2021. Journal of Arachnology 49:324–331

Alternative responses by two species of jumping spiders to unpalatability and toxicity in prey

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Abstract. A key challenge for generalist predators is avoiding toxins in prey. Species-specific strategies range from total avoidance of distasteful (and potentially toxic) prey to the use of physiological mechanisms to metabolize toxins after consumption. We compare two species of jumping spiders, *Habronattus trimaculatus* Bryant, 1945 and *Phidippus regius* CL Koch, 1846. Based on several anecdotal observations and other aspects of their biology, we hypothesized *a priori* that *H. trimaculatus* would be (1) less willing to feed on unpalatable prey and (2) more susceptible to toxins that are consumed compared with *P. regius*. In Experiment 1, we presented spiders of both species with size-matched quinine-dipped crickets. Consistent with our hypothesis, all *H. trimaculatus* attacked and rejected them while all *P. regius* attacked and consumed them. In Experiments 2 and 3, we assigned spiders of both species to experimental feeding treatments with varying levels of toxicity (using toxic springtails, *Folsomia candida*) and assessed effects on their growth. Spiders of both species readily fed on the springtails. Collectively, results from these two experiments suggest that springtails have negative effects on both species, but that these effects are stronger in *H. trimaculatus*. *Habronattus* FO Pickard-Cambridge, 1901 has a unique red retinal filter pigment (not found in *Phidippus* CL Koch, 1846) that likely improves their ability to discriminate reds and oranges. The evolution of this unique visual system may have been driven by their heightened susceptibility to prey toxins, and thus the benefits of avoiding prey that advertise toxins with long-wavelength colors.

Keywords: Salticidae, chemical defense, toxin susceptibility, aposematism, predation

https://doi.org/10.1636/JoA-S-20-066

Generalist predators often encounter a wide variety of prey while foraging. These potential prey items can differ in nutritional value and may range from high quality (increasing predator fitness) to low quality (with little to no nutritional value to the predator) and, in some cases, may even be toxic to a predator (Toft 1999; Toft & Wise 1999a, b; Fisker & Toft 2004; Rickers et al. 2006; Harwood et al. 2009). Given this variation in prey quality, predators must make decisions about how to interact with such prey. For instance, predators may exhibit total avoidance of prey (e.g., Skow & Jakob 2006), attack and reject prey (e.g., Taylor et al. 2016), consume and regurgitate prey (e.g., Paradise & Stamp 1991), or consume toxic prey and metabolize the prey toxins (e.g., Skelhorn & Rowe 2007; Sloggett & Davis 2013). These examples illustrate the considerable variation in how predators react to unpalatable and toxic prey and deal with prey toxins. Even closely related predator species may differ drastically in their responses, yet our understanding of such differences among some key groups of ecologically important species is limited.

Spiders are a large and diverse group of predators that are ecologically important to a variety of ecosystems; most are considered broad generalists and interact with many prey types (Wise 1995). With over 800,000 arthropod species documented to be chemically defended in some way (see table 1 in Berenbaum 1995) the likelihood of spiders encountering toxic prey is high. The small amount of prior work on toxin susceptibility in spiders has shown that eating toxic prey has detrimental effects including (1) inhibiting feeding (Toft 1999), (2) reducing egg sac production (Rickers et al. 2006), (3) reducing nutrient absorption (Strohmeyer et al. 1998), (4) decreasing growth rates (Oelbermann & Scheu 2002; Jespersen & Toft 2003), and (5) increasing mortality rates (Toft 1999; Toft & Wise 1999a, b; Fisker & Toft 2004; Hill 2006; Rickers et al. 2006; Harwood et al. 2009). Despite these negative consequences, many spiders will still readily feed on toxic prey when it is offered to them, making them an interesting group for understanding the species-specific costs and benefits of such decisions. Aside from the literature cited above which focuses mainly on wolf spiders, there is relatively little information about how other spider groups respond to and process toxic prey (but see Strohmeyer et al. 1998: Harwood et al. 2009 for a few exceptions).

Jumping spiders (family Salticidae) are an interesting group to examine species-specific differences in responses to toxic prey. Salticids comprise one of the largest families of spiders with more than 6500 described species (World Spider Catalog 2019) and occupy a variety of habitats on every continent except for Antarctica (Maddison et al. 2008). While some charismatic members of this family are known for specialized feeding strategies, including specializing on other spider species (Penney & Gabriel 2009), mosquitoes (Jackson et al. 2005), ants (reviewed in Cushing 2012), and even plant material (Meehan et al. 2009), most salticids are considered broad generalists with species varying in their diet breadth (e.g., see Jackson 1977; Nentwig & Wissel 1986; Nentwig 1987; Nyffeler et al. 2017). Very few studies have focused on any aspect of toxin susceptibility in this group (but see Strohmeyer et al. 1998; Hill 2006).

