

Nutritional requirements for web synthesis in the tetragnathid spider *Nephila clavipes*

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Abstract. Trap-constructing organisms provide a unique opportunity for the study of resource allocation, because an observer can unambiguously determine the allocation to foraging. In species that synthesize a trap from physiologically important compounds, there is the further advantage that there may be direct trade-offs between allocation of resources to foraging and physiological functions. We examined the ability of the spider *Nephila clavipes* (L.; Araneae: Tetragnathidae) to synthesize resources that are known to be used for both web synthesis and non-foraging physiological functions. We found that choline, required for both web function and physiological function, is an essential nutrient: it is not synthesized by this spider. Under laboratory conditions with a diet of fruit flies, choline is limiting, and the spiders make allocation trade-offs between investing choline in foraging (the web) or in their body.

Key words. Choline, orb-web synthesis, resource allocation.

Introduction

The study of resource allocation in animals poses several difficulties. The primary function of an activity, such as locomotion, is often difficult to determine, and is often classified by the end result (such as the capture of prey) because the original intention is unknowable to the observer. Also, different functions such as foraging, growth, and courtship often involve disparate resources that are not directly comparable.

Studying resource allocation in trap-constructing organisms avoids much of this uncertainty. Resource allocation to foraging is unambiguous in trap-builders such as larval antlions, net-making larval caddisflies and web-spinning spiders. Most trap-building organisms must have a trap in order to forage, and juveniles use their trap only for foraging. Furthermore, web-building spiders synthesize their traps from physiologically important resources (Tillinghast, 1984). Thus, it should be possible to track allocation of a compound that is critical to foraging as well as to somatic maintenance and growth, and to determine the relative investment in these different functions under different circumstances. To take advantage of the unique qualities of this model system, we sought to identify an informative measure for resource allocation in the orb-weaving spider *Nephila clavipes* (L.;

Araneae: Tetragnathidae). The results of this research provide the basis for future work examining resource allocation and its consequences in different environmental regimes.

It is likely that an informative measure of resource allocation would be a resource that was limiting, and the most limiting resources might be those that are nutritional essentials: i.e. compounds that the organism cannot synthesize and must obtain from its food. We identified two components of the orb web that are likely essential nutrients, choline and glycine betaine. Choline is a nutritional requirement by two functional definitions: it is not found labelled when the spiders are fed radioactive glucose as a precursor and it is missing on the web when the spiders are fed an artificial diet lacking choline and glycine betaine. Given that choline is a nutritional essential for these spiders, we then tested whether it is limiting for spiders fed a normal laboratory diet by examining the allocation of choline between the body and the orb-web of the spider.

Materials and Methods

The araneoid orb web

The sticky orb-webs of *N. clavipes* and many araneoid spiders consist of two distinct fractions: a water-insoluble proteinaceous silk, from which the radii, frame, and spiral are constructed, and a water-soluble mixture of low-molecular weight organic and inorganic compounds (Tillinghast, 1984; Vollrath *et al.*, 1990). This mixture is deposited on the spiral in

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droplets with a large glycoprotein, and these droplets make the web sticky (Vollrath *et al.*, 1990; Townley *et al.*, 1991; Tillinghast *et al.*, 1993). The glycoprotein and the low-molecular weight compounds are products of the aggregate glands (Townley *et al.*, 1991). The organic components of the low-molecular weight mixture have been identified from the web of *N. clavipes*. There are seven principal components: glycine, alanine, N-acetyltaurine, putrescine, GABamide, glycine betaine and choline (Tillinghast & Christenson, 1984). Several of these components are also physiologically important, being precursors or derivatives of neurotransmitters (choline, GABamide, N-acetyltaurine, putrescine), vitamins (choline), amino acids, and osmoprotectants (choline, glycine betaine). Thus, among these seven components are several that are necessary for processes other than foraging (building the orb-web). It is unknown whether any of them are sufficiently limiting to require trade-off decisions between foraging and other functions under ecologically meaningful experimental rearing regimes.

