from the front with a probe and all the resulting behaviors were recorded until the spider settled. After 30 seconds of quiescence, the spider was prodded from the rear and subsequent activity was recorded. The order of the stimuli (front, rear prod) alternated between individuals. After testing, all exhibited behaviors were examined and ranked for aggressiveness. The rankings were based on energetic expenditure and potential risk to the test subject. Rankings were adapted from Riechert 1978, 1979 to fit *L. mactans* behavioral repertoire. The frequency and sequence of behaviors were also considered (Vetter 1980). (Table 1)

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strike probe</td>
<td>22</td>
</tr>
<tr>
<td>Grind chelicerae</td>
<td>21</td>
</tr>
<tr>
<td>Rush probe</td>
<td>20</td>
</tr>
<tr>
<td>Lay viscous silk</td>
<td>19</td>
</tr>
<tr>
<td>Web flex</td>
<td>18</td>
</tr>
<tr>
<td>Pump abdomen</td>
<td>17</td>
</tr>
<tr>
<td>Vibrate</td>
<td>16</td>
</tr>
<tr>
<td>Waggle</td>
<td>15</td>
</tr>
<tr>
<td>Palpate web</td>
<td>14</td>
</tr>
<tr>
<td>Come out of retreat</td>
<td>13</td>
</tr>
<tr>
<td>Raise 1st two pairs</td>
<td>12</td>
</tr>
<tr>
<td>Raise 1st pair</td>
<td>11</td>
</tr>
<tr>
<td>Explore</td>
<td>10</td>
</tr>
<tr>
<td>Circle egg case</td>
<td>9</td>
</tr>
<tr>
<td>Wrap legs around egg case</td>
<td>8</td>
</tr>
<tr>
<td>Lay silk</td>
<td>7</td>
</tr>
<tr>
<td>Hop toward probe</td>
<td>6</td>
</tr>
<tr>
<td>Walk toward probe</td>
<td>5</td>
</tr>
<tr>
<td>Turn toward probe</td>
<td>4</td>
</tr>
<tr>
<td>Shift toward probe</td>
<td>3</td>
</tr>
<tr>
<td>Raise body</td>
<td>2</td>
</tr>
<tr>
<td>Widen stance</td>
<td>1</td>
</tr>
<tr>
<td>No movement</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 1: A list of all the behaviours exhibited by *L. mactans* during testing. Behaviours are ranked by potential risk to the individual and energy expenditure with consideration given to frequency and sequential presentation.

Reproductive status experiment

Recently-collected adult females were tested at three stages of reproduction: virgin, mated, and guarding egg case (*N* = 13, 15, 27). Females were tested upon entering the lab (no egg case, *N* = 34). After the initial trial was completed, I kept detailed records of the laying and hatching of egg cases in order to determine whether a particular female had mated prior to collection. After a female laid an egg case, the tests were repeated.

Removal experiment

I removed the egg case from a test female’s web following the completion of measuring its aggressiveness in the presence of its egg clutch. The prod test was subsequently repeated at one minute, five minutes, and ten minutes (*N* = 11, 9, 9) after removal to assess changes in aggressiveness over time. The same procedure was used for these tests as the previously described trials. After the conclusion of the testing, the egg cases were returned, and all mothers immediately began guarding them again.
**F1 Juvenile experiment**

A random sample of the offspring of the parental generation was reared in lab with the trials repeated following the appearance of each molt. After hatching, spiderlings were allowed to molt once on their mother’s web. Then, juveniles were removed from the web and isolated in 59 ml plastic enclosures. Juveniles were maintained on a diet of nymph crickets and termites fed twice weekly. Water was not provided as it was determined to be detrimental to survivorship.

Juveniles were first tested for anti-predator behavior during the second instar following the same methods as the parental generation. A sample of ten juveniles was tested after each subsequent molt. A single individual was tested in no more than three trials and none of the trials were in consecutive molts. The testing of the offspring continued through seven molts. *Latrodectus mactans* is not sexually dimorphic until the fourth to sixth instar, when the males begin to mature. After seven molts, all of the offspring were mature. Virgin females of the F1 generation were tested upon reaching sexual maturity.

**Results.**

*Aggressiveness and reproductive state*

My ANOVA results indicate significant differences between adult females of different reproductive classes (ANOVA: $F_{2,53}=19.29$, $P<0.0001$). Post-hoc Tukey groupings indicate females guarding egg cases were significantly more aggressive than both mated and virgin females, but mated and virgin females were indistinguishable in their aggressiveness (Figure 1).
Figure 1: The aggression scores of adult females at three different reproductive states: guarding egg case (white box), mated (light grey box), and virgin (dark grey box). Filled vertical bars represent 25% quartiles, medians are demarcated by the horizontal lines within bars and vertical lines represent the full range of scores for the measure indicated. Continuous center line represents the grand mean. Different letters indicate there were significant differences between classes at $P < 0.05$ using Tukey tests.

**Aggressiveness and development**

Juveniles in the beginning stages of development displayed low levels of aggressiveness. As they developed, their aggressiveness increased. An ANOVA with a fixed effects test (identity as random effect) indicates the differences between instars are statistically significant ($\text{ANOVA}: F_{5,57}=3.51, P<0.0082$). Figure 2 displays the post-hoc Tukey groupings by instar.
Figure 2: The distribution of aggressive behavior over development. Significant differences are indicated with different letters as established by Tukey tests (P < 0.05).