Two common, abundant, speciose, and relatively well-studied genera of particular interest are *Phidippus* CL Koch, 1846 and *Habronattus* FO Pickard-Cambridge, 1901. While most *Phidippus* will avoid prey known to be chemically defended, such as milkweed bugs (Hill 2006; Skow & Jakob

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2006) and fireflies (Long et al. 2012), recent work has shown that some individuals of *Phidippus regius* CL Koch, 1846 occasionally consume chemically defended milkweed bugs in their entirety without any negative effects (Powell & Taylor 2020). Spiders in this genus have also been documented to consume chemically defended *Junonia* caterpillars (Strohmeyer et al. 1998) and bella moth caterpillars (LAT, unpub. data) with only minor, if any, sublethal effects. Even when *Phidippus* spiders consumed fruit flies artificially laced with the cardenolides ouabain and digitoxin, only a relatively small proportion (22–25%) died in experiments (Hill 2006). By contrast, spiders in the genus *Habronattus* seem to be more conservative in their prey choices, have never been documented to feed on these same chemically defended prey in the field, and have been unwilling to eat them when offered in the lab (Taylor 2012; Taylor et al. 2016, LAT, unpublished data). Moreover, spiders in the genus *Habronattus* have been found to use color to guide their prey choices in ways that would position them to avoid prey toxins; specifically, multiple populations have been found to exhibit prey color biases against red and yellow artificially colored prey (Taylor et al. 2014; Powell et al. 2019) or to increase their avoidance of red when it is presented alongside defensive odors from chemically defended prey insects (Vickers & Taylor 2018; Vickers & Taylor 2020). This particular attention to long-wavelength colors (red, orange, yellow) in prey is likely the result of a red retinal filter pigment that likely gives them enhanced color vision; unlike *Habronattus*, most other jumping spider groups (including *Phidippus*) are currently believed to be have a more limited ability to discriminate long-wavelength colors (Zurek et al. 2015).

Taken together, this information led us to the *a priori* hypothesis that *Habronattus* are generally more susceptible to prey toxins compared with *Phidippus*. In addition to helping to explain the differences in behavior described above, exploring this idea of heightened sensitivity to prey toxins in *Habronattus* may help explain why this group has evolved the enhanced color vision that most other salticids lack. Moreover, it may provide general insight into why several other jumping spider groups in addition to *Habronattus* have independently evolved long-wavelength color vision (Outomuro et al. 2019). The goal of the present study was to test some preliminary predictions of this hypothesis, using two focal species: *Habronattus trimaculatus* Bryant, 1945 and *Phidippus regius*. The first prediction from our hypothesis was that *H. trimaculatus* would be more likely to reject novel unpalatable prey compared with *P. regius*. In Experiment 1, we tested this prediction by presenting both species with bitter quinine-dipped crickets and assessing their willingness to consume them. Quinine has been used in several previous studies to make prey distasteful to predators, including spiders; interestingly, results with spiders show that some but not all spider species reject quinine-treated prey (Bays 1962; Holden 1975; Jakob & Long 2016; Mebs et al. 2019). The second prediction from our hypothesis was that *H. trimaculatus* would be more susceptible to prey toxins generally (resulting in more negative effects when consuming toxic prey) compared to *P. regius*. To test this prediction, we ran Experiments 2 and 3 in which we randomly assigned spiders of both species to experimental feeding treatments with varying levels of toxicity and assessed the effects on their growth. These experiments were accomplished using *Folsomia candida* Willem, 1902, a toxic but apparently palatable prey species that many arachnids will readily feed on (even with experience) but that has well-documented negative effects (Toft 1999; Toft & Wise 1999a, b; Oelbermann & Scheu 2002; Jespersen & Toft 2003; Fisker & Toft 2004; Hvam & Toft 2005; Rickers et al. 2006; Harwood et al. 2009).

Jumping spiders are important predators in both natural and agricultural food webs (Young & Edwards 1990; Pekár 2012). Understanding how they respond to and process toxins from chemically defended prey may provide insight into the selection pressures they place on these prey defenses. Moreover, it may help us better understand their potential roles as biocontrol agents and may even predict how they will respond to non-dietary toxins, such as pesticides (Roach & Moore 1988; Pekár 2012).

