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UNA PRUEBA DE PLASTICIDAD EN ARAÑAS DIMINUTAS: CONSTRUCCIÓN DE TELAS ORBICULARES POR NINFAS DE *LEUCAUGE ARGYRA* (ARANEAE: TETRAGNATHIDAE) EN ESPACIOS REDUCIDOS.

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar por el grado y título de Maestría Académica en Biología.

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Dedicatoria

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Tabla de contenidos

Lista de cuadros

Lista de figuras

RESUMEN

La regla de Haller se conoce para muchos taxones en todo el reino animal (Eberhard & Wcislo 2011): animales pequeños poseen un cerebro proporcionalmente más grande que animales más grandes. Mantener un cerebro proporcionalmente grande probablemente representa altos costos metabólicos para animales pequeños. La hipótesis de la limitación en comportamiento por tamaño (Eberhard 2007, Eberhard 2001) predice que, debido a que el sistema nervioso se encuentra bajo la presión selectiva de minimizar su consumo de energía y generar comportamientos adaptativos (Niven et. al 2007, Niven & Laughlin 2008, Hasenstaub et. al 2010) y debido a los costos de mantener cerebros proporcionalmente más grandes, animales pequeños -ya sea inmaduros o adultos con tamaños corporales pequeños- tendrán limitaciones en su comportamiento, cometerían más errores o serán menos precisos que animales más grandes. En el presente estudio, probé esta hipótesis determinando si las ninfas de *Leucauge argyra* (Walckenaer 1842), que son hasta 750 veces más pequeñas que las adultas (en cuanto a peso), son capaces de realizar los mismos ajustes comportamentales extremos realizados por las adultas de estas misma especie en telas orbiculares construidas en espacios reducidos (Barrantes & Eberhard 2012).

En el laboratorio, coloqué los cuatro tamaños más pequeños de ninfas de *L. argyra* en espacios reducidos que representaban de un 5 a un 13% del espacio en el que usualmente construyen sus telas orbiculares. Encontré tres tipos de evidencia en contra de la hipótesis de la limitación en comportamiento por tamaño: (1) las ninfas fueron capaces de hacer todos los ajustes que hicieron las adultas en sus telas orbiculares en espacios reducidos; (2) las ninfas no demostraron menos precisión en los ajustes a espacios reducidos que las adultas;
 (3) las ninfas más pequeñas fueron capaces de realizar los mismo ajustes en sus telas orbiculares en espacios reducidos que las ninfas más grandes.

Algunos caracteres fueron más comunes en telas en espacios reducidos, pero raramente encontrados en telas más grandes tanto en adultas como en ninfas, como por ejemplo radios colocados directamente al sustrato y centros del meollo sin remover. Debido a éstos caracteres, las telas de las ninfas en los espacios reducidos formados por cilindros de 9 mm de diámetro, fueron muy parecidas a las telas orbiculares modificadas construidas por otras especies de arañas más derivadas, que viven en condiciones de poca disponibilidad de espacio.

Los ajustes observados en este estudio son complejos, y posiblemente representan comportamientos neurológicamente más demandantes que los documentados en arañas diminutas en estudios previos (Eberhard 2007, Hesselberg 2010, Eberhard 2011): las ninfas de *L. argyra* ajustaron sus telas orbiculares a espacios de 8 a 20 veces más pequeños de los que usualmente utilizan para construir sus telas. La complejidad en los ajustes utilizando tamaños extremos de arañas que hacen telas orbiculares, me permitió proveer la prueba más sensible realizada hasta el momento a la hipótesis de la limitación en comportamiento por tamaño. Por medio de esta prueba reforzé la conclusión de que las arañas que construyen telas orbiculares no se comportan según sus predicciones. Sin embargo, son necesarios estudios futuros relacionando comportamiento y el balance entre los costos y beneficios que han influenciado la miniaturización del cerebro, con el

fin de entender cómo los animales pequeños son capaces de mantener capacidades comportamentales similares a las de los animales más grandes, pagando los altos costos metabólicos de poseer un cerebro proporcionalmente más grande que el de los animales grandes.

Complex plasticity in miniature spiders: adjustments of orb web building

behavior to constrained spaces by nymphs of *Leucauge argyra* (Araneae:

Tetragnathidae)

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Abstract: The size-limitation hypothesis predicts that small animals, because of the implications of maintaining a proportionally large and expensive brain, will have relatively limited behavioral capacities. I tested this hypothesis by comparing whether *Leucauge argyra* (Walckenaer 1842) nymphs were able to make the same extreme behavioral adjustments in their orb webs to adapt them to constrained spaces as reported in conspecific adults. I confined the four smaller sizes of *L. argyra* nymphs in spaces representing from 5 to 13 % of the space in which they usually spin their webs. I found three types of evidence against the size-limitation hypothesis: (1) nymphs were able to perform all the orb web adjustments to constrained spaces adults did; (2) nymphs did not show less behavioral precision on their adjustments than adults and (3) The smallest nymphs were able to make the same behavioral adjustments as larger nymphs. Some traits were common in webs built in reduced spaces (small web traits) but rarely found in larger orbs in both nymphs and adults, such as radii attached to the substrate and un-removed hubs centers. Mostly because of these traits, webs of nymphs in 9 mm cylinders resemble modified orb webs built by other more derived species of spiders that live in cramped spaces. The complex adjustments observed using extreme sizes of orb weaving spiders allowed me to provide the most sensitive test of the size-limitation hypothesis yet performed, reinforcing the conclusion that orb weavers do not behave as to its predictions.

Key words: behavioral size-limitation hypothesis, orb webs, spider behavior, small web traits

Introduction

Haller´s rule, which states that small animals have relatively larger brains than bigger ones, is widely applicable throughout the animal kingdom. This body-brain scaling relationship has been studied in a wide range of sizes in animals. Different taxonomic groups of both vertebrates (Striedter 2005) and invertebrates have different grades of this allometric relationship between the size of the body and the size of the brain (Eberhard & Wcislo 2011), with only very occasional

exceptions (Van der Woude et al. 2013). However, most research regarding the size of the brain and its relationship with behavior have focused on understanding the behavioral capacities of animals with large body sizes. The behavioral capacities of miniaturized animals with absolutely small but relatively larger brains in practically unknown (Eberhard & Wcislo 2012). On the other hand, the morphological implications of relatively large brain sizes in tiny animals have recently started to be studied.

For example, there are several studies demonstrating that having a large brain and little space in which to house it sometimes leads to morphological modifications of the brain. For example, in the smallest species of Ptiliid beetles, part of the brain has been displaced to the prothorax (Polilov & Beutel 2009) and in strepsipteran larvae the brain has been displaced to the posterior part of the thorax and the anterior part of the abdomen (Beutel et al. 2005). In the only 0.6 mm long chalcidid wasp, the brain even extends into the coxae, (Hustert 2012) as also happens in some small spiders which weigh less than 0.005 mg (Quesada et. al 2011). Proportionally large brains could involve other morphological modifications of the body. For example, the cephalothorax in at least one species of tiny spiders, also has a ventral bulge of the sternum (Quesada et. al 2011), probably to harbor the brain (Madrigal et al. in prep).

Maintaining a relatively large brain also implies relatively high metabolic expenses. Neurons require a high energetic investment just to maintain the membrane potential at rest and the conduction of action potentials. These processes are possible thanks to the ionic interchange through the neural membrane, which is considered to be the most energy-demanding process at the neuronal level (Niven & Laughlin 2008, Hasenstaub et. al 2010).

Tiny insects and spiders have smaller neurons than bigger ones (Beutel et al. 2005, Polilov 2008, Quesada et. al 2011), and this could increase these already high metabolic costs of the brain. Their somas and axons are reaching the minimum functional limits and even anucleate neurons have been reported in a trichogrammatid miniature wasp (Polilov 2012). Reduced neuron size involves an increase in their energy consumption (Niven & Farris 2012). Besides, thinner axons

present in very small arthropods encompass consequences in communication between neurons. In axons less than 0.1 µm in diameter changes in ionic currents generate channel noise. This noise produces spontaneous action potentials that could interfere with the transmission of regular action potentials (Faisal 2005).

The behavioral size-limitation hypothesis (Eberhard 2007, 2011) predicts then, that because nervous systems are under selective pressure to minimize energy consumption and generate adaptive behavior (Niven et. al 2007, Niven & Laughlin 2008, Hasenstaub et. al 2010), small animals -either immature individuals or adults with small body sizes- given the costs of maintaining relatively large and expensive brains will have limitations in their behavior, make more mistakes or be less precise than larger animals. There are two alternative hypotheses. The oversized hypothesis predicts that small animals have the same behavioral capacities as larger animals but pay higher energetic costs maintaining oversized brains. The neuronal trick hypothesis also predicts that small animals have behavioral capacities comparable to those of larger animals but, due to novel modifications of the nervous system to economize energy, have less flexibility in the performance of given tasks (Eberhard 2011, Eberhard & Wcislo 2011).

Previous studies on ants have supported the size-limitation hypothesis (Howse 1974, Wilson 1984, Cole 1985), but had serious problems defining, quantifying and comparing complexity (see discussion in Eberhard 2011). Evidence from more recent studies with orb weaving spiders did not favor this hypothesis. Behavioral imprecision was measured by Eberhard (2007) comparing the consistency of the spaces between the sticky spiral loops in three species of orb weavers (nymphs and adults) that varied by a factor of 10^4 in size; smaller spiders did not show signs of greater imprecision. Three orb web parameters thought to be affected by brain constraints were measured by Hesselberg (2010) in nymphs, juveniles and adults of two species of orb weaving spiders that differed in size, but in neither species did smaller individuals show evidence of more limited behaviors. Nymphs of different size and adults of four species of orb weaving spiders were also compared by testing for correlations between different web traits and analyzing the scatter around these correlations as a measurement of imprecision. One partial

confirmation for the size-limitation hypothesis was found, but most trends did not support it. Small species even showed challenging adjustments to space availability that were not present in larger species, as well as some additional derived traits that were lacking in the larger species (Eberhard 2011), suggesting that smaller individuals are more, not less, plastic, at least when challenged by restricted space.

