



# Behaviour of the Sydney funnel-web spider *Atrax robustus* over different contexts, time, and stimuli

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## ABSTRACT

*Atrax robustus* is an iconic Australian spider because the venom can be lethal to humans. Moreover, some of the venom biomolecules have promise as therapeutic and bioinsecticidal leads. Nonetheless, aspects related to the life history and behaviour of this species, which might influence changes in venom components, have been overlooked. We assessed different behavioural traits (antipredator behaviour, defensiveness and activity) of juveniles and adult females across different contexts (predation, conspecific tolerance and exploration of a new territory) and stimuli (puff of air versus prod) over time. Adults responded to a puff of air faster than juveniles, but in response to a prod, both juveniles and adults become more defensive over time. No differences were observed between adults and juveniles for conspecific tolerance and exploration. Understanding behaviour of venomous species is important because behaviours may affect physiological traits, such as venom, and the ability of spiders to adapt to different conditions.

## 1. Introduction

Australian funnel-web spiders are of considerable research interest because the venom can cause a fatal envenomation syndrome in humans (Nicholson et al., 2004; Nicholson and Graudins, 2002), but also because some of the bioactive components in their venom have shown potential as therapeutic and agrochemical leads (Chassagnon et al., 2017; Ikonopoulou et al., 2018; Nicholson and Graudins, 2002; Schendel et al., 2019). However, ecological, biological, and behavioural aspects of funnel-webs have been largely overlooked.

The study of behaviour is critical for understanding the complexity and variation of venom components because behavioural traits can act synergistically with other factors to affect variation, abundance, and function of spider venoms (Cooper et al., 2015; Hernández Duran et al., 2021). The behaviours associated with venom deployment affect fitness because spiders depend on venom for the acquisition of resources (Boevé et al., 1995; Nelsen et al., 2014), territory defence, predator deterrence, and reproduction (Boevé et al., 1995; Nelsen et al., 2014; Cooper et al., 2015; Zobel-Thropp et al., 2018; Schendel et al., 2019; Herzig et al., 2020a, 2020b). For example, spiders may deploy only small amounts of venom when subduing small prey, but may need to deploy larger

amounts when subduing larger prey, as seen in the tiger wandering spider, *Cupiennius salei* (Boevé et al., 1995).

The ability of spiders to assess threats and modulate their defensive behaviours could explain how spiders use different physiological resources, such as venom, to respond or adapt to different environmental conditions and threats, considering the costs and benefits that these threats could have to their fitness (e.g. high predation risk or competition from conspecifics) (Nelsen et al., 2014). The selection pressures that affect individual responses depend on the context and situation, which involve the survivorship, life stage and reproduction of individuals (Dillon and Pruitt, 2014). To understand how spiders respond in different situations, it is necessary to study their behaviour in different contexts, such as foraging, interactions with conspecifics (social behaviour), antipredator behaviour, and exploration of new environments (Riechert and Hedrick, 1990; Hernández Duran et al., 2021). This information provides insight into individual performance capacities. For example, individuals with faster responses or a higher frequency of defensive behaviours are associated with greater survivorship; aggressive behaviours (more bites) also may help individuals to defend their territory and obtain access to prospective mates (Dillon and Pruitt, 2014).

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The majority of behavioural work on spiders testing threatening stimuli or studying risk assessment has been conducted on species belonging to the infraorder Araneomorphae (Riechert and Hedrick, 1990; Lohrey et al., 2009; Pruitt and Husak, 2010; Dillon and Pruitt, 2014; Nelsen et al., 2014). Less work has been conducted on spiders in the infraorder Mygalomorphae (Jackson and Pollard, 1990; Bengston et al., 2014; Hernández Duran et al., 2021). In Australian funnel-web spiders (Mygalomorphae), behaviour has received less attention. The Sydney funnel-web spider, *A. robustus*, is one of the most venomous species in the world (Nicholson and Graudins, 2002). However, despite its reputation, aspects related to the ecology, biology and behaviour of this species are sparse (Bradley, 1993). Studies on *A. robustus* have mainly focused on the characterisation, identification and mode of action of  $\delta$ -hexatoxin-Ar1a ( $\delta$ -HXTX-Ar1a), the lethal neurotoxin found in the venom of mature males (Nicholson et al., 1996; Alewood et al., 2003; Klint et al., 2012; Wilson, 2016). Mature male *A. robustus* use  $\delta$ -HXTX-Ar1a as defence against vertebrate predators through inhibition of the inactivation of voltage-gated sodium ion channels involved in nociceptive signalling (Herzig et al., 2020b). The lethal effects on humans are seemingly an unfortunate evolutionary coincidence (Herzig et al., 2020b). No studies have explored the behaviour of females, which have a different lifestyle to males (Bradley, 1993), and also show different venom profiles (Nicholson et al., 1996; Wilson, 2016). Females spend their lives in a burrow (Gray, 1981), which they aggressively defend against conspecifics and predators, rarely moving unless forced to do so.