METHODS

Spider collection and housing.—We collected *Habronattus trimaculatus* and *Phidippus regius* from populations around Gainesville, Micanopy, and Ocala, FL, USA. These two species differ in adult size with *H. trimaculatus* being much smaller than *P. regius* (adult size range: 3–6 mm vs. 6–22 mm, respectively; Edwards 1980; Griswold 1987). We housed spiders in the lab following previously published methods (Taylor et al. 2014) and fed them approximately their own mass in juvenile crickets (*Gryllodes sigillatus* Walker, 1869) 3x per week.

Experiment 1: Determining the willingness of spiders to consume distasteful prey.—For Experiment 1, we wanted to determine if our two study species differed in their willingness to consume distasteful prey, and specifically to test our *a priori* prediction that *H. trimaculatus* would be less willing to accept distasteful prey compared with *P. regius*. We presented spiders ($n = 15$, 14 adult and 1 juvenile *H. trimaculatus*; $n = 15$, all juvenile *P. regius*) with quinine-dipped crickets; quinine has been commonly used in other studies to make prey unpalatable to other spiders and insects (spiders: Bays 1962; Holden 1977; Jakob & Long 2016; insects: Mery & Kawecki 2002; Wang et al. 2013, 2018). We tested spiders prior to feeding them (on a regularly scheduled feeding day) meaning that they had not eaten for the two days prior to testing. We chose this feeding regime so that spiders were hungry and motivated to attack the crickets during our tests (but were not starving).

To test our spiders, juvenile crickets (*G. sigillatus*) were size-matched to spiders (using body length) and dipped in a 4% (4g/100ml) solution of quinine sulfate dihydrate (product number QESD10G, Chemsavers, Bluefield, VA, USA) and allowed to dry for 10 minutes. Prior to testing, spiders were placed in a clear 3.5 cm diameter petri dish that served as an acclimation chamber. This chamber was placed in the center of a larger 9 cm diameter petri dish that served as the testing arena. The bottom of the testing arena was lined with filter paper to provide a consistent visual background for foraging. After a 5-minute acclimation period, the lid of the acclimation chamber was removed to allow spiders to exit the chamber and roam freely in the larger petri dish. After the spider exited the acclimation chamber, trials lasted an additional 10 minutes or until the spider attacked the cricket (whichever came first).

Upon attack, we recorded whether the spider rejected or consumed the cricket; in every case, the response was unambiguous (either immediate rejection or complete consumption, see Results). If the spider did not attack the cricket after 10 minutes, we ended the trial, and the spider was retested on the following testing day.

Statistical analyses for Experiment 1.—To determine if the two species of spiders differed in their willingness to consume distasteful quinine-dipped crickets, we used a Pearson's chi-square test to ask whether the instances of prey consumption (vs. rejection) differed between the two species.

Experiment 2: Determining the effects of toxins on spiders once toxic prey have been consumed.—In Experiment 2, we wanted to determine if consuming toxic prey had different effects on our two study species, and specifically to test our *a priori* prediction that *H. trimaculatus* would be more susceptible to prey toxins than *P. regius*. To accomplish this, we needed a prey type that has been shown to be toxic to spiders generally, but that both of our study species used here would readily ingest. We used the springtail *Folsomia candida* because they have been used extensively in toxicity experiments; unlike many other species of springtails, they are toxic to many arachnids (Toft 1999; Toft & Wise 1999a, b; Fisker & Toft 2004; Hvam & Toft 2005; Rickers et al. 2006; Harwood et al. 2009). Moreover, our preliminary observations indicated that both *H. trimaculatus* and *P. regius* would readily feed on *F. candida* in the lab.

Similarly sized spiders of both species (*H. trimaculatus:* $n =$ 48, 9 adult females, 5 adult males, 23 juveniles; *P. regius*: $n =$ 52, all juveniles) were randomly assigned to one of three feeding treatments with varying levels of toxicity, which they remained on for four weeks: (1) springtails (*F. candida*: toxic prey), (2) control (no prey), and (3) crickets (*G. sigillatus*: non-toxic prey). Because the effects of toxins can depend on the size of the spider ingesting them, with toxins affecting smaller individuals more than larger ones (Fisker & Toft 2004), we deliberately used similarly sized spiders of the two species here; because *P. regius* is a considerably larger species than *H. trimaculatus*, similar sizes were achieved by using primarily adult *H. trimaculatus* and juvenile *P. regius*. Spiders in the springtail and cricket diet treatments received an amount of prey that was approximately equivalent to their own mass three times per week, and all three groups were given water on wet cotton balls. Because the springtails were replenished three times per week without removing old (uneaten) prey, springtail numbers gradually increased within the arena over the four-week experiment providing a continuous supply of toxic prey.