Handling of spiders

In autumn 1994, we collected gravid female spiders in Texas and Los Tuxtlas, Veracruz (Mexico), housed them in a glasshouse in Austin, Texas, and collected their egg sacs. In autumn 1995, we collected gravid females at these sites and at Tehuacán, Puebla (Mexico). The glasshouse had controlled lighting, temperature and humidity (LD 12:12 h (daylight supplemented by broad spectrum lighting), 25–27°C day/20–22°C night temperatures, minimum 60% RH). We supplied the adult females with water and one adult or two juvenile crickets daily. This regimen was maintained until the spiders died; the egg sacs were collected and stored as they were produced. We stored the egg sacs in a 16°C incubator until about 2 months prior to the start of the experiment. At that time, we moved the egg sacs into a 30°C incubator with a humidifier (\approx 100% RH), and most egg sacs from Mexico hatched. Losses were heavier for egg sacs from Texas, and we supplemented these by collecting dispersing juveniles in the field in the springs of 1995 and 1996.

Hatching *N. clavipes* juveniles pass through one non-feeding larval stage and moult to the first instar before leaving the egg sac to spin a communal web. The spiderling aggregations were moved into 26 × 24 × 8 cm redwood boxes (screened on the narrow sides, Plexiglas® sliding doors on the wide sides, or screened on one wide side with Plexiglas® on the other). At this stage, the spiderlings were fed *Drosophila melanogaster* killed by freezing (they do not attack live prey; Hill & Christenson, 1981). In 1996, to provide additional nutrition for the small juveniles, their webs were sprayed every other day with a dilute (1 teaspoon/100 ml water) solution of bee pollen (Smith & Mommsen, 1984). Chicken wire hung inside the boxes provided web attachment sites as the spiderlings dispersed from the communal webs and started to spin individual orbs (second and third instar).

Groups of 10–20 dispersing spiderlings from each egg sac were placed in individual boxes with chicken wire web

supports, and open vials of *D. melanogaster* cultures were placed in the boxes three times a week. The spiderlings were held in these small groups until they moulted to the fourth instar (0.4 cm leg I tibia + patella length, slightly smaller for animals from Texas). Upon moulting to the fourth instar, the spiders were randomly assigned to an experimental treatment. Fourth-instar spiders were moved into isolated or pair-wise occupancy of the same boxes (when in pairs, animals were individually marked on a leg with a spot of flat enamel paint). These spiders were fed live *D. melanogaster* each day and were offered distilled, deionized water from a syringe. In 1996, fourth-instar spiders were fed *D. melanogaster* and *D. virilis* on alternate days. Upon moulting to the fifth instar, the experimental treatments were initiated.

Web collection and handling

The orb-webs collected for chemical analysis were collapsed using a clean, sharp scalpel. Webs were wound onto a clean glass rod that was stored under refrigeration (\approx 5°C) suspended from hollowed-out corks inside clear PVC tubing. Webs collected each day from a given spider were wound sequentially along the same rod. Each web was scraped off the rod with a clean razor blade and placed individually into micro-Eppendorf tubes. The razor, scraping surface and forceps used for this process were wiped with a 50% ethanol solution between every web collection to avoid contamination. The Eppendorf tubes were left open and set into Petri dishes inside a desiccator filled with phosphorous pentoxide at atmospheric pressure and ambient temperature to dry. After a week, the desiccated webs were weighed with a microbalance and subsequently washed with 2 × 50 µl distilled, deionized water (at room temperature without agitation; first wash \approx 6 h, second wash \approx 14 h). The pooled washes were stored until analysis at –20°C (no more than 4 weeks later). Subsequent treatment depended upon the experiment.

Labelled precursors

This experiment involved spiders collected from Brazos Bend State Park, Texas (1994–95) and Los Tuxtlas, Veracruz, Mexico (1995). In 1994, we used spiders from two clutches, supplemented by five field-collected juveniles (a total sample size of 25 juveniles). In addition, we analysed webs made by three field-collected adult females from Brazos Bend that had been fed radioactive glucose for another study. In 1995, we repeated the experiment with five animals from one clutch from Los Tuxtlas and one field-collected spider from Brazos Bend.

