Laboratory husbandry of arboreal tarantulas (Theraphosidae) and evaluation of environmental enrichment

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Summary
Environmental enrichment has long been recognised as an integral part of sound husbandry practice for vertebrates, but its impact on invertebrates is relatively understudied. The successful maintenance of arboreal tarantulas (theraphosids) in a laboratory environment requires an effective husbandry protocol coupled with appropriate environmental enrichment. In order to develop such a protocol, three species of tropical arboreal theraphosid were observed over a six month period and detailed husbandry records kept. To examine the effects of environmental enrichment on theraphosids, a study group of *Psalmopeus cambridgei* juveniles were maintained in enriched and un-enriched environments and their activity patterns monitored and behavioural data recorded. Examination of this data revealed that spiders maintained in un-enriched enclosures were more aggressive and exhibited a much stronger flight response than their counterparts. These negative behaviours were a sharp disparity between the positive behaviours exhibited by the enriched spiders which subsequently fared better in the captive laboratory environment.

Introduction
Spiders of the Theraphosidae family (suborder Mygalomorphae) are true tarantulas recognisable by their large size and often hirsute appearance. With a worldwide distribution, there are approximately 937 known theraphosid species from 120 general[1]. Theraphosids have a particularly strong representation in tropical and semi-tropical areas, but their ecological diversity is extensive and includes inhabitation of savannah, desert, rainforest and semi-temperate environments[2]. All spiders are predatory (with the exception of *Bagheera kiplingi*, a neotropical jumping spider which feeds predominantly on leaf tips[3]), but contrary to popular belief, not all spiders spin webs to ensnare prey. Theraphosids depend on their physical strength and rapid acting venoms to overcome prey only producing silk predominantly to form tubular shaped retreats in the case of arboreal species and to line burrow entrances in the case of most terrestrial species[4].

Theraphosids are relative newcomers to the laboratory environment, but as recognition of their potential to provide novel tools for pharmacological research grows so does the animal technician's need to develop effective husbandry protocols and procedures. While certain invertebrate species such as fruit flies (*Drosphila melanogaster*), horseshoe crabs (*Limulus polyphemus*) and cephalopods such as *Octopus vulgaris* have been used in the laboratory for years[5], it would be fair to say that, in general, the effective maintenance of other invertebrates in the laboratory environment remains an understudied field. This is compounded by the dearth of sound invertebrate husbandry information, misconceptions about the needs of invertebrates in the laboratory environment and a common perception that invertebrates fall into the 'lower end' of a welfare scale[6]. Historically, invertebrates have not been deemed to require the same duty of care and attention as the vertebrate species commonly housed within the laboratory – indeed, with the exception of cephalopods, invertebrates are not currently protected by UK law[7]. It is the animal technician's role, however, to ensure that their captive charges – vertebrate or invertebrate – do not simply survive but thrive.

Focusing on three species of tropical, arboreal theraphosid – the Singapore blue (*Lampropelma violaceopes*), Salem ornamental (*Poecilotheria formosa*) and Trinidad chevron (*Psalmopoeus cambridgei*) – this paper describes a captive husbandry protocol and enclosure design for their effective maintenance in the laboratory environment. Animals observed during husbandry protocol development were mixed gender, wild caught specimens of an undetermined age.

Moreover, the effect of behavioural enrichment was examined by recording and comparing the behaviours exhibited by two study groups of enriched and un-enriched *P. cambridgei* juveniles after they were exposed to a series of aversive and non-aversive stimuli. Negative behaviours were linked to a heightened flight response and over-aggressive behaviour. This was coupled with observations on the amount of webbing and type of webs constructed by the juveniles. Theraphosids are, in general, reluctant to leave the security of their web retreats, requiring a significant amount of provocation to do to so[8]. Spiders that had not constructed web retreats or very little webbing were deemed as 'more stressed' and subsequently more likely to exhibit negative behaviours. A behavioural ethogram was developed and the spiders' behaviour analysed.