The previous studies did not find support for the size-limitation hypothesis, but both authors mentioned the need to study more neuronally demanding behaviors (Eberhard 2007, Hesselberg 2010, Eberhard 2011). A possible behavior involving extreme flexibility and that seems likely to be neurally demanding was reported by Barrantes & Eberhard (2012) in the tetragnathid spider *Leucauge argyra* (Walckenaer 1842). Adult females of this species normally construct more or less horizontal orb webs that span approximately 80 to 100 cm; but they built highly altered orb webs when they were confined in constrained spaces that were only 7% of the mean span of webs in the field. Spiders in these constrained spaces made extreme modifications such as reduced spacing between sticky spiral loops, reduced number of frames and increased proportion of radii attached to the substrate, in order to adapt to the space available. These modifications represent extensions of behavioral adjustments made in the field to less extreme space conditions.

Adults of *L. argyra* pass through several immature stages that include very small nymphs up to 12 times smaller (by weight) than the adults. As in other orb weavers, when first instar spiders hatch from the eggs they stay inside the egg sac and molt once before coming out from it (personal observations from egg sacs laid by *L. argyra* females in captivity, Foelix 2010). Second instar youngs or first stage nymphs outside the egg sac already build orb webs. Therefore, both small early instars nymphs and large adult females face the same challenge of building an orb web to capture prey. This large size difference, the facts that both stages perform the same behavior in nature and that adults showed extreme behavioral plasticity when building orbs in constrains spaces, make *L. argyra* a suitable model for comparative studies regarding the size-limitation hypothesis. Furthermore, orb webs represent an easy way to measure comparable behavioral decisions that spiders make during construction (Eberhard 2007). In the present study, I tested

this hypothesis by determining whether nymphs of *L. argyra* are able to perform the same extreme behavioral adjustments and with the same behavioral precision as those reported in orbs built by the adults in constrained spaces (Barrantes & Eberhard 2012).

Methods

Webs in the field

I photographed orb webs of possibly the first five instars (based on body size) of *L. argyra* after coating the webs lightly with talcum powder. I counted as first instar nymphs the first stage spiderlings outside the egg sac. Photographs were taken in a plantation of African oil palm (*Elais guineensis* L.) in Parrita, Puntarenas, Costa Rica (09°30′N, 84°10′W, elev. 10 m) from May to September 2011 and from February to June 2012. A black cardboard was placed behind the web for contrast, and a calibrator or a ruler was placed next to the web to scale the photo. Young spiders from the webs photographed and other individuals from the first five instars were collected from their orb webs during this period and taken to the laboratory.

Nymphs used in this study build horizontal webs that span from 6 to 20 cm between attachments points. They are attached to rigid and non-rigid substrates like veins of dry ferns, dry palm leaves or grass blades. Webs also have a tangle of non-sticky lines above the orb, with threads connecting it to the inner spirals of the hub. This tangle may give support to the web (Triana-Cambronero et al. 2011). The number of threads to the tangle and the size of the tangle decrease as the spiders grow and tangles are absent in the webs of adult spiders (Triana-Cambronero et al. 2011).

Orb webs in constrained spaces

In the laboratory, I placed each nymph in one of the four cylindrical containers with the following diameters: 24 mm diameter x 30 mm long, 18 x 30 mm, 9 x 15 mm and 5 x 15 mm. I put the 5 and 9 mm diameter cylinders on a thin layer of white plasticine with a coverslip on the top of each cylinder, to prevent the

spiders from escaping. The 18 and 24 mm diameter cylinders were covered, both on top and at the bottom, with plastic wrapping material attached around the cylinders with a rubber band. Each cylinder was lined with a black paper that covered nearly half of the inner upper part, to allow the spider to attach threads and to provide a dark background for photographs. The cylinders were kept in 21 x 11 x 9 cm plastic containers with large pieces of wet cotton to provide a humid microenvironment. The spiders were not fed.

Webs built in the cylinders were photographed under a dissecting microscope after being coated with talcum powder. Another photograph was taken after rapping on gently the cylinder to cause the powder to fall from the non-sticky lines and remain only on the sticky spiral loops (Fig. 3). Most orb webs were constructed in the lower part of the cylinders where there was no black construction paper and these were photographed from below. Spiders built their orbs from the day after being introduced into their containers and up to 15 days after (I observed complete orb webs in some nymphs all along this period. Therefore, despite their time in the laboratory nymphs were able to build orb webs, even not all of them did). I kept the nymphs in the cylinders until they died. Spiders that died within the first three days of being confined in the cylinders without building any type of web were excluded from the study. Spiders that died after the fist three days without building an orb web were counted as nymphs less likely to build an orb in constrained spaces. In any case a spider was used more than once. All spiders were preserved in alcohol for later body measurements.

Size categories of nymphs

I estimated the size of the spider by measuring: (1) the length of the ventral edge of the tibia of each nymph, using digital photographs made under the microscope of the right leg I fixed on a slide (Fig. 1). In a few cases in which the right leg could not be used, the left one was used instead; and (2) the width of the cephalotorax of each spider, using digital photographs made under the stereoscope of each cephalothorax placed in a layer of paraffin and covered with alcohol. Both measurements showed a continuum in which no clear size groups could be

established (Fig. 2). Because the measurement of the size of the cephalothorax could be affected due to small movements while being photographed in alcohol whereas the tibia was fixed and therefore immovable, the latter was used to determine size groups. I visually grouped a set of individuals that varied in body size in four groups of spiders with similar sizes. Then I examined their tibia lengths, and with those values and the curve of variation obtained from all 271 individuals measured (Fig.2), I established the limits between groups. The four groups were: nymph size (1) 0,12- 0,33 mm of tibial length; nymph size (2) 0,34- 0,49 mm of tibial length; nymph size (3) 0,50- 0,72 mm of tibial length and nymph size (4) 0,73- 1,03 mm of tibial length (Fig. 3). Group of size 1 nymphs probably included the first two instars outside the egg sac because two subgroups could be observed. However, they were very similar in body size and therefore grouped together. Data on nymphal growth is only available for a distant species (*Tengella radiata,* Tengelidae), and indicates the growth during the first three instars is slow compared with the rest of the instars (Barrantes & Madrigal 2008). As group of size 1 nymphs probably included two subgroups, they represent almost half of the spiders measured.
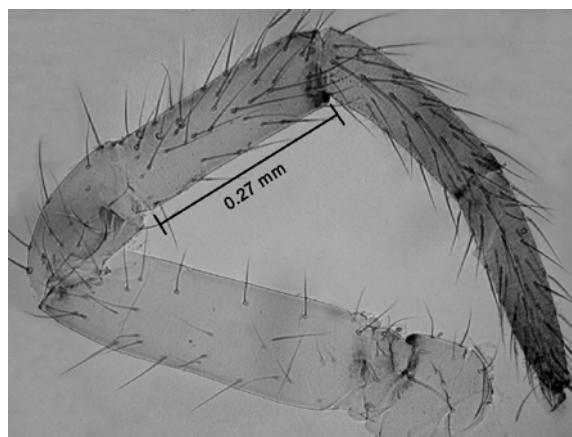
Fig. 1: The ventral margin of the tibia was measured as an estimation of the spider size.
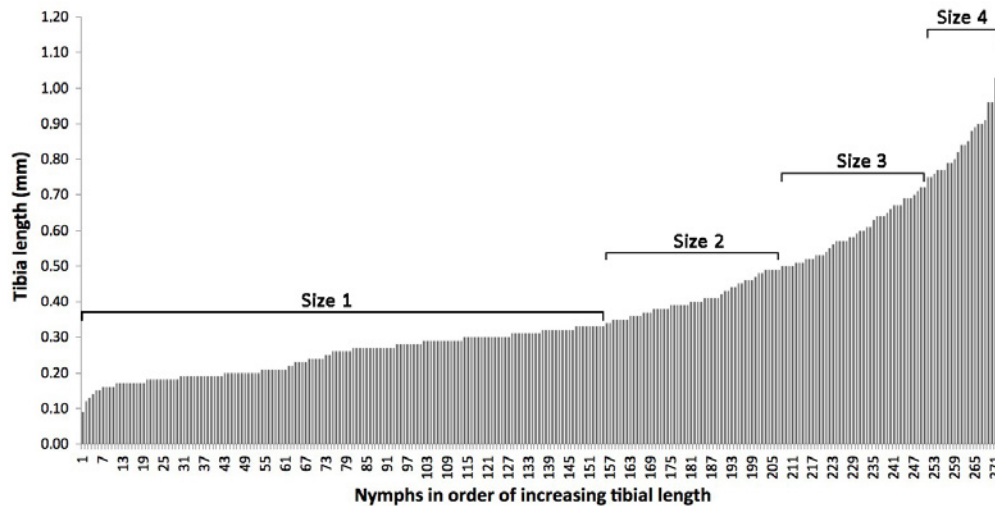
Fig. 2: Tibia lengths in order of increasing size for 271 *L. argyra* nymphs, showing a continuum where no clear size groups could be defined. Four groups of nymphal boby sizes establish based on both visually similarities in body size and in this curve of variation in tibial length are indicated in the figure.

Orb web measurements

I measured the following variables using the program Image Tool (Wilcox et al. 2002): 1) the total area (area enclosed by the outer sticky spiral loop); 2) the capture area (area enclosed by the outer and the inner sticky spiral loop); 3) the free zone area (area enclosed by inner sticky spiral loops and the outer spiral loop of the hub); 4) the hub area (area enclosed by the outer spiral loop of the hub); 5) the number of frames (only frames with radii); 6) the number of frames with sticky spirals attached beyond them; 7) the number of radii; 8) the number of subsidiary radii (radial lines that arose from other radii and that had sticky spirals loops attached to them) (Zschokke 1999); 9) the mean number of radii per frame; 10) the number of radii attached directly to the substrate; 11) the number of spiral loops in the hub; 12) the web symmetry (length of the opposite to longest radius /length of the longest radius), and 13) whether the webs had the hub center removed or un-removed (after finishing the sticky spiral loop, both the adults and nymphs of *L. argyra* remove the hub center by ingesting the silk that they accumulated during radius construction in the center of the hub, leaving an empty hole. After that,

several draglines are laid across this hole and then held by the spider's spinnerets as she remains in the hub to wait for prey (this behavior was observed only in adults, but hubs of webs of nymphs are very similar). When the hub center had been removed, it lacks the white specks of silk that accumulated during radius construction and sometimes the draglines laid by the spider in the center remain (Fig 3.a) and sometimes they do not (Fig 3.d). When the hub center had not been removed, the white specks of silk remained accumulated in the center of the hub (Fig 3.f)).