We assessed the effects of our treatment diets on the amount of mass that the spiders gained over the four-week experiment by recording the spiders' mass at the start and end of the experiment.

Statistical analyses for Experiment 2.—To assess initial mass differences between the two species of spiders (prior to the start of experiments), we compared pre-trial mass using a Wilcoxen 2-sample test.

To determine if the two species of spiders were affected differently by the toxic prey, we compared mass gains among the three feeding treatments. We used a two-way ANOVA with spider species and feeding treatment (and their interaction) as factors in our model and spider mass gain as our dependent variable. When we found a significant interaction between spider species and feeding treatment (suggesting that the two species were responding differently to the feeding treatments, see Results), we went on to run follow-up analyses using separate ANOVAs for each species. We used planned contrasts to compare differences between the feeding treatments (specifically comparing the springtail group to the unfed control group and the springtail group to the cricket group) (see Ruxton & Beauchamp 2008). The springtails vs. the unfed control comparison allowed us to assess whether the springtails were toxic enough to the spiders that they would be worse than eating no food at all. The springtail vs. cricket group comparison allowed us to assess how springtails compared to a diet that we know from previous studies allow these two species of jumping spiders to thrive in the lab (Vickers & Taylor 2018; Powell & Taylor 2020).

Experiment 3: Determining the extent to which spiders were feeding on toxic prey and re-examining the effects of toxic prey once consumed.—One limitation of Experiment 2 was that while the individuals of the two species were similar in size, they differed in life stage; all *P. regius* were juveniles while *H. trimaculatus* were mostly juveniles plus some adults (see details above). As such, any differences we saw between the two species could be real species-specific differences, or they could be a result of differences between life stages, or some combination of both. We therefore ran Experiment 3 using small juveniles of both species. Experiment 3 had two objectives: (3a) to confirm that both species of spiders were indeed feeding on the toxic springtails in our experimental setup and (3b) to further examine the effects of toxic prey consumption. In objective 3b, we were again testing the *a priori* hypothesis that *H. trimaculatus* would be more susceptible to the toxins from springtails than *P. regius*. Here we used only small juvenile *H. trimaculatus* ($n = 33$) and *P. regius* ($n = 39$) spiders (all 2–3 mm in body length) to ensure that all spiders were in a stage of development where we would expect them to be actively growing.

Spiders were fed three times their body size in crickets, three times per week, until their first molt in the lab. Once spiders molted, we assigned them to one of two treatment groups: (1) 'cricket + springtail' diet or (2) 'cricket only' diet. These two treatment diets were different than those used in Experiment 2 and were chosen so that we could examine the toxic effect of springtails when combined with a cricket diet (as previous work has shown that the toxins in *F. candida* can inhibit the incorporation of nutrients from other prey, see Rickers et al. 2006). Before diet treatments began, we recorded each spider's mass. Spiders were fed their treatment diets one day per week. We presented spiders in the 'cricket only' group with one pinhead-cricket (1/4 of the spider's body size) on their feeding day. Spiders in the 'cricket + springtail' group were given one pinhead-cricket (1/4 of their body size), plus 3–4 springtails on their feeding day. In addition, we gave all spiders a 20% sugar water solution on a cotton ball, as this has been shown to increase survival of jumping spiders at this small size (Carvell et al. 2015). We replaced old cotton balls with fresh 20% sugar water-soaked cotton balls once per week.

A second limitation of Experiment 2 was that we did not assess whether the two species were equally likely to feed on

the springtails during experiments. While our pilot observations showed that both species would eat them, there is the slim possibility that any differences we see between species could be driven by how likely the two species are to eat them (rather than differences in how the two species respond to the toxins after consumption). Therefore, in Experiment 3, to assess whether spiders of both species were indeed actively feeding on the springtails in our experiment, we conducted visual observations for the first 15 minutes of the first feeding immediately after springtails were introduced. During these 15-minute visual observations, we recorded whether or not spiders fed on the springtails. If we did observe the spider feeding on a springtail, the observation ended (and we did not formally observe their springtail-feeding behavior again); however, if spiders did not feed on a springtail during this first 15-minute observation, we observed them again at the beginning of the next feeding. With this method, we were able to confirm whether each spider fed on a springtail at some point during the experiment (but due to logistical constraints, we did not quantify the total number of springtails eaten over the course of the entire experiment). After four weeks on these treatment diets, we weighed spiders to assess any changes in mass.

Statistical analyses for Experiment 3.—To assess any initial size differences between the two species, we compared pre-trial mass using a Wilcoxon 2-sample test. We performed a likelihood ratio chi-square test to examine differences between the two species in how likely they were to consume the springtails.