In this study, we measured different behavioural traits of juveniles and adult females of *A. robustus* across different ecological contexts (response to predation, conspecific tolerance and exploration of a new territory) to assess spider responses over time. We assessed spider responses to different types of predator threat stimuli, a puff of air (simulated approach of an aerial predator such as a wasp or bird) and prodding (mechanical stimulus: simulated encounter with a predator). We also assessed spider responses to conspecifics in the same spatial area, and how spiders responded when placed into a novel environment. This information contributes to understanding the relationships between contexts and the environmental cues that affect the spiders' ability to modulate their behavioural responses under different levels of threat.

## 2. Methods

### 2.1. Study species and husbandry

We collected 18 individuals of *A. robustus* ( $n = 13$  adult females, body length:  $9.75 \pm 1.46$ ;  $n = 5$  juveniles, body length:  $7.09 \pm 1.31$ ) by burrow excavation from the Gosford/Central Coast region, New South Wales, Australia, and transported them alive in small plastic containers with damp cotton wool to the laboratory of the Australian Institute of Tropical Health and Medicine (AITHM), James Cook University Ngumbada (Cairns) campus, Queensland, Australia. Spiders were kept individually in 5 L plastic containers (L: 25 cm; W: 17.5 cm; H: 10 cm) in a climate-controlled room (temperature:  $20 \pm 2$  °C; relative humidity: 60%) on a reverse light:dark cycle (12L:12D; lights on at 6 p.m.). The spiders were acclimated for one month before the start of behavioural assays. Each spider received one house cricket, *Acheta domestica*, and water (spraying the container) once a week.

*A. robustus* has a long life span; females can live for more than eight years, and both males and females generally only reach maturity in the fifth year (Gray, 1981; Levitt, 1961). Adult females were identified by epigyne sclerotisation and the opening of the epigastric furrow (gonoslit, Zhan et al., 2019), which is very apparent in adult females but is absent in juveniles (F. Perez-Miles, pers. comm.). Cephalothorax width was measured to assess spider size. To obtain the size, we photographed the cephalothorax of each spider under a Leica stereomicroscope and processed the images using Image J 1.8.0 Software. To determine whether

the size of individuals was related to their life stage, we ran a repeated measures ANOVA (juvenile vs. adult). We found a significant effect, with juveniles being consistently smaller than adults (Supplementary material Figure S1, Table S1). Then, we assessed the correlation between stage and size, using point-biserial correlation (package lmt; Rizopoulos, 2006), where we found a positive correlation between both variables (Supplementary material Figure S1b, Table S1b). Therefore, we chose to use the life stage as a measurement of size in all further statistical analyses.

The research was conducted within the framework of the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Funnel-web spiders are not a protected species in Australia. Spider collection was carried out in collaboration with the Australian Reptile Park (Somersby, New South Wales).

### 2.2. Behavioural assays

After the one-month acclimation period, spiders were starved for two weeks prior to behavioural testing (see below). Thereafter, following modified methods of Riechert & Hedrick (1990) and Stankowich (2009), we assessed antipredator behaviour and defensiveness of all individuals in the following contexts: response to predation, conspecific tolerance and exploration of a new territory. To avoid habituation, only one test was carried out each day, allowing the spiders to rest for 24 h between tests. While tests were conducted on consecutive days for each spider, the order of tests was randomised for each spider, except for the prod test, which was always presented first due to venom collection (venom profiles were not included in this study). Behavioural tests were repeated three times for each individual, one month apart, to measure changes over time. All assays were carried out between 6 a.m. and 12 p.m. during the dark phase, and were video recorded under red light (Supplementary material Table S). Behaviours were analysed using BORIS version 7.8.2 (Friard and Gamba, 2016).