The following measurements were taken along the longest radius, the radius opposite the longest radius, and the two radii perpendicular to the longest radius: 14) the number of sticky spiral loops attached to the radius; 15) the spaces between sticky spiral loops and 16) the consistency of the sticky spiral spacing (calculated using the formula $(space_n)/(space_{n-1} + space_{n+1})/2)$ (Barrantes &Eberhard 2012). In cases where a measurement could not be taken in one of the mentioned radii it was taken in an adjacent radii. Most measurements were chosen in order to compare them with those peblished for the adults (Barrantes & Eberhard 2012).

In webs built in the cylinders not all radii had spirals attached to them (Fig.3.f and 3.g). For that reason, I counted two kinds of radii in each web 1) Hub radii: all radii to which the outermost spiral of the hub was attached and as a consequence, deviate slightly when leaving the hub, or any radii that seemed to arise from the outermost spiral of the hub, whether or not they had sticky spirals attached to them (Fig.9.3). Lines attached to any of the inner spirals of the hub (any hub spiral except for the outermost one), going upwards or downwards to the substrate, a mesh or other threads were not counted as hub radii. These lines were considered as threads resembling those connecting the inner spirals of the hub with an upper tangle in field webs; 2) Spiral radii: only hub radii with sticky spirals attached to them (Fig.9.3). Attachments of sticky spirals to a radius were easily recognizable because they deviate slightly from the radius line at attaching points. The number of hub radii and spiral radii in all webs was different (paired t test: t=-6.981, df=131, p <0.00001). However, the number of hub radii was used for all statistical analyses. The latter in order to include webs without sticky spirals (that

did not have spiral radii) in statistical analyses where the number of radii was used as an independent variable.

Statistical analysis

a) Comparisons between nymph webs and adult webs

Orb web adjustments to constrained spaces

To determine the effect of the different sized containers on the measured variables in webs of nymphs I performed a Manova. To determine if nymphs were able to perform behavioral adjustments to constrained spaces, I regressed each web variable against the total web area. As in the previous study with the adults (Barrantes & Eberhard 2012), a behavioral adjustment was considered as a significant slope when a web parameter was regressed against total web area (a non-significant slope would mean no change of a web variable from the field to the constrained spaces). As Barrantes & Eberhard (2012), I used total web area as the independent variable because it could be measured precisely and because it is limited by the space available to build a web, becoming a direct representation of the different sized containers and the field. To include webs without sticky spirals in which there was no capture zone, I conducted another set of regressions using the number of radii as the independent variable. I also compared the slopes between nymphs and adults for 14 web variables regressed against total web area with unpaired t- tests (data of adults were obtained from G. Barrantes and W. Eberhard), in order to compare the magnitude of the adjustments. Captive and field webs used for most regressions are referred in the text as all webs. In all regressions I used all webs, otherwise is indicated in the text (were I only used field webs).

Barrantes & Eberhard (2012) used two criteria in order to propose that some of the designs in webs built by the adults in unnaturally small spaces were extensions of adjustments made in less constrained circumstances in the field: 1) no statistical difference between the slopes of field webs and all webs in variables regressed against total web area and 2) statistical significance of the slope in only field webs on variables regressed against total web area. Therefore, in order to assess if the adjustments made by nymphs were as in the adults, continuations of

adjustments from the field to the constrained spaces, I also compared the slope of all nymph webs with that of only nymphal field webs with unpaired t- tests.

Precision in the orb web adjustments

Given that variation can be an indicator of the behavioral ability to make precise modifications in certain behaviors (higher variability, less precise modifications), in order to compare the precision between nymphs and adults in the orb web adjustments, I evaluated the proportion of the variability explained by the independent variable (r^2) in web variables regressed against total web area in both stages. I assumed precise modifications would have a smaller scatter around the slope and therefore a higher r^2. Web variables that differed in less than 0.10 between the r^2 for nymphs and for adults were considered as modifications with a very similar precision in both stages; the ones that differed between 0.10 and 0.14 as with a similar precision and the ones with a difference of more than 0.14 as with a different precision. All web variables were compared between both stages, even those that did not qualified as adjustments to constrained spaces.

I also regressed the absolute value of the residuals obtained from regressions of each web variable with total web area, against total web area as independent variable. Then, I compared the slopes of nymph's residuals and adult's residuals from 13 web variables.

b) Nymph size and hub removal (only for nymphs)

I also compared the slopes from web variables regressed against total web area between the smallest nymphs (nymphs 1) and the rest of the nymphs (nymphs 2, 3, 4) grouped together. Besides total web area, I regressed each web variable against nymph size and both total web area and nymph size as independent variables. Then, the Akaike information criterion (AIC) was used to choose which regression explained more variability. Differences by two units in the AIC values were interpreted as not different values. In cases where the AIC values were the same, models with fewer variables were chosen.

I used three logistic regressions to test whether total area, nymph size or both were related to whether nymphs removed the center of the hub or not. An AIC was then used to compare between regressions. All analyses were made using the R statistical language (Version 2.14.0, The R Foundation for Statistical Computing). In all statistical analyses variables were $\log_{10}$ transformed to approach residuals to the normal distribution for the regression analyses. All area values were used as the square root of the actual values. All means are followed by ± standard deviation.

Results

Only two nymphs in the 5 mm containers built structures resembling an orb web. Therefore, all the analyses were made using 9, 18 and 24 mm cylinders. Representative webs built by nymphs in each size of container are shown in Figure 1. The size of the cylinders explained a large part of the combined variation (MANOVA: $F_{69,177}$ = 6.09, Pillai test = 2.11, p < 2.2E-16) in almost all variables. The mean of all measured variables for all webs was different between the different spaces available to build a web, except for the proportion of subsidiary radii/total radii and the consistency of sticky spiral spacing. Variations between the measurements in each cylinder size and in the field are shown in Table 1.
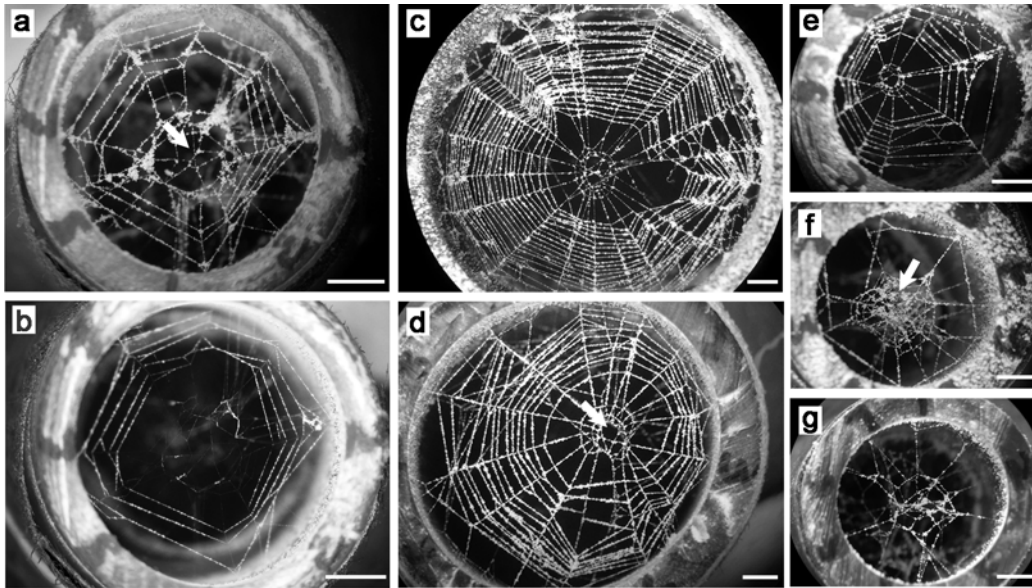
Fig. 3: Orb webs built by nymphs of *L. argyra* in containers of different diameters that represent from a 5 to a 13 % of the space in which they usually spin their webs. Orb webs built in a 9 mm diamater cylinder a) after being coated with talcum powder, b) after rapping on gently the cylinder, causing the powder to stay only on the sticky spiral loops. Orb webs built in: c) 24 mm diameter cylinder, d) 18 mm diameter cylinder and e, f, g) 9 mm diameter with, e) many sticky spiral loops, f) only one sticky spiral loop and, g) no sticky spiral. White arrows point hub centers, a) removed by spiders by ingesting the silk accumulated after radius construction in the center of the hub, where draglines laid by the spider after that remained, d) where the draglines did not remain, f) Hub center un-removed with white specks of silk accumulated after radius construction. All scale bars are 2 mm.

Table 1. Mean values of the 23 web variables measured in orbs built by *L. argyra* nymphs in containers of 9, 18 and 24 mm diameter and in the field. Also included are F-tests for the means (H_0: differences between means of different sized containers and the field=0) of each web variable between the different sized containers and the field. L=longest radius. L+O= Longest radius plus radius opposite to longest radius.

Variable	Means in captivity			Means in field	F	df	p
	Span = 9 mm	Span = 18 mm	Span = 24 mm	Span = 186.92 ± 46.35 mm			
Total area (mm^2)	38.5 ± 7.9	148.9 ± 37.4	230.7 ± 56.8	3930. 5 ± 2143.7	470.0	3/79	<0.00001
Capture area (mm^2)	14. 5 ± 9.0	115.3 ± 36.9	179.1 ± 60.7	38.2 ± 21.0	474.2	3/79	0.00001
Free zone (mm^2)	18. 1 ± 8.5	27.2 ± 10.9	38.2 ± 16.7	79.7 ± 46.5	29.6	3/79	<0.00001
Hub area (mm^2)	4.9 ± 3.7	5.1 ± 3.1	6.8 ± 5.1	8.5 ± 6.7	5.1	3/79	0.003
Number of (hub) radii	12.9 ± 0.8	15.3 ± 3.5	18.2 ± 4.1	31.8 ± 6.1	69.2	3/79	<0.00001
Number of (spiral) radii	6.5 ± 5.1	12.9 ± 5.8	16.5 ± 3.8	31.6 ± 6.0	113.2	3/79	<0.00001
Prop. secondary radii/total radii	0.01 ± 0.03	0.03 ± 0.04	0.04 ± 0.05	0.1 ± 0.1	2.2	3/79	0.091
Mean No. sticky spiral loops	2.7 ± 1.9	10.5 ± 4.7	12.1 ± 4.0	49.4 ± 13.9	193.1	3/79	<0.00001
No. sticky spiral loops L+O	5.2 ± 3.7	21.4 ± 10.6	25.0 ± 8.4	99.4 ± 29.3	170.3	3/79	<0.00001
Mean sticky spiral space (mm)	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	29.6	3/79	<0.00001
Sticky spiral space L (mm)	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	25.6	3/79	<0.00001
Mean Consistency (mm)	1.1 ± 0.5	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	0.6	3/79	0.635
Consistency L (mm)	1.6 ± 0.5	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.0	0.5	3/79	0.690
No. hub loops	3.0 ± 0.9	3.0 ± 0.5	3.2 ± 0.7	3.5 ± 0.6	3.4	3/79	0.023
Web symmetry	0.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	11.31	3/79	<0.00001
Number of frames	5.2 ± 2.7	7.0 ± 3.6	8.6 ± 3.8	13.1 ± 3.6	8.3	3/79	0.0001
Mean radii/frame	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 1.1	2.5 ± 0.7	82.2	3/79	<0.00001
Prop. frame w. single radius/total frames	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	0.4 ± 0.2	39.2	3/79	<0.00001
Prop. spirals beyond frames/total	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.2	0.04 ± 0.06	10.7	3/79	<0.00001