**Egg case removal**

The egg case was removed, and the decay of the aggression level was measured at intervals over ten minutes. ANOVA results indicate significant differences between the time intervals (ANOVA: $F_{3,24}=3.92$, $P<0.0136$). After five minutes, the aggression level was indistinguishable from the average level of females not guarding egg cases.
Figure 3: The distribution of aggressive behavior after the removal of an egg case. No egg case data are presented as a baseline for comparison. Significant differences are indicated with different letters as established by Tukey tests (P < 0.05).

**Discussion.**

*Brooding Females.* Modification of aggressive behavior on the part of reproducing females has been shown in other types of organisms (e.g., rodents and cichlid fishes) (Neil and Elwood 1985). In fact, most spiders show some degree of maternal care. Constructing silken egg sacs, guarding egg sacs, and even feeding spiderlings are a few examples (Futami and Akimoto 2005). Egg case guarding is one of the most primitive forms of parental care (Huang 2006), and
a better understanding of this behavior in spiders may lead to evolutionary insights into the strategy in other animals.

Females that were not guarding egg cases had significantly lower levels of aggression regardless of whether they were mated. Females guarding egg cases were nearly twice as aggressive as their unburdened counterparts. The mere presence of the egg case elicits a marked change in aggressiveness. This indicates that the resource value of the egg case is very high, probably because the egg case is approximately 40% of the spider’s preparturition mass (personal observation). Maternal care of eggs and juveniles has been shown in many types of spiders (Sub-social species: Matlack and Jennings 1977; *Uloborus ferokus*: Patel and Bradoo 1981; Orb-weaving spiders: Hoffmaster 1982; Clubionidae: Pollard 1983; Green lynx: Fink 1987; *Uloborus glomosus*: Cushing 1989; Araneidae: Hieber and Uetz 1990; *Coelotes terrestris*: Horel and Gundermann 1992, Gundermann et al. 1997; Salticidae: Mooney and Haloin 2006). However, each species has its own variations indicating that the process is unique and context dependent for each group (Tanner and Adler 2009).

The aggression levels exhibited by female black widows decline very quickly after their egg cases are taken from them. The behavior is indistinguishable from a female not guarding an egg case within five minutes. This return to baseline occurred significantly faster than I expected. As the results of the first experiment indicate that the resource value is very high, I anticipated that the decay of aggressive behavior would be slower in order to prevent mistakes. These could occur if an egg case were shifted into a recoverable position, but not removed. A mother whose aggressiveness is declining so rapidly may not recover her egg case. However, in nature it is probably unlikely that these cases are the norm. The rapid decay may be an evolved mechanism to allow females to begin obtaining the resources necessary to produce a second egg case.
Perhaps here, not in the reproductive state experiment, is where the effect of a second oviposition is truly observed. This rapid decay is seen in other iteroparous species. For instance, Oliveira and Almada (1998) found that when the brood is removed from the mouth of a cichlid fish, the level of aggression declines within 24 hours. By this time coloration and behavior patterns in brooding females cannot be distinguished from nonbrooding females.

*Juveniles.* Juvenile *L. mactans* showed significant temporal variation in levels of aggressiveness associated with instar. Post hoc analyses showed that the sixth instar juveniles were significantly more aggressive than the second and third instar individuals. The seventh instar (mature) individuals were not significantly more aggressive than their younger counterparts, but also showed a much greater variance.

I hypothesized that juveniles would become more aggressive as they aged because of their increased levels of venom. However, this is not the only potential cause of escalating aggressive behavior. The changes could be due to ontogenetic niche shifts resulting from a significant change in size (Rudolf and Armstrong 2008). Abrupt changes in body size of young spiderlings are often related to cannibalism of siblings (personal observation, unpublished data). The rate of cannibalism combined with an individual’s body size is very likely to affect decisions to escalate an aggressive encounter or to flee. Raihani et al. (2008) found that juvenile aggressiveness was correlated with adult dispersal patterns in juvenile pied babblers. It could be that in spiders, as the frequency of aggressive encounters increases, inter-individual distance increases by necessity, thus providing a mechanism for dispersal. There are many potential causes and correlations to investigate with regard to the aggressiveness of juvenile spiders. I recommend this as an area for future research.
Within the seventh instar, there appears to be greater variation than in previous instars. This could be a result of the spiders developing their adult temperaments or personalities. Sih et al. (2004) discusses an aggression syndrome in which some individuals are more aggressive, and others are less so, across many different contexts. Behavioral syndromes often explain individual variation. It is doubtful that the expression of the variation correlating with maturation, and thus a set of new selection pressures, is purely coincidental. Assessing the personality of individuals was beyond the scope of this study, but this method could provide an interesting way to track changes in aggressiveness more closely.

Aggressiveness is affected by a variety of factors, both contextual and developmental. *Latrodectus mactans* appears to be affected differently by the factors in its different life stages. Adults respond to environmental factors, whereas juveniles respond to developmental cues. More extensive testing of juveniles may shed light upon their increasing aggressiveness. Possible future research could investigate the effects of venom, body mass and developmental environment upon aggressiveness.

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