To examine how our diet treatments affected spider mass gains, we used a two-way ANOVA, with species and feeding treatment (and their interaction) as factors in the model, and mass gained during the experiment as our dependent variable. Despite the lack of a significant species*treatment interaction, we performed an exploratory analysis to further analyze data from the two species separately. For each species, we ran t-tests to determine the effect of feeding treatment on spider mass gains.

Because we had higher than expected mortality in *H. trimaculatus* (13 out of 33 spiders died over the course of the experiment), we performed a likelihood ratio chi-square test to determine whether there were differences between treatment groups in how likely they were to die. We also used a survival analysis to compare time to death between spiders in the 'cricket + springtail' and 'cricket only' treatments.

Finally, because of the unexpected high mortality in *H. trimaculatus* that did not occur with *P. regius*, we performed a *post hoc* exploratory test (likelihood ratio chi-square) to determine if there were differences between the two species in how likely they were to die over the course of the experiment.

RESULTS

Data Availability.—The data generated during this study are available in the Dryad repository (available online at https://doi.org/10.5061/dryad.qnk98sfdx).

Experiment 1: Determining the willingness of spiders to consume distasteful prey.—We found a highly significant difference between the two species: all *H. trimaculatus* attacked and rejected quinine-dipped crickets while all *P. regius* attacked and consumed them ($X^2 = 30.00$, df = 1, $P<0.0001$).

Table 1.—Results of ANOVA for Experiment 2 examining the effects of feeding treatment (springtails, unfed control, or crickets) and species (*Habronattus trimaculatus* or *Phidippus regius*) on spider mass gains (g) after four weeks. Significant *P* values are shown in bold.

	df	*F*	*P*
ANOVA (mass gains)			
Species	1,65	25.53	**<0.0001**
Feeding Treatment	2,65	79.60	**<0.0001**
Species*Feeding Treatment	2,65	41.90	**<0.0001**

Experiment 2: Determining the effects of toxins on spiders once toxic prey has been consumed.—Before trials began, the two species did not differ in mass ($Z = 0.93$, $P = 0.35$), suggesting that any differences we see between the two species are unlikely to be due to size alone.

There was a significant interaction between the effects of spider species and feeding treatment on spider mass gains (Table 1) suggesting that the two species responded differently to the diet treatments. When examined separately, the mass gains of both species were affected by diet (*H. trimaculatus*: $F_{2,32} = 6.88$, $P = 0.003$; *P. regius*: $F_{2,36} = 104.98$, $p<0.0001$; Fig. 1), but in different ways. For *H. trimaculatus*, spiders in all three treatments lost mass after four weeks; however, springtail-fed spiders lost significantly more mass compared to control (no prey) spiders ($P = 0.03$) and compared to cricket-fed spiders ($P = 0.003$, Fig. 1), suggesting that the springtail prey was both less profitable than cricket prey and also toxic to the spiders (i.e., worse than no prey at all). For *P. regius*, the springtail-fed spiders did not differ from the control (no prey) group ($P = 0.70$) but they lost more mass than the cricket-fed group ($P<0.0001$, Fig. 1); this suggests that for *P. regius*, the springtails are inferior prey compared to the crickets, but not so toxic that they are worse than no prey at all.

Experiment 3: Determining the extent to which spiders were feeding on toxic prey and re-examining the effects of toxic prey once consumed.—The two species did not differ in pre-trial mass ($Z = 1.40$, $P = 0.16$), suggesting that any differences we see between the two species are unlikely to be due to size alone.

We found no significant difference between the two species in how likely they were to consume springtails during our experimental observations (88% of *H. trimaculatus* and 100% of *P. regius* fed on springtails during observations, $X^2 = 0.52$, $P = 0.47$). These results suggest that both species were likely to feed on springtails during the four-week feeding treatments; these are conservative estimates because our observation periods were only 15 minutes long and we only recorded whether they fed on springtails or not (without recording the total number of springtails each spider ate).