frames

Prop. radii attached to substrate/total radii	0.4 ± 0.2	0.4 ± 0.3	0.30 ± 0.2	0.00 ± 0.01	89.1	3/79	<0.00001
Prop. capture area/total area	0.4 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.1 ± 0.0	69.5	3/79	<0.00001
Prop. free zone/total area	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.02 ± 0.01	22.4	3/79	<0.00001
Prop. hub area/total area	0.2 ± 0.1	0.04 ± 0.03	0.03 ± 0.02	0.002 ± 0.001	3.6	3/79	0.016

a) Comparisons between nymph webs and adult webs

Orb web adjustments to constrained spaces

All slopes from variables regressed against total web area that were significant in adults were also significant in nymphs (Table 2, Fig.4). Therefore, all variables that represented adjustments to constrained spaces in adults also did in nymphs. Two other variables did not represent adjustments (did not have significant slopes) in adults but did in nymphs: the number of hub loops and the proportion of free zone area/total area. Neither adults nor nymphs modified the consistency of sticky spiral spacing into the constrained spaces (slope was not statistically significant) (Fig.4.k). These results illustrate that nymphs were able to perform all the adjustments to constrained spaces adults did and even two additional ones. An additional variable that was measured only in nymphs corresponded to an adjustment: the proportion of spirals beyond the frame/total frames. Also as in adults, all four web variables regressed against the number of radii in nymphs, represented an adjustment because they had significant slopes (Fig.5).

In both nymphs and adults almost all variables increased with total web area excepting for the proportion of frames with a single radius/total frames, the proportion of radii attached to the substrate/total radii, and the proportion of hub area/total area (Fig. 4, Table 2). Two variables decreased with total web area only in nymphs: the proportion of free zone are/total area and the proportion of spirals beyond the frame/total frames. None of the slopes in nymphal webs differed significantly from the slopes in adult webs in any of the 14 variables (unpaired t-tests) (Table 2, Fig. 4 a-k).

Regarding if the adjustments made by nymphs were as in the adults extensions of adjustments made in less constrained circumstances in the field, almost all variables in both stages accomplished the first criterion of no statistical difference between the slopes of all webs and only field webs, of variables regressed against total web area. Comparing the slope for nymph webs with that of only field webs showed no significant difference in 18 of the 19 compared variables (unpaired t-tests). Only the slope of the proportion of radii attached to

substrate/total radii was different (Table 2). Similarly in the adults, the slope for all webs built by adults compared with only field webs showed no significant difference in any variable.

On the other hand, several variables did not accomplish the second criterion of statistical significance of the slope in only field webs, on variables regressed against total web area (Table 2), therefore they did not qualify in this sense as continuations of adjustments. Web variables that did not satisfy the second criterion in both nymphs and adults were: web symmetry (Fig. 4g), mean radii per frame (Fig. 4j), proportion of frames with a single radius, proportion of radii attached to the substrate (Fig. 4i) and only in nymphs, proportion of spiral over frames/total frames and proportion of free zone area/total area. However, as argued by Barrantes & Eberhard (2012), those web variables do not correspond to new designs, because they were also found in the field, but in a much lower frequency.

According to both criteria, variables that were extensions of field adjustments in both nymphs and adults were: capture (Fig. 4c), free zone (Fig. 4d) and hub area (Fig. 4e), number of radii (hub and spiral in nymphs) (Fig. 4f), number of sticky spiral loops (Fig. 4a) and proportion of capture area/total area. The only variable that was an extension of a field adjustment only in adults and not in nymphs (because the slope in field webs was not statistically significant, violating the second field statistical criterion) was the sticky spiral space in the longest radii (Table 2, Fig. 4b). However, the mean sticky spiral space was an extension in nymphs and the mean of four radii is possibly a better representation of this variable than the measurement it in only one radius. Therefore, the statistical data evidencing that the sticky spiral space was not an extension in nymphs might be loose. In sum, all extensions of adjustments that occurred in adults also occurred in nymphs. Furthermore, three additional variables were extensions only in nymphs: number of hub loops, number of frame lines and the proportion of hub area/total area (Fig. 4h).

In the case of nymphs, the proportion of radii attached to the substrate did not follow either criteria, and therefore could represent a new design in webs built

in reduced spaces. The possible evolutionary importance of radii attached to the substrate in small containers will be discussed later.

Table 2. Slope values, F-test for the slopes (H_0: b=0) and proportion of the variability explained by the independent variable (r2) when each web variable was regressed against total area and number of radii as independent variables. Also included are comparisons of slopes between all nymphal webs (an) and nymphal field webs (f) and between all nymphal webs (an) and all adult's webs (aa) by t-tests. L=longest radius. L+O= Longest radius plus radius opposite to longest radius.

| Variable (* = only in nymphs) | All webs **ADULTS** | | | | | All webs **NYMPHS** | | | | | Field webs **NYMPHS** | | | | | Test between slopes | | | |
| | | | | | | | | | | | | | | | | ALL NYMPHS vs FIELD NYMPHS | | ALL NYMPHS vs ALL ADULTS | |
	Slope(b)	p	r^2	F	df	Slope(b)	p	r^2	F	df	Slope(b)	p	r^2	F	df	$t(b_{an}-b_f)$	$p(b_{an}-b_f)$	$t(b_{an}-b_{aa})$	$p(b_{an}-b_{aa})$
Total area as independent																			
Capture area	1.39	<0.00001	0.96	2235.0	1/105	1.19	<0.00001	0.96	2725.0	1/112	1.01	<0.00001	0.99	90430.0	1/42	1.10	0.273	0.90	0.370
Free zone	0.86	<0.00001	0.66	200.0	1/105	0.33	<0.00001	0.63	189.8	1/111	0.67	<0.00001	0.48	38.8	1/42	0.93	0.351	1.29	0.199
Hub area	0.23	<0.00001	0.46	89.2	1/105	0.13	<0.001	0.10	12.6	1/111	0.71	<0.0001	0.33	21.1	1/42	1.34	0.184	0.42	0.675
Number of (hub) radii	0.40	<0.00001	0.75	291.7	1/99	0.40	<0.00001	0.75	328.5	1/107	0.37	<0.001	0.31	17.9	1/40	0.09	0.927	0.01	0.989
Number of (spiral) radii*						0.50	<0.00001	0.83	512.6	1/107	0.35	<0.001	0.29	16.5	1/40	0.45	0.650		
Mean No. sticky spiral loops*						1.16	<0.00001	0.88	795.2	1/112	0.70	<0.00001	0.45	34.7	1/42	1.16	0.246		
No. sticky spiral loops L+O	0.89	<0.00001	0.89	409.1	1/98	1.17	<0.00001	0.86	601.9	1/101	0.72	<0.00001	0.43	27.1	1/36	1.05	0.298	0.93	0.353
Mean sticky spiral space *						0.22	<0.00001	0.35	60.4	1/110	0.30	0.010	0.15	7.2	1/42	0.23	0.822		
Sticky spiral space L	0.42	<0.00001	0.46	82.0	1/102	0.24	<0.00001	0.35	55.0	1/102	0.26	0.063	0.09	3.7	1/38	0.06	0.949	0.65	0.517
Mean Consistency *						-0.02	0.254	0.01	1.3	1/104	0.00	0.588	0.01	0.3	1/42				
Consistency L	0.03	0.123	0.02	2.4	1/96	-0,02	0.381	0.01	0.8	1/104	0.01	0.424	0.02	0.0	1/42				
No. hub loops	0.30	0.137	0.02	2.3	1/102	0.08	<0.001	0.02	15.6	1/111	0.32	0.000	0.29	16.9	1/42	0.77	0.442		
Web symmetry	0.44	<0.00001	0.32	45.1	1/97	0.37	<0.00001	0.30	46.0	1/108	0.12	0.069	0.08	3.5	1/46	0.72	0.476	0.19	0.851
Number of frames	0.41	<0.00001	0.24	28.4	1/92	0.45	<0.00001	0.34	54.6	1/104	0.55	0.001	0.25	12.2	1/37	0.22	0.822	0.10	0.923
Mean radii/frame	0.43	<0.00001	0.72	234.5	1/92	0.26	<0.00001	0.62	173.1	1/104	-0.16	0.157	0.05	2.1	1/37	1.16	0.247	0.78	0.439
Prop. frame w. single radius/total frames	-0.46	<0.00001	0.33	45.1	1/92	-0.35	<0.00001	0.47	93.4	1/104	0.29	0.178	0.05	1.9	1/37	1.28	0.203	0.36	0.722
Prop. spirals beyond frames/total frames*						-0.21	0.002	0.09	10.4	1/104	-0.14	0.520	0.01	0.4	1/37	0.14	0.891		