Methods

All procedures were performed in accordance with the principles of the Animals (Scientific Procedures) Act, 1986, even though arachnids are not currently protected under the Act. Microbiological status of animals was not examined.

Theraphosids are robust arachnids with a large abdomen (opisthosoma) connected by the pedicel to the prosoma (Figure 1). Like all spiders, they possess spinnerets enabling them to manipulate the silk as it is produced, are venomous and have fangs attached to the chelicerae. They have eight legs that often appear hairy due to the setae and also possess a pair of pedipalps used for prey capture and walking. These are enlarged in the male on maturity as they are used to collect and transfer sperm during reproduction.

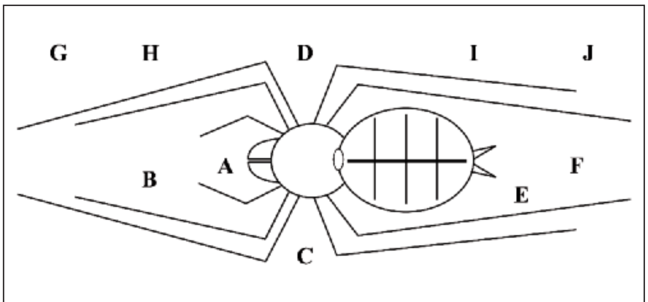

Figure 1. Theraphosid anatomy

A. Chelicerae
B. Pedipalps
C. Prosoma
D. Pedicel
E. Opisthosoma
F. Spinnerets
G. Leg I
H. Leg II
I. Leg III
J. Leg IV

Theraphosids are described as being either 'old world' or 'new world' due to their geographical distribution. New world theraphosids have the ability to flick urticating hairs as a means of defence which causes inflammation of the skin and mucus membranes (urtication) in vertebrates. Two of the three species featured in the paper (*L. violaceopes and P. formosa)* are old world theraphosids and, therefore, lack this ability. They compensate for this by being much more aggressive when provoked, frequently threat posturing and ultimately lunging and biting would-be attackers if provocation persists. *P. cambridgei* is a new world theraphosid but – as is the case in all *Psalmopoeus* species[2] – it lacks the urticating hairs associated with new world theraphosids. Its defence therefore, also focuses on posturing and aggressiveness.

L.violaceopes and *P. formosa* are Asian theraphosids located in Singapore and Malaysia, and in India respectively while *P.cambridgei* is endemic to the West Indies, specifically Trinidad. Adult female and immature arboreal theraphosids are predominantly sedentary and, as such, construct web retreats behind loose bark and in tree nooks to hide in during the daytime, usually only emerging at night to wait in ambush for prey. Males also build web retreats but in their quest to find females are peripatric and are more active in their enclosures.

Husbandry protocol

Enclosure Design – adult and sub-adult arboreal theraphosids; Due to the climbing nature of arboreal theraphosids, it is important that accommodation focuses on height rather than width[4]. The arboreal theraphosids were housed in transparent, front-opening, 8 litre polypropylene boxes (Really Useful Products Ltd.) with a series of nine 4mm ventilation holes drilled into each side at the top of the box and a further six 4mm ventilation holes drilled into the front (Figure 2). When stood vertically, these open front boxes are particularly useful as they provide front and top access. A retreat is provided in the form of a short (15-20 cm) section of UPVC roundline guttering (Wickes Ltd.) to simulate the rot holes and loose bark that arboreal theraphosids would utilise in the wild to construct their hides[9]. The guttering is scoured with a sharp implement to facilitate climbing as the spiders can sometimes struggle to grasp the slippery outer surface. It is then secured vertically with a 4mm diameter aluminium rod (Wickes Ltd) which is threaded through the sides of the box via two strategically placed holes. To further aid climbing, a plastic, leafy branch cut to the appropriate size (Concrete Jungle Ltd) is threaded through one of the ventilation holes at the top of the box and twisted back on itself so the spider cannot pull it down. The length of the plastic branch must be long enough so that the spider can easily reach it with one of its legs if it is at the bottom of the enclosure. A strip of white plastic runner (18mm high (Wickes Ltd), is cut to size and adhered to the bottom of the box with electrical tape: this creates a barrier preventing loose substrate from falling out of the box when the front lid is opened. Lastly, a 5cm diameter water bowl (Vanishing World) is provided at the bottom of the enclosure.