#### 2.2.1. Predation

The variation in behavioural responses to an antipredator stimulus has been linked with fitness components in different species (Dillon and Pruitt, 2014; Nelsen et al., 2014). Spiders use their different senses (visual, chemical, mechanical) to acquire information from the environment, habitat and they also use previous experiences to help them respond to different types of stimuli (Eiben and Persons, 2007). To measure spider responses to different types of antipredator stimuli, we used two types of aversive stimulus: the puff of air stimulus and the prodding stimulus. Both stimuli represent a predatory cue (e.g. avian predator; Riechert and Hedrick, 1990; Dillon and Pruitt, 2014), but the puff of air produces an avoidance response because the spider is not physically touched (Riechert and Hedrick, 1990), whereas the prod provokes a defensive response because the spider is physically touched (Nelsen et al., 2014). The puff of air was conducted in each individual's home container. Lifting the first pair of legs, moving the fangs and expelling venom are common behavioural responses to predatory threats in Australian funnel-web spiders (Wilson and Alewood, 2004, 2006). We use fang movements to measure spider responses to this aversive stimulus as frequency of fang movements was positively correlated with lifting the first pair of legs (Kendall's tau = 0.744,  $z = 6.8145$ ,  $P < 0.001$ , and negatively correlated with huddling (Kendall's tau = -0.51,  $z = -4.710$ ,  $P < 0.001$ ). Each spider was gently moved out of its retreat using tweezers. After a 60 s acclimation period (a longer period resulted in spiders returning to the burrow), we applied three rapid puffs of air on the spider's anterior prosoma using a camera air blower and recorded fang movements for 420 s (Jackson et al., 1990; Riechert and Hedrick, 1990).

The prod test was also conducted in the home container and followed the modified methodology of Bengston et al. (2014). The individual was moved out of the retreat, as for the "puff test", and after a 60 s acclimation period, we gently prodded the first pair of legs with blunt

tweezers continuously for 240 s. Thereafter, we recorded the number of fang movements of the individual for a further 360 s, totalling 600 s. Using the same behavioural response (frequency of fang movements) for the two different stimuli (puff of air and prod) allowed us to directly compare if the stimulus affected the spiders' behaviour in a different way.

### 2.2.2. Conspecific tolerance

*A. robustus* is usually a solitary species; however, it is possible to find female retreats located spatially close to one another (Gray, 1992), suggesting that competition might occur between females for food. In addition, males wander in search of females, and female aggression towards males may lead to precopulatory sexual cannibalism (Johnson and Sih, 2005). To test defensiveness towards conspecifics we followed the modified protocol of Bengston et al. (2014). We placed an individual in a new arena (L: 23 cm; W: 16.5 cm; H: 10.5) that was divided into two equal halves with a mesh barrier (mesh diameter:  $2 \times 2$  mm). This prevented direct contact between individuals, reducing the risk of fighting and death, but the mesh allowed spider to detect olfactory (and possibly mechanical cues) from other individuals. We randomly selected pairs of spiders (we attempted to size match but this was not always possible) and video recorded the whole arena for 1800 s. We measured the number of times the spider climbed the barrier as a measure of a conspecific defence response, which usually occurred when one spider perceived the other and tried to move closer by climbing the barrier. We previously observed that defensive spiders tried to cross the barrier and attack the other spider. Therefore, we chose climbing as a measurement of defensiveness against conspecifics. While this test could potentially measure a spider's propensity to escape using the mesh barrier, initial observations indicated that some spiders never approached or climbed the mesh, regardless of whether a conspecific was present or not, and others only moved towards the mesh when a conspecific was present, often displaying aggressive behaviours (e.g. leg waving and lunging) towards them. Therefore, we suggest that this test measures a response to conspecifics rather than a motivation to escape.

### 2.2.3. Exploration of a new territory

The willingness of spiders to disperse or move could affect spider survival because females can relocate their burrow in order to acquire resources, colonise new areas and avoid predators (Nakata and Ushimaru, 2013; Bengston et al., 2014). We assessed exploration of a new territory using activity level as the individual's willingness to explore a new environment (fear of novel objects or environment; Bengston et al., 2014). We placed an individual spider in a novel arena (L: 34.5 cm; W: 22 cm; H: 10.7 cm). We then measured the activity of the spiders as the time the individual spent moving around the container for 900 s.