There was no significant interaction between the effects of species and feeding treatment on spider mass gains (Table 2), suggesting that the two species responded similarly to the feeding treatments. There was a significant main effect of feeding treatment with the 'cricket + springtail' group gaining less mass than the 'cricket only' group, suggesting that both species suffered negative effects from feeding on springtails (Table 2). In our exploratory analyses where we looked at each species separately, *H. trimaculatus* gained significantly less

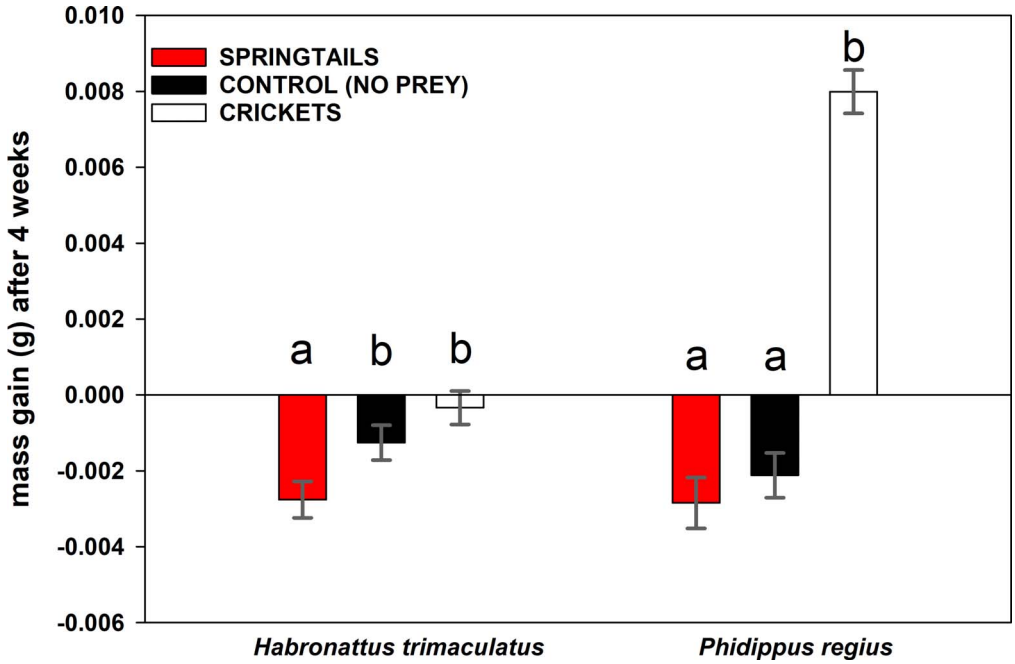

Figure 1.—Results of Experiment 2 showing the change in mass (g) of *Habronattus trimaculatus* and *Phidippus regius* after four weeks on treatment diets that differed in toxicity. Different letters indicate significant differences between feeding treatments for each species. Bars indicate the mean ± standard error for each treatment.

mass on the 'cricket + springtail' diet, compared with those on the 'cricket only' diet ($t_{18} = 2.47$, $P = 0.02$; Fig. 2). In contrast, *P. regius* did not differ depending on their diet treatment ($t_{32} = 1.39$, $P = 0.17$) although the non-significant effect was in the same direction as that for *H. trimaculatus* (Fig. 2).

In our survival analysis with *H. trimaculatus,* 4/15 spiders (27%) died in the 'cricket only' diet group compared to 8/17 spiders (47%) that died in the 'cricket + springtail' group during the four-week experiment; the likelihood of death did not differ between treatment groups ($X^2 = 0.87$, $P = 0.35$), nor did we find a significant difference in the time to death ($X^2 = 1.51$, $P = 0.22$). Due to low mortality in the *P. regius* (only a single individual died), we did not run this same analysis for them (see Methods section above).

We found that *H. trimaculatus* were significantly more likely to die over the course of the experiment compared to *P. regius* ($X^2 = 17.38$, $P<0.0001$).

DISCUSSION

Here we show that *Habronattus trimaculatus* and *Phidippus regius* respond differently to both unpalatable prey and toxic prey in ways that offer support for our hypothesis that the two species differ in toxin susceptibility. In Experiment 1, all *H. trimaculatus* attacked and quickly rejected unpalatable quinine-laced crickets compared to all *P. regius* that attacked and completely consumed them. This result is consistent with our hypothesis: if *H. trimaculatus* are more susceptible to prey toxins, we would expect them to be more wary and less likely to feed on prey with a novel unpalatable taste, as unpalatability is often (but not always) an indicator of toxicity (reviewed in Skelhorn & Rowe 2010; Marples et al. 2018). Both *H. trimaculatus* and *P. regius* fed readily on the springtails offered as part of our experimental feeding treatments, yet the two species responded to the toxins in these springtails in different ways. Our hypothesis predicted that the springtails would be more toxic to the *H. trimaculatus* than the *P. regius* and our data mostly followed this pattern. In Experiment 2, *H. trimaculatus* fed springtails fared worse than spiders given no prey at all, suggesting a toxic effect. By contrast, for *P. regius,* a springtail diet was similar to no prey at all, suggesting a lack of toxicity. However, because the *P. regius* were all juveniles and the *H. trimaculatus* were a combination of juveniles and adults, our results could be explained by either real species-specific differences, differences in life stages, or both. Experiment 3 helped us tease apart these effects by comparing small juveniles of both species. Here the presence of springtails in the diet reduced both species' mass gains (the lack of an interaction term in our model suggests that both species responded similarly to the springtails). However, further exploration of the data suggests a stronger effect in *H. trimaculatus* compared to *P. regius*.