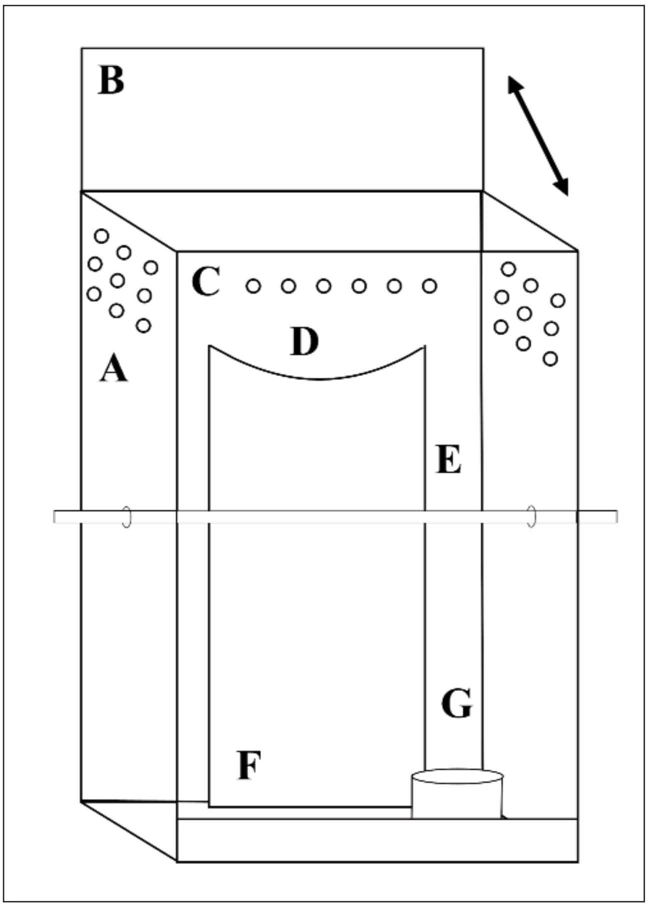

Figure 2. Enclosure design for adult and sub-adult arboreal theraphosids

A. Ventilation
B. Top lid
C. Front lid
D. Guttering retreat
E. Rod to secure retreat
F. Substrate barrier
G. Water bowl

Substrate – Vermiculite (Peregrine Livefoods Ltd.) is the substrate of choice as it can be moistened to the species' requirements and helps maintain relative humidity in the spiders' enclosures. Vermiculite is a particularly 'clean' substrate as it is inert, has a neutral pH and is less likely to harbour pathogens and micro-organisms[4]. It has been deemed as a particularly suitable substrate for large collections as it is also relatively inexpensive and easily obtainable. The colour of vermiculite also makes it easier for the animal technician to spot boluses, uneaten prey items and other detritus in the enclosure.

Humidification – As the species outlined in this paper are from tropical regions, it is necessary to ensure that they are maintained with the appropriate level of humidity. This is achieved by lightly misting the enclosures using a 5l Hozelock pressure water sprayer (Homebase) in the morning every couple of days. It is important not to overspray the enclosure or directly spray the spiders as they are strongly averse to this[4] and the enclosure should be cleaned, and substrate replaced should an odour start to develop.

Photoperiod – theraphosids are nocturnal and are therefore somewhat photophobic. Care should be taken not to place theraphosid enclosures next to sources of bright light as this will inevitably cause the spiders significant stress. They do not require UV lighting as is sometimes purported[4]. The lab is typically light for 9 hours a day with a 3 hour twilight period before the onset of darkness.