Upon reaching the fifth instar, spiders were moved into individual boxes (if not already isolated) and fed live *Drosophila melanogaster* and water for 5 days. After 5 days, they were fed a solution containing a radiolabelled precursor: either D-[C¹⁴(U)] glucose (ICN Pharmaceuticals, Inc.) with unlabelled acetic acid or C¹⁴-(U) acetic acid (ICN Pharmaceuticals, Inc., Irvine, CA, U.S.A.) in 0.5 M glucose solution

(4 µl solution = 1 µCi, trace acetate $\text{apx} = 3.3 \times 10^{-4}$ M). In 1995, the spiders were all given D-[C¹⁴(U)] glucose in 0.5 M glucose solution. After receiving the radioactive solution, the spiders were not fed, but were provided with water each day. On the day that a spider was fed radioactivity, its web was collapsed but not collected (to encourage recycling). All subsequent webs were collected. The webs were collected each day until the spiders ceased spinning. If spinning occurred for more than 7 days in 1994, an additional dose of radioactive precursor was administered. In 1994, spiders were killed after 10 days if they had not stopped spinning earlier; in 1995 they were killed after 7 days by freezing.

After the webs had been collected and washed (see above), the non-water-soluble portion (silk and glycoprotein) and 10 µl of the wash were counted on a scintillation counter to determine levels of radioactivity. The web washes were dried completely in a speed-vac. If the individual web washes had low radioactivity (<1000 cpm per 10 µl sample), sequential pairs of web washes were pooled before drying was completed. All water-soluble samples were resuspended in 8 µl of distilled, deionized water.

Two-dimensional miniature thin-layer chromatography (5.7 cm square 0.1 mm precoated cellulose plates; polyester-backed in 1994 and glass-backed in 1995) was run using 3 µl of each specimen (1st dimension 45:30:5:20 v/v pyridine:acetone:ammonium hydroxide:water; 2nd dimension: 75:12.5:12.5 v/v 2-propanol:formic acid (88%):water; Townley & Tillinghast, 1988). Glycine (1 µl of 0.01 M) was used as a standard on all plates. After drying, the plates were stained with ninhydrin (0.2 g in 100 ml 98% ethanol) and read. They were then exposed directly to Kodak XAR autoradiography film for 10 days. If the ninhydrin stain or the autoradiography revealed no compounds then washes from up to the entire complement of webs from a given individual spider were pooled, dried, resuspended in 5 µl of distilled deionized water, and the analysis was repeated with the pooled specimen.

Artificial diet

Not all synthetic pathways involve glucose or acetate. Therefore, the fact that choline and glycine betaine were never found to be radiolabelled is a necessary, but not sufficient, basis for concluding that these two compounds are not synthesized by the spiders but are nutritionally essential. To provide the spiders with a broader diet from which to synthesize choline and/or glycine betaine, spiders reared as described above were assigned randomly to receive a liquid diet based on glucose and the chicken egg protein vitellin (a complete protein).

Fifth-instar spiders (eight field-collected spiders from Brazos Bend and five spiders reared from a female collected at Los Tuxtlas) were fed *Drosophila* for 5 days following the moult. Orb size (maximum vertical diameter) and mesh size (number of viscid and nonviscid spiral strands per cm measured 1 cm above the lower edge) were measured during this time. After 5 days, food was withheld and water given *ad libitum* while webs were collected daily on glass rods. Food

was withheld until either the orb produced was one half of its pre-fasting dimension (either diameter or mesh size) or, failing that, after 8 days. At this time, we randomly assigned the spiders to receive one of two artificial diets (spiders from both populations were included in each treatment): (A) 0.5 M glucose, vitellin (0.5% by weight; Sigma, from chicken egg) + glycine betaine (2×10^{-6} M, Sigma) + choline chloride (2×10^{-6} M, Sigma), (B) 0.5 M glucose and vitellin (0.5%) alone.

The spiders were fed what they would accept, up to a maximum of 5 µl of the artificial diet. Web size and mesh size were measured each day while on the artificial diet, and the webs were collected daily. The treatment continued until the spiders no longer spun webs or until eight webs had been collected.