## 2.3. Statistical methods

All statistical analyses were conducted using the statistical software R version 4.1.0 (R Core Team, 2021; <https://www.rproject.org>).

### 2.3.1. Changes in behaviour over time

We used rank-based non-parametric analyses for longitudinal data instead of generalized linear mixed models (GLMM) to test for changes in behaviour between individuals over time. This analysis offers a robust framework for non-continuous variables, small sample size and skewed data (Noguchi et al., 2012). We used this analysis to assess the effects of life stage (juvenile and adult) on the behavioural variables (fang movements, number of climbs and duration of activity) measured in each context (antipredator behaviour, conspecific tolerance and exploration of a new territory), and over three time periods (T1, T2, T3). The design used was F1-LD-F1 in the nparLD package (Noguchi et al., 2012). The first F1, refers to the number of factors in each group, in this case juveniles and adults (whole-plot factor group) of *A. robustus*. LD refers to the term longitudinal data, and the last F1, refers to the time level

(sub-plot factor). The random effect of individual identity was included as a subject in this model. We performed a Bonferroni correction to adjust *p*-values for repetitions. We also used the F1-LD-F1 design to test for differences between the type of stimulus (puff of air and prod) used, considering fang movements as the response variable in the antipredator context. For each group (stage), the rank mean of overall rank, observations (Nobs), the point estimates of the relative treatment effect (RTE), and confidence intervals for each behavioural variable are shown in the Supplementary Material Table S2.

To validate our hypotheses related to the effects of repetition and differences between stages over each behavioural variable in each context, we conducted a randomization test (1000 times) for each longitudinal model (nparLD), where the ANOVA-Type statistic was used to assess whether the values obtained in the tests were simply a result of chance or were indicative of true significant differences (Supplementary material Figure S2).

## 3. Results

### 3.1. Changes in behaviour over time

For response to predation, the frequency of fang movements of all individuals was compared over time in response to the puff of air (Table 1). We found no significant difference over repetitions (ATS = 0.270; *df* = 1.711; *P* = 0.729). However, there was a significant difference between adults and juveniles (ATS = 9.157; *df* = 1; *P* = 0.002, Table 1; Fig. 1a; Supplementary material Figure S2a). Adults showed more fang movements than juveniles at T1 and T3. In contrast, adults and juveniles showed a similar frequency of fang movements at T2 (Fig. 1a).

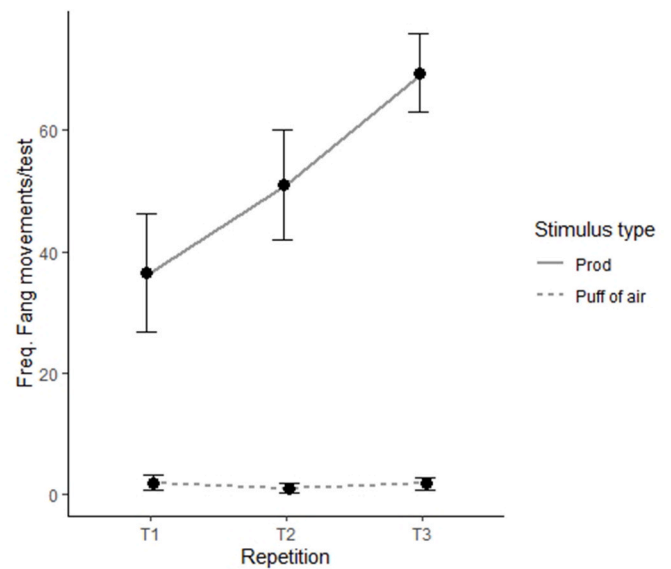
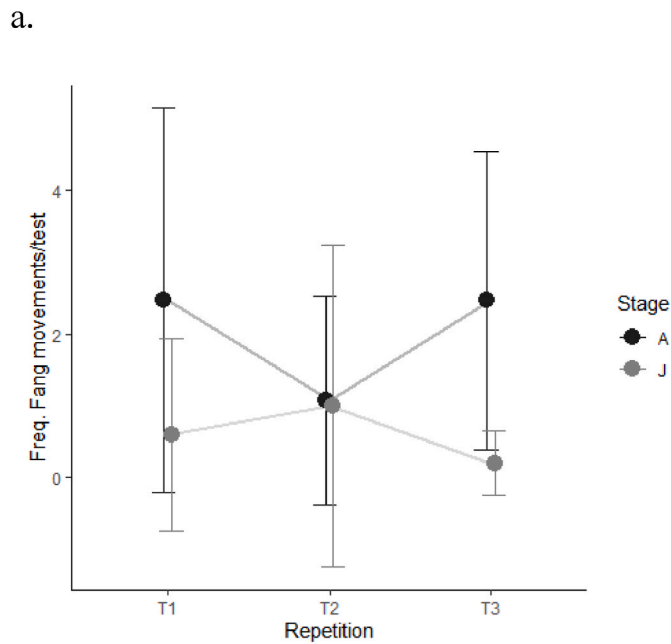
In contrast, when we measured the frequency of fang movements of all individuals over time in response to the prod stimulus (Table 1), we found a significant increase in the frequency of fang movements from repetitions T1 to T3 (ATS = 26.308; *df* = 1.989; *P* < 0.001; Fig. 1b). However, we did not find differences in the frequency of fang movements between adults and juveniles (ATS = 2.247; *df* = 1; *P* = 0.195; Table 1; Supplementary material S2b).