Feeding – Theraphosids feed on a wide variety of invertebrate and small vertebrate prey items in the wild[9]. In the laboratory they are fed on 3-4 large, gut-loaded black crickets (*Gryllus* sp.) or a couple of *adult locusts* (*Schistocerca gregaria*) every couple of weeks. Theraphosids are prone to obesity if they are overfed which is characterised by an almost balloon shaped, overextended opisthosoma. It is important to monitor theraphosids to prevent them from gaining too much weight.

Behavioural Enrichment – *Psalmopoeus cambridgei* juveniles

Enclosures for the juveniles were 360ml polystyrene extra wide mouth containers, (Fischer Scientific) with a flexible plastic lid. 2mm holes were drilled all around the top of the tub and pin prick holes in the lid to increase ventilation. The un-enriched enclosures were barren of any hides and a thin layer of blue roll was placed at the bottom of the tubs so it could be dampened to maintain humidity as this is critical for their survival. The enriched enclosures were given a 2cm deep layer of moistened vermiculite as a substrate and artificial retreats were made by drilling a hole into 35mm camera film canisters just large enough for the spider to pass through. Plastic leaves were placed next to the camera film retreats and a

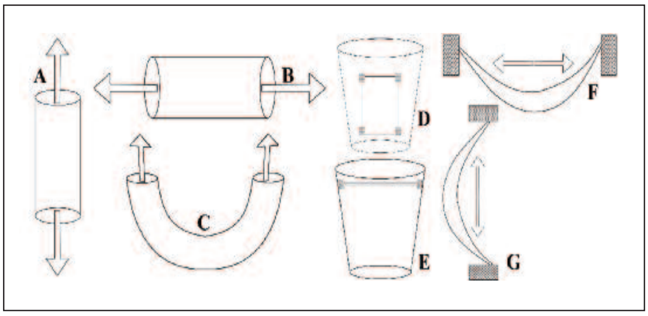

Figure 3. Webbing types: tubes, sheets and canopies

A. Vertical tube web
B. Horizontal tube web
C. U-shaped tube web
D. Vertical sheet web within enclosure
E. Horizontal sheet web within enclosure
F. Horizontal canopy web
G. Vertical canopy web

small water bowl (bottle top) was also placed at the bottom of the enclosures. Particular attention was paid to web construction and types of webs made during the study. A spider that produces a web or a silken retreat, is displaying natural behaviours and therefore deemed to be unstressed. Web building is a very important part of arboreal theraphosid ecology. There are many different types of webs that arboreal theraphosids construct (Figure 3) – the most common are the tubular web retreats. These are constructed by juveniles and adults alike. Juveniles, however, also produce canopy and sheet webs. Three distinct types of webbing with two orientations were recorded during the study: tube, sheet and canopy.

Discussion

Six *P.cambridgei* juveniles, of undetermined sex were used in the behavioural study. All six spiders were captive bred at Venomtech and had moulted three to four times. Average weight (0.53 +/– 0.25g), length (1.8 +/– 0.3cm). To obtain these measurements, it was necessary to first anaesthetise each animal with CO_2 to prevent getting bitten[10] as well as minimise the risk of spiders autotomising limbs. Length measurements were taken from the chelicerae to the base of the spinnerets.

Spiders were divided into two study groups: group A were housed in un-enriched enclosures while group B were housed in enriched enclosures, as described previously. The spiders were given a week to acclimatise to their new enclosures before being exposed to five different stimuli: four aversive and one non-aversive. For each test, focal sampling was carried out for each individual spider wherein the animal was observed and its behaviour (Figure 4) recorded for a three minute period following the stimulus. Notes on web construction were taken during each behavioural study.