Prop. radii attached to substrate/total radii	**-1.00**	<0.00001	0.75	294.3	1/96	**-0.75**	<0.00001	0.70	253.9	1/107	**0.05**	0.223	0.04	1.5	1/40	2.67	0.008	0.77	0.443
Prop. capture area/total area	**0.27**	<0.00001	0.65	195.4	1/105	**0.23**	<0.00001	0.37	64.7	1/112	**0.01**	0.002	0.21	11.3	1/42	1.28	0.204	0.17	0.865
Prop. free zone/total area	**0.03**	0.372	0.01	0.8	1/105	**-0.22**	0.00001	0.46	94.1	1/111	**0.09**	0.167	0.05	2.0	1/42	1.05	0.295	1.10	0.271
Prop. hub area/total area	**-0.19**	<0.00001	0.62	171.7	1/105	**-0.13**	<0.0001	0.13	16.5	1/111	**0.27**	0.003	0.19	9.8	1/42	1.17	0.243	0.26	0.794
Number of radii as independent *																			
Hub area						**0.32**	<0.00001	0.15	21.9	1/122	**0.58**	0.033	0.11	4.9	1/40	0.45	0.652		
Web symmetry						**0.65**	<0.00001	0.19	28.6	1/121	**0.22**	0.026	0.12	5.4	1/39	0.92	0.361		
Mean radii/frame						**0.93**	<0.00001	0.54	150.0	1/125	**0.71**	0.002	0.20	11.2	1/44	0.42	0.678		
Prop. radii attached to substrate/total radii						**-1.31**	<0.00001	0.57	173.6	1/129	**-0.07**	0.319	0.02	1.0	1/48	3.00	0.003		
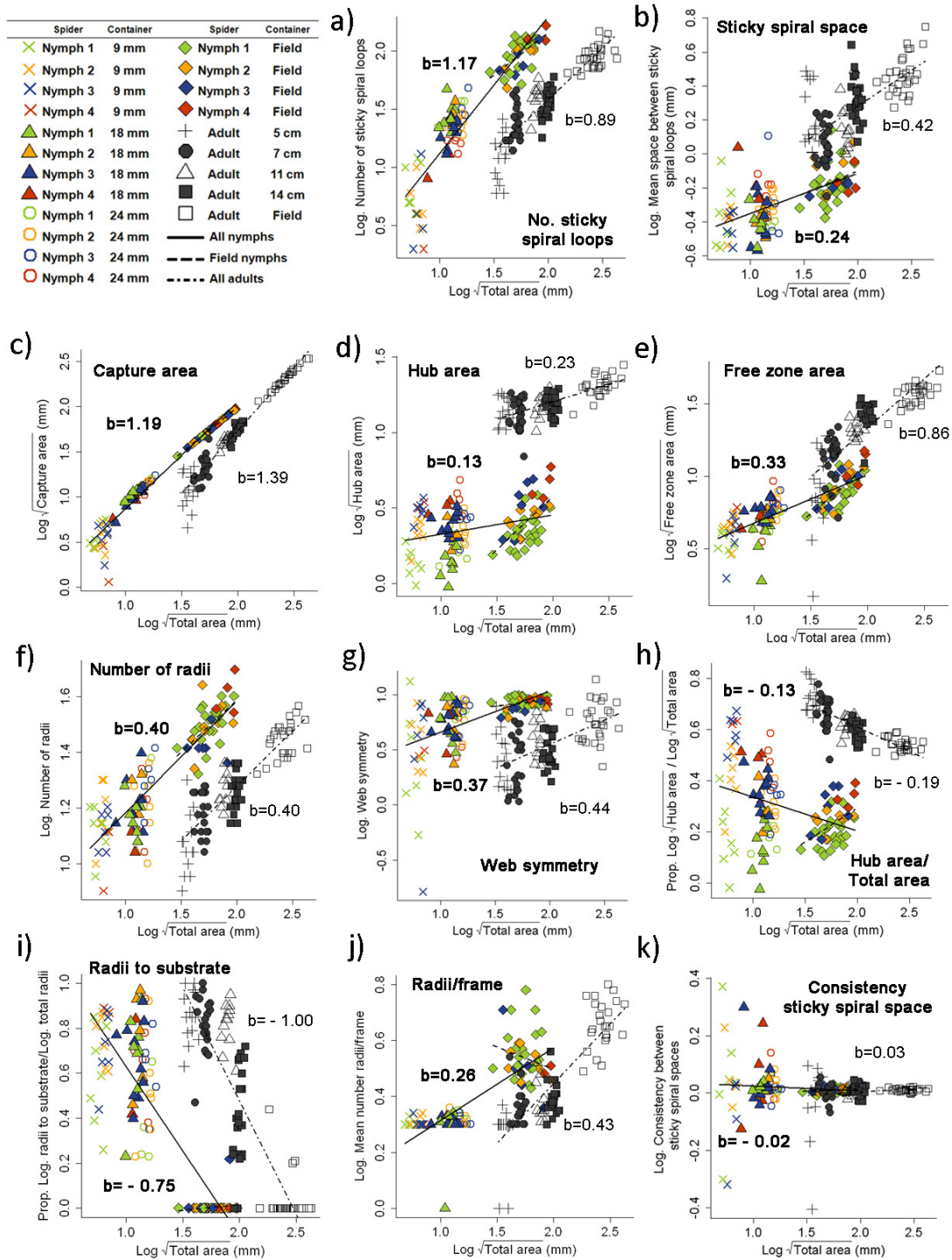
Fig. 4. Relationship between total web area and other measured variables in orb webs built in cylinders of 9, 18 and 24 mm of diameter and in the field by *L. argyra* nymphs (solid lines) and in containers of 5, 7, 11 and 14 cm of diameter and in the field by *L. argyra* adults (dotted lines). Dashed lines correspond to the orb webs built by nymphs in the field. The slope value (b) for all nymph webs is in bold, and for all adult webs in ordinary typeface.
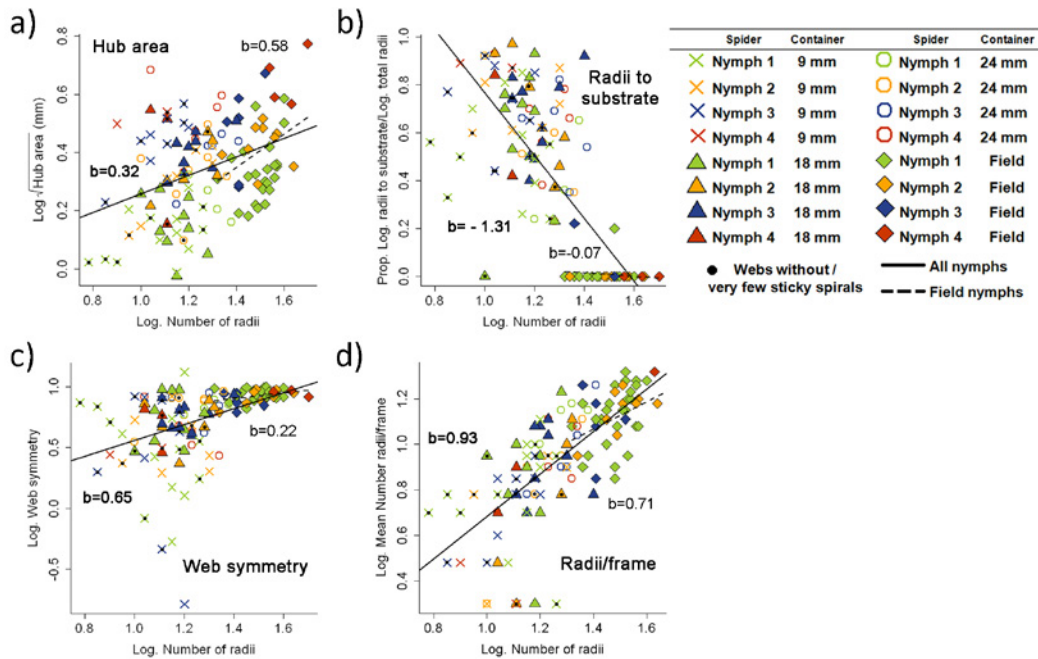
Fig. 5: Relationship between number of radii and other measured variables in orb webs built in cylinders of 9, 18 and 24 mm of diameter and in the field by *L. argyra* nymphs. The slope value (b) is included for all nymph webs in bold, and for nymph field webs in ordinary typeface. Webs with few (three or less) or no sticky spiral loops (that were not included in Fig. 4) are represented with a black dot.

Precision in the orb web adjustments

Evaluating the proportion of the variability of different web parameters explained by the total web area (r^2) between nymphs and adults, showed very similar precision in eight of the 16 compared variables (Table 2). Four other variables, the spaces between sticky spiral loops, the mean radii per frame, the number of frames and the proportion of frames with a single radius/total frames presented similar precision. The hub area, and the three variables regarding the proportion of the web dedicated to the capture area, the free zone and the hub area presented very different precision between nymphs and adults. Nymphs were less precise adjusting three of these variables: the hub area, the proportion of the web dedicated to the hub and to the capture area (Table 2).

Residuals of almost all variables decreased with total web area except the mean radii per frame in both nymphs and adults (Table 3). Comparison between slopes of nymphs and adults of the residuals of different web variables regressed

against total area showed no statistical differences between the slopes in any of the 13 variables compared (unpaired t -tests) (Fig. 6, Table 3).