For differences in fang movements between the type of stimulus, puff of air and prod in the context of a predatory cue, we found significant differences in the frequency of fang movements between the different types of stimuli (puff vs. prod; ATS = 220.673; *df* = 1; *P* < 0.001), and over repetitions (ATS = 10.495; *df* = 1; *P* < 0.001). Spiders showed a significantly higher frequency of fang movements when prodded than when exposed to the puff of air (Fig. 2), and there was a significant increase in fang movements over time in response to the prod stimulus

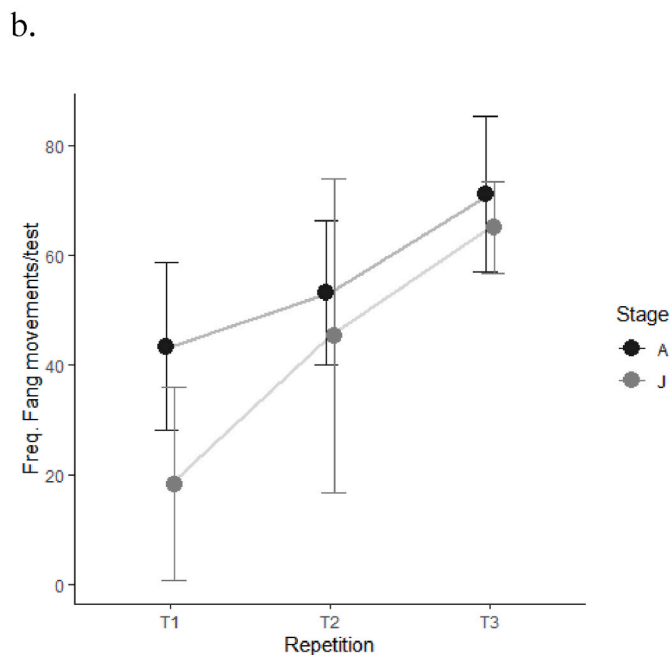
**Table 1**

Output of rank-based non-parametric analyses for longitudinal data models of different spider behavioural traits, and the effects of repetitions and life stage. The \* refers to results that are significant at the  $\alpha = 0.05$  level.

ANOVA-Type Statistic (ATS)			
Model	Frequency of fang movements (puff of air) ~ Repetition + Stage, subject = factor (ID)		
	Statistic	df	p-value
Stage	9.157	1	0.002 *
Repetition	0.270	1.711	0.729
Model	Frequency of fang movements (prod) ~ Repetition + Stage, subject = factor (ID)		
Stage	2.247	1	0.195
Repetition	26.308	1.989	<0.001 *
Model	Frequency of climbs ~ Repetition + Stage, subject = factor (ID)		
Stage	0.516	1	0.472
Repetition	0.468	1.828	0.607
Model	Activity ~ Repetition + Stage, subject = factor (ID)		
Stage	0.145	1	0.703
Repetition	1.590	1.723	0.207



**Fig. 2.** Mean frequency of fang movements made by *Atrax robustus* in response to either a puff of air (dotted line: antipredator behaviour) and/or a prod stimulus (solid line: defensiveness) over three-time repetitions (T1-T3).



**Fig. 1.** Frequency of fang movements made by adult female and juvenile *Atrax robustus* in response to (a) a puff of air and (b) a prod stimulus.

from T1 to T3 (Fig. 2).