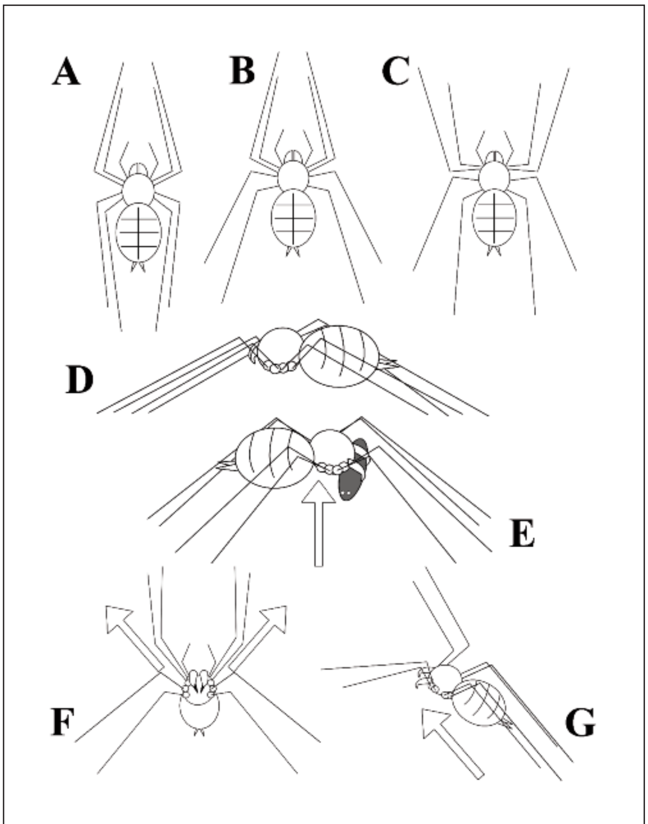

Figure 4. Examples of theraphosids at rest, ambulatory and defence postures

A & B. Resting
C. Alert
D. Walking: prosoma and opisthosoma slightly elevated
E. Feeding: prosoma and opisthosoma raised high off the ground, prey tightly held between chelicerae and fangs
F. Threat display frontal view: note exposed fangs, raised pedipalps and raised legs I and II
G. Threat display side view: note the steep angle at which both the prosoma and the opisthosoma are raised, chelicerae pushed forward to expose fangs.

Behavioural studies

I. Reaction to enclosure rotation – Each enclosure was systematically placed on a work bench and gently rotated a full 360 degrees twice causing vibrations in the enclosure.

II. Reaction to prey item – Each spider was fed a 3rd instar banded brown cricket (*Acheta domestica*). Crickets were placed into the enclosures using tweezers and dropped in on the opposite side to where the spider was located.

III. Reaction to spraying – Spraying was administered using a 5l Hozelock pressurised water sprayer (Homebase) and the nozzle was introduced to the opposite side of the spider. A spray of water was emitted for 2-3 seconds and spray was allowed to fall on the spider in order to act as a direct stimulus.

IV. Reaction to tapping – The enclosures were tapped down on the work bench three times.

V. Reaction to stroking – Spiders were gently stroked on the back legs (legs IV) using the soft end of a small paintbrush until it responded. Each spider was stimulated twice 1.5 minutes apart.

After each study, the spiders' behaviours were recorded, categorised and an ethogram derived (Table 1). In total fourteen different behaviours were identified, half of which were deemed as 'negative' behaviours – the spider exhibited a very strong flight response or aggressive reaction to the stimuli. Given that the majority of the spiders were in their webs and at rest (because it was daytime), it would take a lot to get a contented spider to come out of its retreat. Un-enriched spiders, that had built far less webbing, reacted much more violently to stimuli.

Behaviour	Definition
Rest	Legs I and II pulled tightly together outstretched in front of spider and legs III and IV pulled tightly together outstretched behind spider
Alert	All legs splayed
Leg raise*	Raises pedipalps and legs I and II off ground, elevates the prosoma, pushes chelicerae forward and exposes fangs
Ground tap*	Leg raise followed by rapidly tapping legs I and II down on the ground.
Lunge*	Jumping forward, grabbing threat with pedipalps, legs I and II, and biting
Flinch*	Legs twitch and body jerks away from ground briefly, raises limb(s) briefly before placing them back on ground, jerk backwards/ forwards but then settles
Saltate*	Jumping and running away
Hide*	Moving underneath, into or behind something, or retreating into web
Chase	Pursues prey around enclosure
Pounce	Pounces forward at prey and grabs hold of it with pedipalps and legs I and II
Feed	Prey grasped between pedipalps and held up in chelicerae to mouth
Climb	Vertical ascent or descent using the enclosure sides or items in enclosure
Walk	Forward movement at slow to moderate pace
Run*	Forward movement at fast pace