Fig. 6. Relationship between total web area and the residuals of web variables regressed with total area in orb webs built in cylinders of 9, 18 and 24 mm of diameter and in the field by *L. argyra* nymphs (solid lines) and in containers of 5, 7, 11 and 14 cm of diameter and in the field by *L. argyra* adults (dotted lines). The slope value (b) for all nymph webs is in bold, and for all adult webs in ordinary typeface.
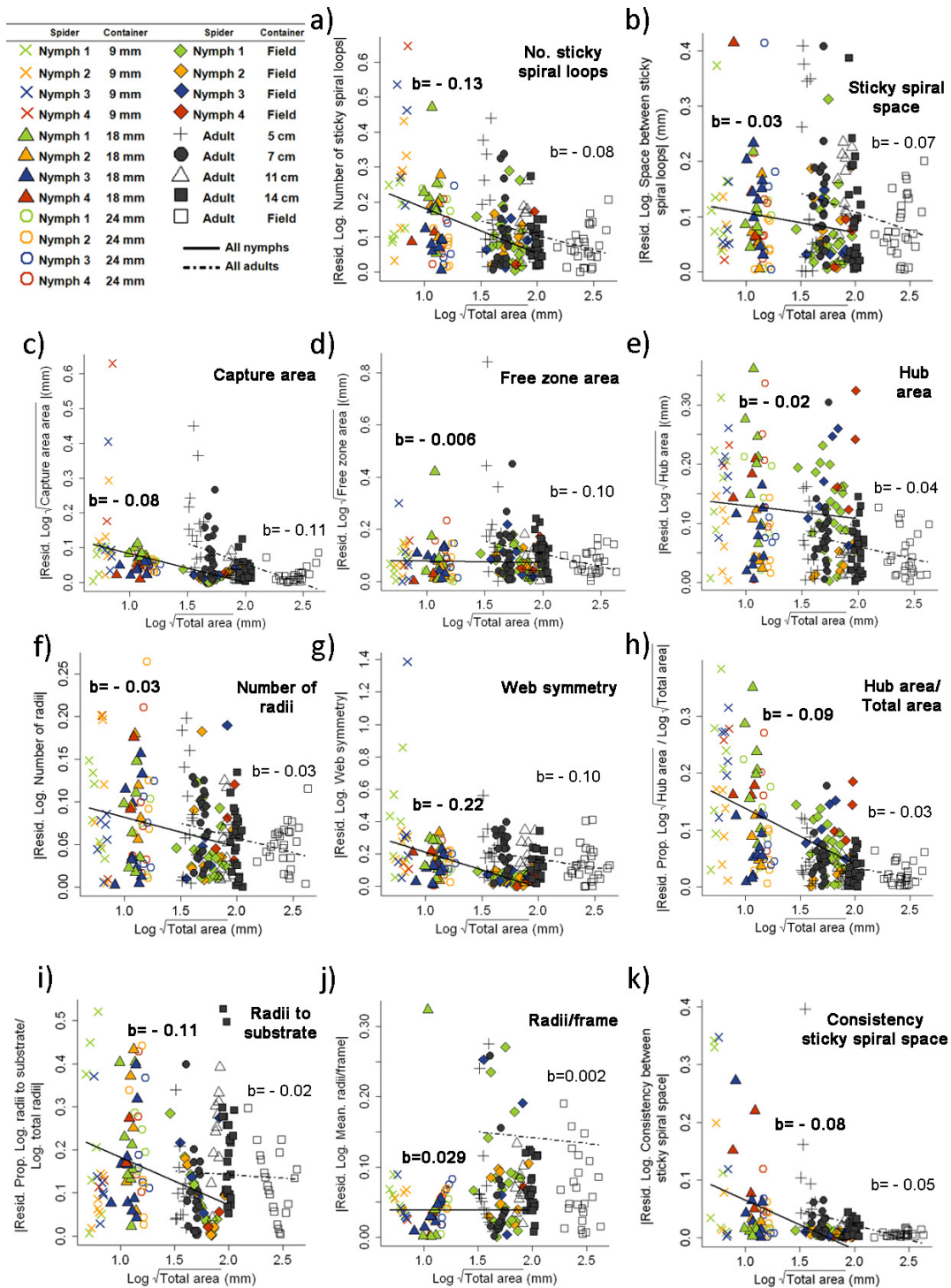
Table 3. Slope values, F-test for the slopes (H_0: b=0) and proportion of the variability explained by the independent variable (r^2) when the absolute value of the residuals obtained from regressions of each web variable with total web area, was regressed against total web area as independent variable. Also included are comparisons of slopes between residuals of all nymph webs (rn) and all adult webs (ra) by t-tests. L=longest radius. L+O= Longest radius plus radius opposite to longest radius.

Variable	Residuals **ADULTS**					Residuals **NYMPHS**					Test between slopes	
	Slope (b)	p	r^2	F	df	Slope (b)	p	r^2	F	df	$t(b_{rn}-b_{ra})$	$p(b_{rn}-b_{ra})$
Total area as independent												
Capture area	-0.12	<0.00001	0.24	33.0	1/105	-0.08	<0.00001	0.19	26.3	1/112	0.17	0.864
Free zone	-0.10	0.004	0.08	8.8	1/105	-0.01	0.703	0.00	0.1	1/111	0.41	0.680
Hub area	-0.04	0.004	0.08	8.8	1/105	-0.02	0.264	0.01	1.3	1/111	0.11	0.912
Number of (hub) radii	-0.03	0.009	0.07	7.0	1/96	-0.03	0.011	0.06	6.7	1/106	0.00	0.998
No. sticky spiral loops L+O	-0.08	0.002	0.09	9849.0	1/98	-0.13	<0.00001	0.18	22.5	1/101	0.20	0.843
Sticky spiral space L	-0.07	0.026	0.05	5136.0	1/98	-0.03	0.088	0.03	3.0	1/102	0.15	0.884
Consistency L	-0.05	<0.001	0.13	14.7	1/96	-0.09	<0.00001	0.23	31.5	1/104	0.20	0.843
Web symmetry	-0.10	0.004	0.08	8.7	1/97	-0.22	<0.00001	0.24	34.7	1/108	0.44	0.659
Mean radii/frame	0.00	0.909	0.04	4.7	1/104	0.03	0.033	0.00	0.0	1/92	0.15	0.879
Prop. radii attached to substrate/total radii	-0.02	0.651	0.00	0.2	1/96	-0.11	0.000	0.13	16.3	1/107	0.38	0.708
Prop. capture area/total area	-0.07	<0.00001	0.20	26.8	1/105	-0.11	<0.00001	0.23	32.5	1/112	0.24	0.813
Prop. free zone/total area	-0.07	<0.001	0.11	13.3	1/105	-0.06	<0.001	0.12	15.3	1/111	0.09	0.928
Prop. hub area/total area	-0.03	<0.001	0.12	14.4	1/105	-0.10	<0.00001	0.21	29.5	1/111	0.39	0.697

b) Nymph size and hub removal (only for nymphs)

All variables that represented adjustments to constrained spaces in nymphs size 1 also did in nymphs size 2,3 and 4 grouped together (slopes for all variables were significant for both group of sizes of spiderlings). The only exception was the proportion of the web dedicated to the hub area that did not qualified as an adjustment in nymphs size 1 (Table 4). In all measured variables, slopes using the webs of size 1 nymphs did not show significant differences when compared with corresponding slopes in the webs of nymphs size 2,3 and 4 grouped together (Table 4).

For 12 of 21 web variables, regression models including both the total web area and the nymph size as independent variables were better for explaining the behavior of the web variables than models including only total web area or only nymph size. For the rest of the web variables, models including only total web area explained more variability than models using both total web area and nymph size or only the size of the spider. In any case, models including only nymph size were more explicative than the other two (AIC tests) (Table 5). Taking into account the webs with few (three or less) or no sticky spiral loops illustrated that most of these webs were built by different sized spiderlings mostly in the smaller cylinders (9 mm diam.) (Fig. 5). No equivalent data of the size of the spiders is available for adults; therefore no comparisons were done.

Table 4: Slope values, F-test for the slopes (H_0: b=0) and proportion of the variability explained by the independent variable (r^2) when each web variable was regressed against total web area for nymphs size 1 and nymphs size 2,3 and 4 grouped together. Also included are comparisons of slopes between webs of size 1 nymphs (1) and nymphs of sizes 2,3 and 4 grouped together (2,3,4) by t-tests. L=longest radius. L+O= Longest radius plus radius opposite to longest radius.

Variable	NYMPHS **1**					NYMPHS **2,3,4**					Test between slopes	
	Slope(b)	p	r2	F	df	Slope(b)	p	r2	F	df	t(b_1-$b_{2,3,4}$)	p(b_1-$b_{2,3,4}$)
Total area as independent												
Capture area	1.12	<0.00001	0.99	4143.0	1/42	1.23	<0.00001	0.94	1145.0	1/68	0.46	0.65
Free zone	0.36	<0.00001	0.75	123.9	1/42	0.35	<0.00001	0.67	134.6	1/67	0.01	1.00
Hub area	0.24	<0.00001	0.52	46.3	1/42	0.12	0.002	0.14	10.8	1/67	0.43	0.67
Number of (hub) radii	0.40	<0.00001	0.83	198.8	1/40	0.40	<0.00001	0.69	143.2	1/65	0.01	0.99
Number of (spiral) radii	0.47	<0.00001	0.89	333.8	1/40	0.51	<0.00001	0.78	233.6	1/65	0.18	0.86
Mean No. sticky spiral loops	1.04	<0.00001	0.90	387.5	1/41	1.22	<0.00001	0.87	461.7	1/69	0.56	0.58
No. sticky spiral loops L+O	1.02	<0.00001	0.89	321.6	1/40	1.27	<0.00001	0.85	337.7	1/59	0.71	0.48
Mean sticky spiral space	0.24	<0.00001	0.54	47.8	1/41	0.22	<0.00001	0.30	28.4	1/67	0.06	0.95
Sticky spiral space L	0.24	<0.00001	0.40	26.4	1/39	0.25	<0.00001	0.33	30.3	1/61	0.04	0.97
Mean Consistency	0.02	0.498	0.01	0.5	1/40	-0.02	0.410	0.01	0.7	1/62		
Consistency L	0.03	0.300	0.03	1.1	1/40	-0.01	0.825	0.00	0.0	1/62		
No. hub loops	0.07	0.024	0.12	5.5	1/42	0.09	0.001	0.15	11.8	1/67	0.11	0.91
Web symmetry	0.40	<0.0001	0.37	24.4	1/41	0.35	<0.0001	0.23	19.7	1/65	0.13	0.90
Number of frames	0.33	<0.001	0.28	15.1	1/39	0.52	<0.00001	0.37	36.7	1/63	0.46	0.65
Mean radii/frame	0.29	<0.00001	0.62	63.3	1/39	0.22	<0.00001	0.62	104.2	1/63	0.30	0.77
Prop. frame w. single radius/total frames	-0.36	<0.00001	0.44	30.5	1/39	-0.31	<0.00001	0.47	55.9	1/63	0.15	0.88
Prop. spirals beyond frames/total frames	-0.25	0.015	0.14	6.5	1/39	0.18	0.062	0.05	3.6	1/63	0.16	0.88
Prop. radii attached to	0.65	<0.00001	0.69	88.5	1/40	-0.77		0.71	160.0	1/65	0.33	0.74

substrate/total radii							<0.00001					
Prop. capture area/total area	0.16	<0.00001	0.47	37.9	1/42	0.27	<0.00001	0.35	36.6	1/68	0.43	0.67
Prop. free zone/total area	0.18	<0.00001	0.45	33.7	1/42	-0.22	<0.00001	0.45	55.4	1/67	0.15	0.88
Prop. hub area/total area	0.04	0.233	0.03	1.5	1/42	-0.19	<0.00001	0.32	31.1	1/67	0.90	0.37

Table 5: Evaluation of models according to the Akaike Information Criterion (AIC). Model 1 including only total web area, model 2 including total web area and nymph size and model 3 using only nymph size as independent variables regressed against other 21 measured variables in all webs built by *L. argyra* nymphs in cylinders of 9, 18 and 24 mm of diameter and in the field.