For conspecific tolerance, there were no significant differences in the climbing frequency over time (ATS = 0.471;  $df = 1.827$ ;  $P = 0.606$ ) and life stage (ATS = 0.544;  $df = 1$ ;  $P = 0.460$ ) (Supplementary material S2c). Similarly, in the context of exploration of a new territory, we found no significant differences in activity over time (ATS = 1.589;  $df = 1.722$ ;  $P = 0.206$ ) and life stage (ATS = 0.145;  $df = 1$ ;  $P = 0.702$ ) (Supplementary material S2d).

#### 4. Discussion

This is the first study to assess changes in behavioural traits of *A. robustus* over different contexts and time. We found that adult female

spiders made more fang movements than juveniles in response to a puff of air over all time periods. Juveniles appeared to show a different behavioural response compared to adults. We observed that juveniles tended to huddle rather than move their fangs, whereas adults tended to lift the first pair of legs and move the fangs in response to the puff of air. Although we must treat these differences with caution because of the small sample size of juveniles, the trend is maintained, even with randomization (Supplementary material Figure S2). The different responses between adults and juveniles could be a consequence of the developmental stage, reproductive status (e.g. females are more aggressive when they reach maturity to deter predators and conspecifics), and/or investment in offspring (e.g. sexually mature females are more aggressive as they invest more energy into egg production) (Assi Bessékon and Horel, 1996; Mooney and Haloin, 2006). Broadly, for spiders, juveniles tend to respond to a threatening stimulus by fleeing or huddling (to blend in to the environment or feign death), reducing the likelihood of being predated or being injured (Stankowich, 2009), which in turn can also reduce the metabolic costs related to the use of venom (Cooper et al., 2015). In contrast, adults are more likely to take risks to defend their burrow or web and guard their offspring (Mooney and Haloin, 2006). Adults are also more likely to show a higher number of defensive behaviours when threatened because they are larger than juveniles, and have different concentrations of toxins (Escoubas et al., 2002) that could help them to deter predators (Herzig et al., 2020b), conspecifics and a diverse range of parasitoids (Stankowich, 2009).

In contrast, when we assessed the spiders' responses to the prod stimulus, we found that both juveniles and adults increased the number of fang movements over time, suggesting that they became more defensive. This type of stimulus likely affects the behavioural response because it is more similar to a direct conspecific or predator encounter (Jackson et al., 1990; Stankowich, 2009) than the puff of air, which elicits another type of antipredator response (predator escape). In support, we found that spiders responded differently to the different types of stimuli, showing an increased frequency of fang movements in response to the prod compared to the puff stimulus. Our results are consistent with those found in *Pholcus phalangioides*, where larger juveniles and adult females whirled for longer periods in response to a mechanical stimulus compared to when they received a puff of air (Jackson et al., 1990).

Spiders can optimise their responses to different threatening stimuli

(Cooper et al., 2015) depending on their life stage, size or the approach of the stimulus (e.g. how fast the threatening stimulus is moving, or how close it is to the spider) (Lohrey et al., 2009; Stankowich, 2009). In addition, antipredator behaviour can change over time (Glaudas et al., 2006). Animals may be more flexible if they are continuously exposed to a threat, where they can learn to discriminate between potential harmful and non-harmful threats (Glaudas et al., 2006; Lohrey et al., 2009; Nelsen et al., 2014). For example, in populations of *Agelenopsis aperta* with high predation pressure, the latency to return to foraging was faster compared to the populations with lower predator pressure (Riechert and Hedrick, 1990).

Spiders can also modulate their behaviour according to the level of threat that they are facing (Lohrey et al., 2009; Stankowich, 2009; Nelsen et al., 2014). For example, in the black tunnel-web spider, *Porrhothele antipodiana*, individuals display a gaping behaviour, which consists of raising the body and moving the fangs into a defensive position against conspecifics and predators (Jackson and Pollard, 1990). Funnel-webs also show similar gaping displays, lifting the first pair of legs and deploying venom from their fangs (Wilson and Alewood, 2004, 2006). The ability to use biochemical weapons might also influence the way spiders assess the level of threat (Nelsen et al., 2014; Cooper et al., 2015). Spiders can choose to expel venom as a mechanism of defence when they do not see a way of escaping the threat (Cooper et al., 2015). For example, an aggravation stimulus of prodding in Australian funnel-web spiders (personal observation) and species of mouse spider, *Missulena* (Herzig et al., 2008), resulted in large amounts of venom being expended in initial bites. However, the spiders tended to reduce the amount of venom expended in subsequent bites in response to the same stimulus. Furthermore, some spiders (e.g. mouse spiders, black widows) can use dry bites instead of deploying venom when they are threatened, reducing the metabolic costs of expending venom (Nelsen et al., 2014; Cooper et al., 2015). In our study, although not quantified here, venom expenditure appeared to be lower in juveniles than adults, as juveniles rarely deployed venom on the fangs, whereas adults readily deployed venom.