The difference between the two species' willingness to consume quinine-laced prey was unambiguous and consistent with our *a priori* expectations. By tasting and immediately

Table 2.—Results of ANOVA for Experiment 3 examining the effects of feeding treatment ('cricket + springtail' or 'cricket only') and species (*Habronattus trimaculatus* or *Phidippus regius*) on spider mass gains (g) after four weeks. Significant *P* values are shown in bold.

	df	*F*	*P*
ANOVA (mass gains)			
Species	1,57	0.10	0.75
Feeding Treatment	1,57	9.10	**0.0039**
Species*Feeding Treatment	1,57	2.38	0.13

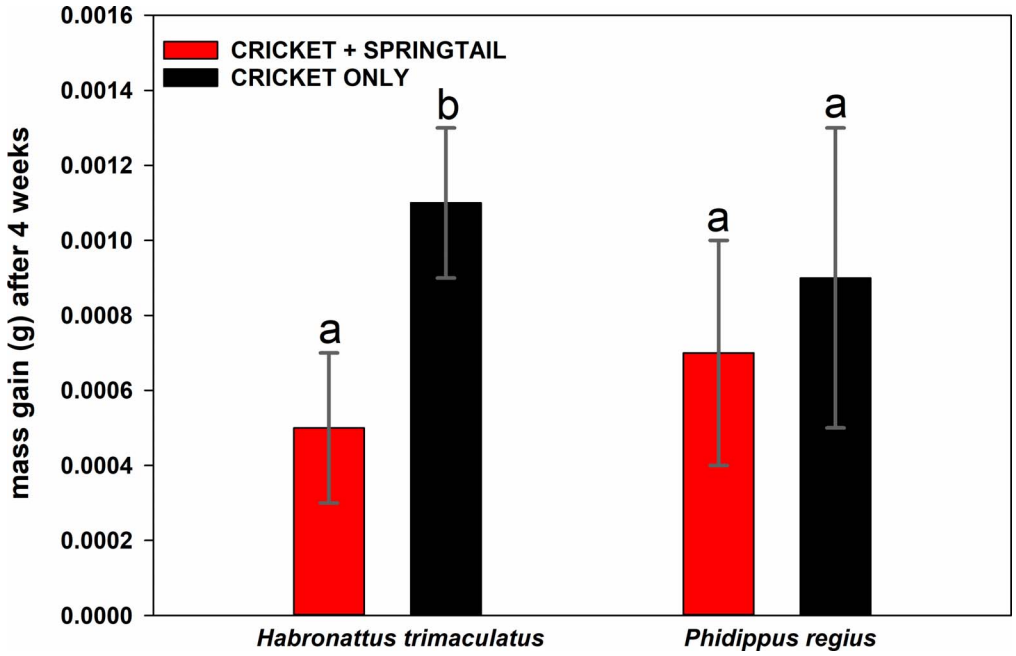

Figure 2.—Results of Experiment 3 showing the change in mass (g) of *Habronattus trimaculatus* and *Phidippus regius* after four weeks on treatment diets that differed in toxicity. Different letters indicate significant differences between feeding treatments for each species. Bars indicate the mean ± standard error for each treatment.

rejecting unpalatable prey, *H. trimaculatus* may be less likely to ingest toxins from chemically defended prey (Skelhorn & Rowe 2006). By contrast, *P. regius'* willingness to accept unpalatable prey may reflect their increased ability to metabolize ingested toxins that have been shown to be harmful to other spiders (Toft 1999; Toft & Wise 1999a, b). For *P. regius,* the small cost of ingesting some toxin (especially if they can metabolize it) may be outweighed by the benefit of being able to take a larger range of prey in the field (Halpin et al. 2014). Indeed, anecdotal accounts, including images on sites such as iNaturalist, suggest that *Phidippus* feed on a much broader range of prey types in the field compared with *Habronattus* (pers. obs.); systematic surveys of such datasets could prove useful in understanding the diet breadth of these two groups, and how likely each are to accept unpalatable prey. Another way to examine such ideas would be to employ DNA gut content analysis on these two species of jumping spiders to assess the diet breath in the field (e.g., Krehenwinkel et al. 2017). In the present study, we only examined one type of unpalatable prey, quinine-laced crickets. However, more work is needed to assess whether the same general findings from this study will hold up when examining other types of unpalatable prey. For example, Winsor et al. (2020) recently showed that *Habronattus pyrrithrix* (Chamberlin, 1924) will taste and reject prey treated with the odorless bittering agent, Bitrex (denatonium benzoate). It would be useful to examine whether *Phidippus* would be more willing to accept Bitrex-treated prey (compared with *Habronattus*), as we would predict based on our findings in the present study.