** indicates a negative behaviour*

Table 1. Ethogram of behaviours exhibited by enriched and un-enriched *P.cambridgei* juveniles in response to aversive and non-aversive stimuli

Results

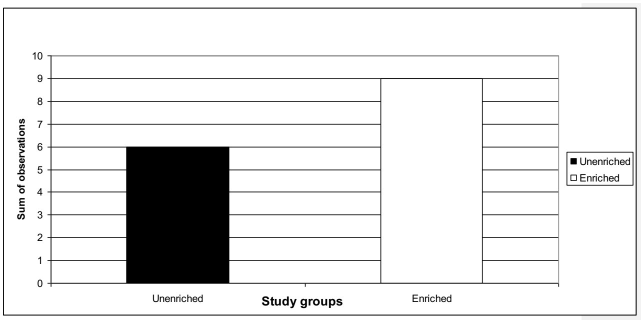

Figure 5. Web construction

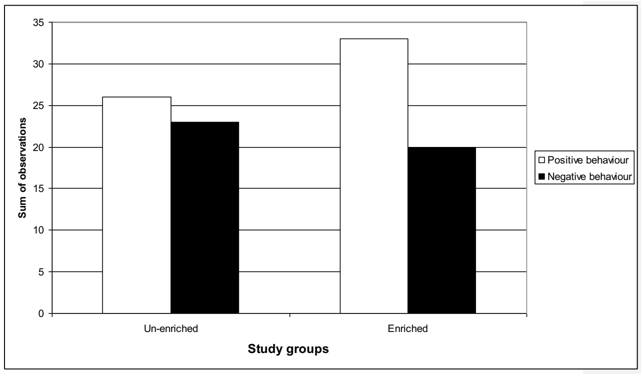

Figure 6. Evoked response of juveniles to all stimuli

Conclusions

Environment enrichment affects natural behaviours in juvenile *P. cambridgei* such as web building; 33% more web was observed in the enriched group. The lack of web construction in the un-enriched group potentially has a synergistic effect on behaviour as un-enriched juveniles also exhibited more negative behaviours and less positive behaviours than the enriched juveniles. These effects are difficult to isolate due to the effect environmental enrichment has on web production.

The enriched spiders had built web tubes so they were observed more often at rest. Stimuli, for the most part, prompted flinching and hiding behaviour. For the un-enriched spiders that were much more exposed, stimuli provoked considerably more saltation, running and leg-raising behaviour. Defensive leg-raising, lunging and biting, observed mainly in un-enriched juveniles and once in the enriched juveniles, are postures and behaviours exhibited by theraphosids when they are under particular duress[11] – the preferred means of defence, certainly in the case of enriched juveniles, is to retreat.

Enclosure rotation – one of the gentler behavioural experiments – had an interesting effect on the un-enriched juveniles, all of which had yet to construct webs. As the enclosure was rotated, the resting un-enriched juveniles assumed an 'alert' position with one of the juveniles running around the bottom of its enclosure and hiding under the blue roll. The enriched spiders remained in the resting position throughout this experiment.

Feeding evoked similar behaviours between the study groups. Upon introduction of the cricket into their enclosures, both enriched and un-enriched juveniles reacted to the prey item. The enriched spiders were quick to pounce and feed on the prey item, but the un-enriched juveniles, while becoming alert, did not react negatively. The time of 3 minutes observation was not adequate enough to record feeding behaviours. It took some spiders longer to actively hunt and catch their prey so they fell out of the 3 minute focal sampling observation time. All spiders, with the exception of one un-enriched juvenile which shed its skin the following day, did eat their cricket. This observation is significant because spiders do not feed in pre-ecdysis[12].