For the contexts of conspecific tolerance and exploration of a new territory, we did not find differences between life stages and repetitions in climbing behaviour and activity level, which suggests that both behaviours are likely not affected by life stage, and do not change over time. This could be due to both juveniles and adults sharing similar types of prey and microhabitats, and engaging in the same types of con- and interspecific interactions (Riechert, 1984). Spiders under natural conditions can alter their agonistic behaviour when resources are manipulated (Riechert, 1978). It would be worthwhile in the future to test spiders in their home containers, as aggressiveness against conspecifics could be affected by their fidelity to a burrow or territory.

In this study, we followed established methodology that largely required active, mechanical stimuli, and found variation in behavioural responses to these stimuli. However, chemical cues also play an important role in how spiders respond behaviourally to predators and potential prey (Eiben and Persons, 2007; Montiglio and DiRienzo, 2016). Studies that have explored personality in spiders with consideration of chemical cues have all been conducted on Araneomorph spiders that build webs, which serve as an extended phenotype (Montiglio and DiRienzo, 2016). To our knowledge, chemical cues have not yet been explored in the context of personality in Mygalomorphs. Female funnel-webs remain in their burrows for their whole lives (Gray, 2010), so future studies into whether chemical cues elicit behavioural changes, or whether behavioural responses to these chemical cues are repeatable over time, would be a valuable area for future research.

Another avenue that needs more exploration is the relationship between venom and different behaviours. These associations will provide important information for understanding the effects of venom and its function on the natural prey of spiders, as well as the role played in other contexts such as mating and intraspecific competition (Hernández Duran et al., 2021; Schendel et al., 2019). Behavioural and ecological

factors can influence the quality of venoms (as has been observed in bees *Apis mellifera*; Scaccabarozzi et al., 2021), having a direct or indirect effect on an individual's fitness. For example, aggressive individuals could experience higher metabolic costs associated with venom expenditure, but aggression could also be associated with higher venom concentration and quantity when they are exposed to predators and when the level of threat is higher (Westermann et al., 2015; Lira et al., 2017; Hernández Duran et al., 2021), increasing the likelihood that they can mount a stronger aggressive response. Studying these relationships will provide insights into understanding how venoms evolve, and could contribute to the identification of molecular changes in toxins, which are relevant in pharmacological applications and drug discovery (Herzig et al., 2020a; Schendel et al., 2019).

In summary, this study highlights how juveniles and adult females of *A. robustus* show variation in behavioural responses when different types of threatening stimuli are assessed over time and across different contexts. Differences in behaviour were observed between life stages, suggesting that previous experiences, physiological traits and/or environmental conditions may trigger or inhibit responses in juveniles and adults differently. Adults were more likely to show a defensive response when they received a puff of air or prod, and this could be related to hormonal changes and/or energy invested in egg production when spiders reach sexual maturity. More studies are required to assess the relationship between venom components, hormones, metabolic rates, and behaviours in different contexts. We suggest that changes in behavioural responses could potentially affect variation in venom components, and how these biological weapons are used over different developmental stages. This is an exciting avenue for future investigation.

#### Author contributions

Linda Hernandez Duran: Conceptualization, Investigation, data collection, data Formal analysis, Methodology, Visualization, Writing – original draft preparation. Writing editing. David Wilson: Methodology and data analysis, writing-reviewing, editing, Supervision. Tasmin Rymer: Writing-reviewing and editing, Supervision.

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#### Ethical statement

Spiders were observed daily and monitored weekly. Experimental procedures did not have any negative effects on the animals. Due to funnel web spiders are not a protected species in Australia, the Department of Environment and Science of Queensland Government advised that a scientific permit was not required. However, our research was conducted within the framework of the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013).

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.toxcx.2022.100093>.

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