| | Most explicative model according to AIC | | |
Variable	Total web area	Total area and nymph size	Nymph size
Capture area		X	
Free zone		X	
Hub area		X	
Number of (hub) radii	X		
Number of (spiral) radii		X	
Mean No. sticky spiral loops		X	
No. sticky spiral loops L+O		X	
Mean sticky spiral space		X	
Sticky spiral space L	X		
Mean Consistency	X		
Consistency L	X		
No. hub loops	X		
Web symmetry	X		
Number of frames	X		
Mean radii/frame		X	
Prop. frame w. single radius/total frames		X	
Prop. spirals over frames/total frames	X		
Prop. radii attached to substrate/total radii		X	
Prop. capture area/total area	X		
Prop. free zone/total area		X	
Prop. hub area/total area		X	

The probability of removing the center of the hub by ingesting the white specks of silk accumulated after radius construction, was lower in webs with smaller web areas than in webs with larger webs areas (x^2=36.89, df=1, p=3.18E-07, Fig.7.a), and most un-removed hub centers were found in constrained spaces. On the other hand, there was no difference in the probability of removing the center of the hub according to the size of the spiderlings (x^2=3.03, df=1, p=0.086, Fig.7.b). The total web area was more important in determining the probability of removing the center of the hub than the size of the spider. The logistical regression model using

(1) only total web area as independent variable (AIC=119.90) was better than the ones including (2) both the total web area and the size of the spider (AIC=120.33) and (3) only the nymph size (AIC=153.75). The hub area of all field and laboratory webs showed a positive relationship with tibia length ($F_{1,111}$ = 91.66, r^2 = 0.45, p <0.00001). No equivalent data of the frequency of removing the center of the hub is available for adults; consequently no comparisons were done. However, un-removed hub centers were also observed in adults in constrained spaces in captivity but not in the field (Barrantes & Eberhard pers. comm.).
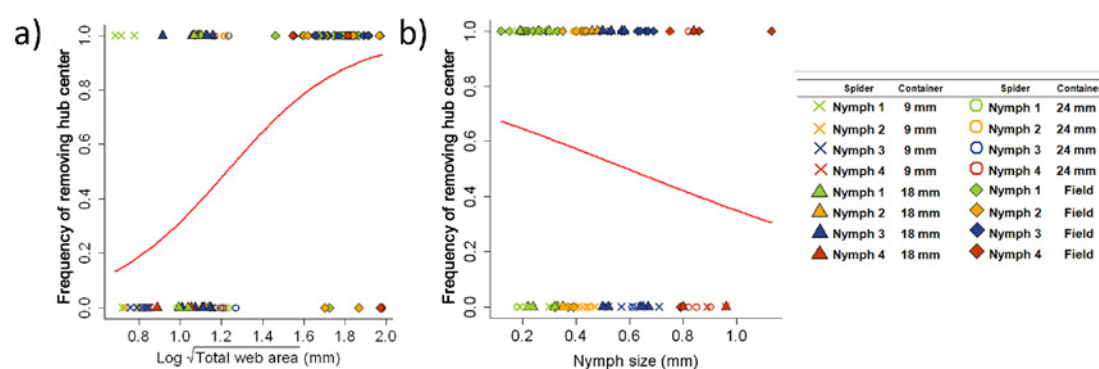

Fig. 7: Frequency of removing the center of the hub as a function of a) total web area and b) nymph size, in orb webs built in cylinders of 9, 18 and 24 mm of diameter and in the field by *L. argyra* nymphs. The red line represents the predicted probability of removing the hub calculated by a logistic regression. Symbols above the line indicate hub centers that were removed and below the line hub centers that were un-removed.

Regarding the tendency of nymphs to build a web in constrained spaces in captivity, the percentage of spiders that built an orb was higher in larger containers in all sizes of spiderlings. Furthermore, smaller nymphs in all three containers generally built proportionally fewer webs than larger ones, except for the size 4 nymphs, whose building rate slightly was lower than that of size 3 nymphs (Fig. 8). No equivalent data of the frequency of building an orb web in the constrained spaces given to the adults in captivity is available; consequently no comparisons were done.
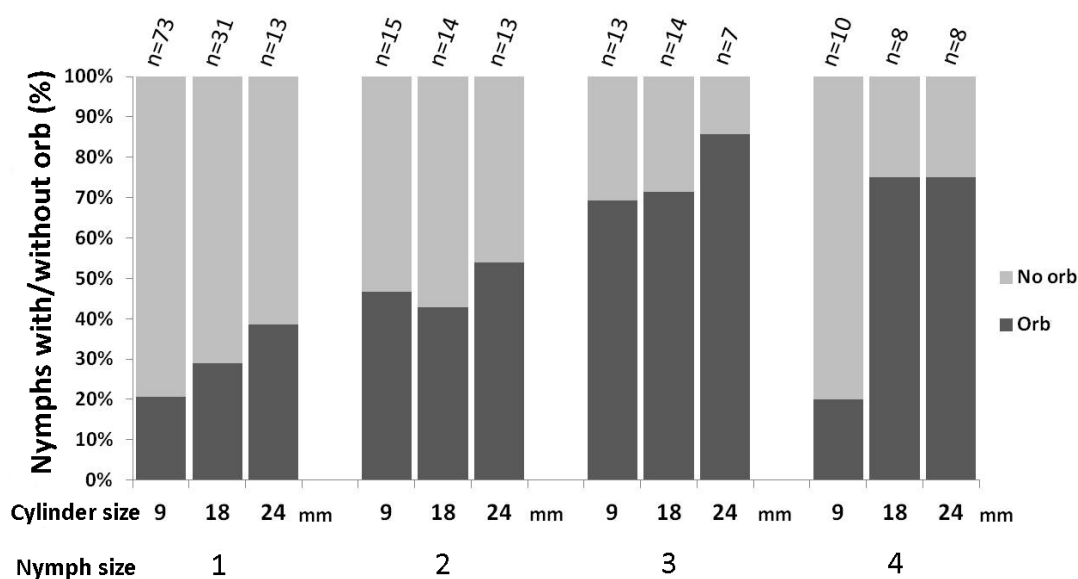
Fig. 8: Percentage of *L. argyra* nymphs of all sizes that built and did not build an orb web in cylinders of 9, 18 and 24 mm of diameter.

Discussion

Comparisons between webs of nymphs and adults

Were the nymphs able to adjust their web construction behavior to constrained spaces as well as adults? Data in this study indicates yes. Nymphs were able to perform all the adjustments to constrained spaces that adults did, and showed no sign of being less behaviorally plastic. The adjustments observed in this study are more complex, and may represent more neurally demanding behaviors than the ones documented in tiny spiders in previous studies (Eberhard 2007, Hesselberg 2010, Eberhard 2011): *L. argyra* nymphs adjusted their orb webs to spaces from 8 to 20 times smaller than their normal web size. This constitutes the first evidence against the size-limitation hypothesis, which is perhaps the strongest to date, as the behavioral adjustments performed by the nymphs would seem to be especially demanding because they involved many different aspects of web construction (for a discussion of independence of characters, see Barrantes & Eberhard 2012).

In addition to the fact that the nymphs adjusted their orb webs to constrained spaces as adults, the relationships between web variables and total web area were also similar, although at a different grade. Besides, the magnitude (slope) of the adjustments did not show differences between nymphs and adults in all cases. However, this results indicating lack of difference in the magnitude between nymphs and adults might be loose. It is possible that some differences exist, but that they are covered by the high scatter around both lines. Also, the modifications to small spaces constituted extensions of adjustments seen in webs built in less extreme spatial conditions in the field in similar web variables in both nymphs and adults.

Regarding the precision in the orb web adjustments made by nymphs and adults to constrained spaces, both stages presented very similar precision in most web variables. Taking in to account only web variables not involving proportions, nymphs showed greater variability in only in one variable: the hub area. However, this higher variability in nymphs could be related to the fact that the size of the spider might have great influence on the size of the hub (hub area increased with tibia length in *L. argyra* nymphs). This result could be then a consequence of the variety of sizes in nymphs studied (adults probably varied less in body size), and might not implicate higher imprecision in the adjustment made by nymphs. In sum, nymphs did not show less behavioral imprecision on their adjustments to cramped spaces than adults, constituting thus another evidence against the size-limitation hypothesis. This result is consistent with the findings in other species of orb weavers, where no greater imprecision when building their orb webs was found in very small species of spiders compared to larger ones (Eberhard 2007, Eberhard 2011).

Another phenomenon was observed in cramped spaces in both nymphs and adults: as web area decreased the variability of web parameters regressed against web area increased (Fig. 6 a-k). That is, the variability in web traits modifications increased in reduced spaces. This means that for most of the measured web variables, less precise behavioral modifications were made in cramped spaces in both nymphs and adults. This variability showed a surprisingly similar pattern of

variation in nymphs and adults: all slopes between the residuals of a variable regressed against total web area showed no difference between the two stages.

Small space traits (characters associated with small spaces) showed by adults in reduced spaces were present in nymphs as well: higher proportion of radii attached directly to the substrate, fewer frames, fewer radii per frame and higher proportion of frames with single radius. An additional small space trait was observed in nymphs: higher proportion of frames with sticky spirals attached beyond them. This modification seems to be a violation of the general pattern of behavior in orb weavers of attaching the outermost loop of the sticky spiral to the distal part of radii (Eberhard 1982) and even sometimes to frames, but not beyond frames. Sticky spirals beyond frames would loose their usual attachment to two radii, and this might affect the transmission of vibrations between them and the spider, if a prey hits those sticky threads. On the other hand, spirals beyond frames in constrained spaces could be instead an adjustment to take advantage of the space available, and cover as much as possible with sticky lines.

Hub center removal

Another apparent small space trait observed in nymphs was non-removal of hub centers, which was independent of the sizes of nymphs. As in *L. argyra,* many other tetragnathids and other orb weaving spiders (see list in Eberhard 1972, Eberhard 1981, Eberhard 1982, Eberhard 1987a) remove the center of the hub. However, only a few functions for this behavior have been proposed, though experimental tests are yet lacking. One study states it could possibly decrease the overall sum of tensions on the radii and tend to reduce the differences in tensions between them (Eberhard 1981). Another study mentions it could be an adaptation to help the web yield when a prey hits the web (Briceño & Eberhard 2012), perhaps by lowering or equilibrating the tensions on different radii (Eberhard 1972).