The differences between the two species in how they metabolize prey toxins (Experiments 2 and 3) were largely consistent with our hypothesis but should be examined further. In most predator-prey interactions, predators quickly stop feeding on prey that make them sick (Paradise & Stamp 1991) which makes assessing the longer-term effects of toxicity difficult, especially in predators like spiders that mostly feed on live prey (Foelix 2011). Our experiments took advantage of an unusual prey item, the springtail *F. candida,* that is known to be toxic to several arachnid species yet continues to be consumed by these arachnids throughout experiments (Strohmeyer et al. 1998; Toft 1999; Toft & Wise 1999a, b; Oelbermann & Scheu 2002; Jespersen & Toft 2003; Fisker & Toft 2004; Hvam & Toft 2005; Hill 2006; Rickers et al. 2006; Harwood et al. 2009). Future work should continue to test the patterns we show here, but it may be difficult to find other toxic prey species that spiders will continue to eat throughout an experiment. It may be useful to explore methods similar to those used by Hill (2006) where palatable prey (fruit flies) were injected with toxic cardenolides that *Phidippus* jumping spiders apparently could not detect and did not avoid (and were therefore poisoned by). Another way to examine toxin susceptibility more generally would be to expose these two species of jumping spiders to non-dietary toxins, such as pesticides (Roach & Moore 1988; reviewed in Pekár 2012) to see if the same pattens of toxin susceptibility hold up.

Our study highlights the difficulty of comparing something as complicated as toxin susceptibility across species. There are difficulties in attributing differences to species (and not differences in size, etc., that also vary between species). For example, adult *P. regius* are two to three times larger than adult *H. trimaculatus* (pers. obs.), which makes comparing similarly sized spiders that are also in the same life stage difficult (see Experiment 2). Moreover, toxin susceptibility might differ seasonally for some species or may differ depending on what other prey types these spiders are eating in the field alongside toxic prey. Toxin susceptibility might

also be affected by stressors in the lab (that affect species differently); the fact that we saw higher overall mortality in *H. trimaculatus* compared with *P. regius* highlights this difficulty. It would be fascinating to explore variation in toxin susceptibility across a larger phylogenetic scale, but the experimental protocols used to do this would need to be considered carefully.

Our results show that *H. trimaculatus* and *P. regius* respond differently to unpalatable prey and likely use different physiological mechanisms to deal with toxic prey. Interestingly, Zurek et al. (2015) recently showed that *Habronattus* possesses a unique mechanism of long-wavelength color vision not found in *Phidippus*. Using a retinal filter, *Habronattus* can likely see and discriminate reds and oranges better than many other salticids (Zurek et al. 2015). While speculative, this raises the intriguing possibility that the inability to detoxify prey and the resulting need to avoid toxins in their diet may have driven the evolution of this unique color vision system in *Habronattus*. Moreover, if the need to avoid toxic prey makes *Habronattus* females particularly attentive to long-wavelength warning colors during foraging, this may help explain why males in this genus have so often incorporated such colors into their courtship displays (Elias et al. 2006 2012; Taylor et al. 2011, 2017; Echeverri et al. 2017). The use of such colors in male displays may exploit a female's innate and/or learned aversions to these colors in the context of foraging (Taylor et al. 2014, 2016), allowing males to both capture female attention and reduce cannibalism. Again, these ideas are speculative at this stage but warrant further study. Future work should also examine such ideas more broadly across the salticid phylogeny, asking whether increased toxin susceptibility in other jumping spider genera may also help explain their enhanced color vision and increased reliance on colorful signals in courtship (e.g., *Maratus*: Girard et al. 2011, 2015, 2018; Outomuro et al. 2019).

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (IOS-1557867 and IOS-1831751 to LAT), including a Research Experiences for Undergraduates supplement that supported MLH, and by grants from the United States Department of Agriculture (Hatch project 1016166 and McIntire-Stennis project 1017978 to LAT).

SUPPLEMENTAL MATERIALS

Data generated during this study are available in the Dryad repository (online at https://doi.org/10.5061/dryad.qnk98sfdx).

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Manuscript received 2 September 2020, revised 20 October 2020.