Physical provocation of the juvenile spiders evoked particularly negative behaviours with salutation, running and hiding widely exhibited in both study groups regardless of whether they were resting in a web or not. Interestingly one of the enriched spiders lunged and bit the paint brush before running away, but this could be because it mistook the brush for a prey item initially. Tactile stimulus does have a marked effect on juvenile spiders and, out of all the aversive experiments, evoked the greatest number of negative reactions from both groups.

Discussion

The behavioural studies undertaken in this paper have clearly shown that theraphosids do benefit from behavioural enrichment and that web construction is an indication of the spiders' well-being in captivity. Arboreal theraphosids of all ontogenetic stages will construct webs but there are some key differences between adult and juvenile web construction. Previous studies conducted on wild theraphosids have found that adult spiders frequently inhabit tree trunks and branches a considerable distance above the ground. They construct predominantly tubular retreats behind the bark and in holes and do not use leaves in their web construction. The adult theraphosids in the laboratory all constructed pouch like web retreats behind the guttering hides in their enclosures. The only time they were observed using the leaves in their enclosures was when they were climbing or hunting prey. Immature spiders, however, are generally located much lower down in vegetation growing close or next to tree trunks. The majority of theraphosids – with a few exceptions – are cannibalistic and therefore live a solitary lifestyle[2] so single housing is essential in the laboratory. The tendency to inhabit lower growing vegetation by the juveniles is most likely an aversion tactic to ensure they are not devoured by larger members of the species. They 'tack' leaves together to form tube retreats, inhabiting them for a short period of time (usually first or second instar) before relocating and constructing a new retreat[9]. This natural proclivity to build transient tube webs supports the results observed. The enriched spiders had access to plastic leaves and each enriched animal had used those leaves at some point for web construction. The un-enriched spiders that did not have access to plastic leaves were forced to try and construct webs either on the sides or at the top of the enclosure resulting in a paucity of webbing.

When theraphosids are at rest, they conceal themselves in their tube webs or sometimes they will rest with just the tips of legs I and II poking out from their retreat. The slightest disturbance will quickly make the spider withdraw completely into its web tube[9]. Previous studies on tunnel web spiders have shown that when a spider is concealed in its retreat, stroking it and prodding it elicits little response but when it is forced from its hide, it will assume a threat posture, tapping the ground with it legs, lunging and biting if the attack is sustained[11]. This is also true of arboreal theraphosids to an extent. The initial response of leaping and running away is the preferred defensive mechanism in juveniles, but it can take a significant amount of provocation to coax a spider to leave its retreat. It would be interesting to conduct the same experiment on adults to examine whether they are more likely to flee or fight.

Evoked stimuli experiments were conducted during the daylight phase but further studies on lighting sources (such as red or sodium) need to be conducted to see if the same study can be repeated in the nocturnal phase. This would also allow observation of non-evoked stimuli which may also be affected by enrichment.

Artificial climate for tropical theraphosids has been suggested as approximately 29˚C (85F) and approximately 80-90% relative humidity[9]. More recent observations have concluded that in fact temperature stability is of far more importance than precise temperature and that the best way to maintain a large collection of theraphosids is to heat the room as opposed to the enclosures[4]. In the laboratory, the ambient temperature over a period of two months (May – June 2011) was 25 +/– 0.6˚C. The arboreal theraphosid enclosures are not directly heated but placed on top of the laboratory's terrestrial arachnid shelving units which are heated typically at 26 +/– 3˚C. Over the study period of six months, two adult male arboreal theraphosids died and accurate cause of death was unable to be determined. It is important to point out that the life span of an adult male theraphosid is considerably shorter than that of the female (dependent on species males usually live 3-5 years and females anywhere from 6-30 years)[2] and that in both cases, the males were wild caught so age was indeterminable.

The key elements of successful laboratory culture for terrestrial theraphosids have previously been determined but this is the first description of laboratory husbandry techniques for arboreal Theraphosidae species. Appropriate temperature and relative humidity, suitable feeding regimes with high quality prey items, and good levels of hygiene[13] are common to all theraphosid husbandry. The authors of this paper would like to add species-specific behavioural enrichment as well as appropriate temperature stability to this list of key elements.

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