The fact that un-removed hubs were more common in cramped spaces in this experiment may support this idea: unlike in field webs, in cramped spaces there is probably no need to release tension in the orb because no large wind currents would deform it. Also, it is unlikely that webs in restricted spaces would receive high

impacts by flying preys, as it could happen in open spaces. Un-removed hub center are present in the species of at least two genera of tetragnathids that build webs between rocks near the ground: *Glenognatha* and *Cyrtognatha* (Eberhard 1981). Orb webs in small containers seemed to be tenser that webs on the field, although no measurements of web tensions were done. This could also be associated with the reduced number of frames in constrained spaces, since frames probably increase the extensibility of the web, enabling it to absorb more stress (Denny 1976, Eberhard in prep).

Regarding the relationship between the size of the hub and the size of the web, there was a striking difference between webs built by nymphs in the field and in constrained spaces. In field webs, the proportion of the web dedicated to the hub increased with total web area (Table 2, Fig.4.h). Nevertheless, when webs in containers were added, this proportion decreased with web area. In adults, the proportion of the web dedicated to the hub also decreased when all webs (field and captivity webs) were regressed against total web areas. The same negative relationship was found in *Nephila clavipes:* adult spiders confined in reduced spaces built webs with hubs that were significantly larger than in field webs (Hesselberg 2010). One possible explanation for the negative correlations in these species is that the hub may have a minimum size that the spiders cannot decrease, perhaps related to the size of the spider (tibia length was highly correlated with hub area in *L. argyra* nymphs) and its position in the hub (Briceño & Eberhard 2012). This hypothesis is in accord with a recent study with the uloborid *Zosis geniculata* (Olivier 1789), which found the opposite trend when adults were confined in unnaturally small spaces: the proportion of the web dedicated to the hub increased with web area (Barrantes & Eberhard in prep). This could be because in this distantly related species, field webs have relatively large hubs compared with the spider's size, and therefore the spider can decreased the hub size without compromising its function in the web.

Nymph's tendency to build a web

All sizes of *L. argyra* spiderlings were more likely to build orb webs in larger containers, which reinforces the idea that space availability in one of the many traits that constrains web building (Vollrath et al. 1997). Among the nymphs enclosed in small spaces in the laboratory, the degree of constraint was greater for larger nymphs. However, bigger nymphs built proportionally more webs even in the 9 mm cylinders, meaning smaller nymphs were less likely to build an orb web. This might be taken as support for the behavioral size-limitation hypothesis, but the hypothesis is framed in terms of differences in capabilities, rather than in disposition to perform a behavior.

Nevertheless, smallest nymphs did not demonstrate signs of behaving differently compared with larger ones when building an orb web in constrained spaces. First, they were able to perform all the web adjustments (significantly slopes when web variables were regressed against total web area) to constrained spaces as the rest of the nymphs grouped together. Second, the magnitude of the adjustments did not show any differences between the two groups of nymphs (slopes for size 1 nymphs were not different from the slope for the rest of the nymphs in all variables).

In addition, AIC tests demonstrated that the size of the spider influenced some behaviors performed by nymphs in constrained spaces, but that it was not crucial in models explaining the variability in web traits. In conclusion, the fact the smallest nymphs were able to do the same behavioral adjustments as larger ones yields an additional evidence against the size-limitation hypothesis.

Convergence with orbs of other species that build in naturally cramped spaces

The small web traits of webs built by *L. argyra* nymphs in smaller containers, especially the smallest (9 mm cylinders), resemble the webs of the tiny (1 mm) European mysmenid *Trogloneta granulum* (Simon 1992). This species lives in the spaces between stony debris in caves, at depths of 30-70 cm. They build tiny, highly modified orb webs that consist of a hub, an average of 8.6 radii and transverse threads that connect radii (Hajer 2000, Hajer & Řeháková 2003). The transverse

lines resemble the sticky spiral loops of an orbicular web, but they are laid in two different arrangements: near the hub they connect all radii, forming concentric circles, but distally, they only connect two or rarely three radii. A conspicuous space is left between both kinds of arrangements (Hajer 2000, Fig. 9.1) and some webs do not have any of these transverse lines (Fig. 9.2). Sticky droplets are found on all components of the webs except for the hub. The center of the hub is left intact and has specks of silk (Hajer 2000), probably accumulated during radius construction. From a drawing in Hajer (2000), it appears that either each radius ends on a very small frame line, or that the radii are attached directly to the substrate (Fig. 9.1 and 9.2).
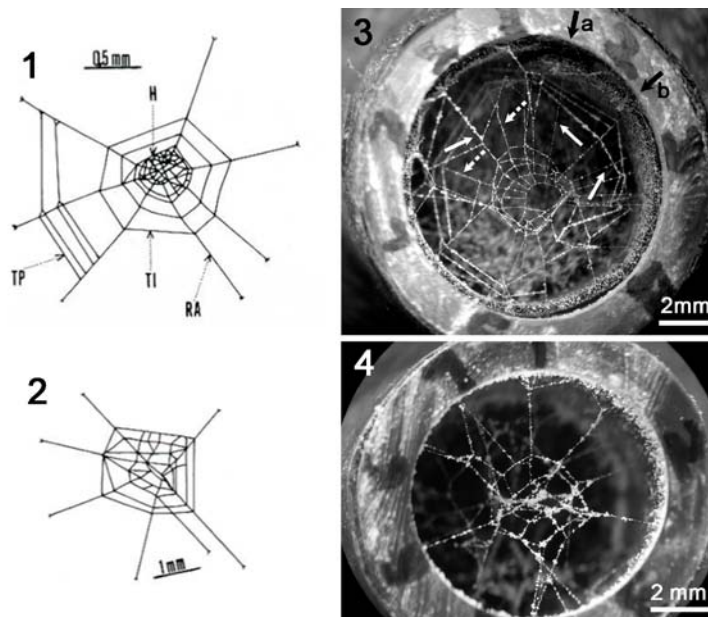
Fig. 9. 1-2: Webs of *Trogloneta granulum,* a mysmenid that builds webs in small spaces in stony debris. H= hub, RA= radial threads, TI=inner transverse threads, TP= peripheral transverse threads. Reproduced from Hajer (2000). 3-4: Orb webs of *L. argyra* (Tetragnathidae) nymphs built in constrained spaces (9 mm dia. cylinders). Full white arrows indicate radii arising from the hub going downwards to the substrate; dotted white arrows indicate radii arising from the hub going upwards to the substrate. Black arrows indicates a) hub radii: radii without sticky spirals attached, b) spiral radii: radii with sticky spirals attached.

Unlike these webs, orb webs made by *L. argyra* nymphs in 9 mm containers only had sticky droplets on the sticky spiral loops and these sticky lines did not have two arrangements as those of *T. granulum*. Similarities between the webs of *T. granulum* and *L. argyra* nymphs are the un-removed hub centers, the occurrence of some webs without sticky spiral loops, and the presence of some radii attached directly to the substrate or with very small frames. Radii attached directly to the substrate also occur in *Meta menardi* (Latreille 1804), a tetragnathid that builds orb webs that span about 15 cm between rocks near caves (Yoshidai & Shinkai 1993). The web of *Anapisona simoni* (Gertsch 1941), a species of the family Anapidae that spans webs of about 10 cm between vegetation near the ground, also present radii attached directly to the substrate (Eberhard 2011). Even no detailed description of

the radii is made in the text, a drawing of the web of another anapid, *Conoculus lyugadinus,* which also builds webs of about 15 cm between rocks near rivers, also shows radii without frame lines (Shinkai & Shinkai 1988). Anapidae and Mysmenidae are closely related (Lopardo et al. 2010) and they both belong to the Symphytognathoid group, which is more derived in some aspects than Tetragnathidae.

Another similarity is that webs of *T. granulum* are three-dimensional. Some radii extend upward and downward from the hub to anchoring points on the substrate (Hajer 2000). The same kind of radii were found in some *L. argyra* webs in reduced spaces. Some webs had radii going from the hub to anchoring points upwards, downwards and in the same plane as the hub (Fig. 9.3). Sometimes, spiders failed to attach sticky spirals to some of these radii (see discussion in Methods regarding hub radii and spiral radii). In most of the webs in 9 mm containers, the majority of radii extended from the hub downwards to anchoring points on the substrate, giving the web the form of an upward directed cone. This shape was probably induced also by the presence of the lines attached to the inner spirals of the hub going upward toward the substrate or other threads, as often happens in the field due to the lines that connect the hub with the upper tangle. Webs of the anapid *Anapisoma simoni* are also conic with radial lines running upward from the hub (Eberhard 1987b, Eberhard 2011).

The unexpected similarities between orb webs built by *L. argyra* nymphs in constrained spaces and webs made by other species in natural small spaces, and the fact that some small space traits showed by *L. argyra* resemble those of a more derived group, could help elucidate the evolution of web adaptations to live in cramped spaces. More recent studies are showing that adults of *Zosis geniculata*, a spider of the family Uloboridae, which is less derived in some respects than Tetragnathidae (Coddington & Levi 1991), present very similar responses to *L. argyra* when confined in constrained spaces (Barrantes & Eberhard in prep.), suggesting that those traits could have evolved earlier than reported.

Summary of conclusions

Three kinds of evidence were found against the size-limitation hyphothesis: (1) *L. argyra* nymphs made multiple adjustments to constrained spaces that were similar to those made by adults; (2) less precise behavioral modifications were made in cramped spaces in both nymphs and adults, but nymphs did not show less behavioral imprecision on their adjustments to cramped spaces than adults and (3) the smallest nymphs were also able to make the same behavioral adjustments as bigger nymphs.

How small animals are able to maintain the same behavioral flexibility as that of larger individuals remains unclear. One possibility is that miniaturization could improve efficiency by reducing transmission times, thus reducing energy consumption (Faisal 2005). Nonetheless, studies relating behavior and the cost-benefit trade offs that influenced the evolution of brain miniaturization need to be addressed, in order to understand how small animals are able to maintain behavioral capacities similar to those of larger animals, and pay the high metabolic costs of their proportionally large nervous system.

Web building behavior can provide important cues regarding phylogenetic relationships (Eberhard 1982, Eberhard 1990). For example, some of the behaviors described here, as small webs traits such as radii attached directly to the substrate and un-removed hub centers, represent possible adaptations that other more derived families perform in extreme natural conditions of space. These observations open the way for future studies of the relationship between size-related and behavioral plasticity and the evolution of fixed adaptive behaviors -patterns that are performed under all conditions.

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