Each glass rod containing all of the webs collected from a given spider was relabelled with a number. Scoring the presence of choline was done without knowledge of the treatment. The webs were washed as described above, and the web washes of sequential pairs of webs from each individual were pooled and dried completely in a speed-vac (maintaining starvation webs separate from webs spun while on the artificial diet). These washes were resuspended in 8 µl of 25% ethanol (to increase drying speed and reduce diffusion; ethanol diluted with distilled deionized water), and 3 µl was run in one-dimensional miniature thin-layer chromatography (TLC; 21 by 27 mm 0.1 mm precoated cellulose glass plates; solvent system 8:2:1:3 v/v n-butanol:95% ethanol:acetic acid:water). Occasionally, concentrations were too high (resulting in large or diffuse spots) or the results were ambiguous, and the TLC was repeated with adjusted amounts of the web washes.

Choline allocation

Spiderlings were reared in the spring of 1996 from egg sacs laid by two females collected at Tehuacán in 1995, as described above. Upon reaching the fourth instar, they were assigned randomly to one of five quantitatively different diet treatments. Spiders were offered prey totalling 2–25% of their initial weight (postmoulting fourth instar weight), 0.5–4 *Drosophila* each day. All spiders were fed on alternate days, and all received 50% *D. melanogaster* (0.86 mg mean weight) and 50% *D. virilis* (1.4 mg mean weight).

All spiders were reared through the fourth instar on the assigned diet. After moulting to the fifth instar, they were kept on the same diet for 5 additional days (three feedings). On the sixth day, they were fed 3 µl 1–2 C¹⁴ choline chloride (NEN-DuPont; 1.5 µl Ci in 0.5 M glucose) and the orb-web was collapsed. The next two webs were collected, and then the spider was killed by freezing at –20°C for at least 20 min, but not more than 1 h. The webs were washed as described in the general methods, but not weighed. The spiders were dissected into cephalothorax plus legs (muscle, nervous system, venom glands) and abdomen (fat bodies, silk glands). These two body parts were homogenized in 1.0 ml distilled deionized water. After mixing on a vortex, a 0.1-ml aliquot was used to determine radioactivity (with 5 ml of scintillation fluid;

Complete Counting Cocktail, Research Products International Corporation, Mt Prospect, IL, U.S.A.). A 10- μ l aliquot of the 100 μ l web wash was used to determine the radioactivity of the orb-webs. The total amounts of radioactive choline in each body part and in each web were calculated from these samples prior to statistical analysis. The amount of radioactivity in the second orb wash was uninformative (no variation across the treatments). Therefore, we compared the proportion of radioactivity (arcsin transformed) in the first orb, the cephalothorax and the abdomen.

Results

Labelled precursors

The five amine compounds present in the water-soluble portion of the orb-web were reliably separated and detected (Fig. 1). To determine whether either precursor was being placed on the web, both were run as standards in addition to glycine on control plates. To determine whether choline or glycine betaine was being synthesized from the labelled

precursors, each was run with standards of the other compounds to determine the relative locations on the plate with this solvent system (use of Dragendorff's stain preceded Ninhydrin staining). Choline and glycine betaine were not distinguishable with this solvent system, but did separate from the amine compounds. The area of each plate where choline and glycine betaine were found was checked for radioactivity after the autoradiograms were developed.

There was no qualitative or quantitative difference between spiders fed C^{14} glucose and C^{14} acetate: all amine compounds in the water-soluble portion of the orb-web were radioactively labelled (glycine, GABamide, N-acetyltaurine, putrescine, and alanine). The webs spun by juveniles from Brazos Bend did not always include detectable quantities of all five compounds, and not all five were labelled on each web. Table 1 presents summary data of the presence and radioactivity of the most common water-soluble components from all webs spun by each individual in 1994. Neither spiders fed glucose nor spiders fed acetate produced radioactively-labelled choline or glycine betaine. The adult females and 1995 study animals, while smaller samples, showed the same patterns of synthesis. Glycine, alanine and GABamide were usually labelled,

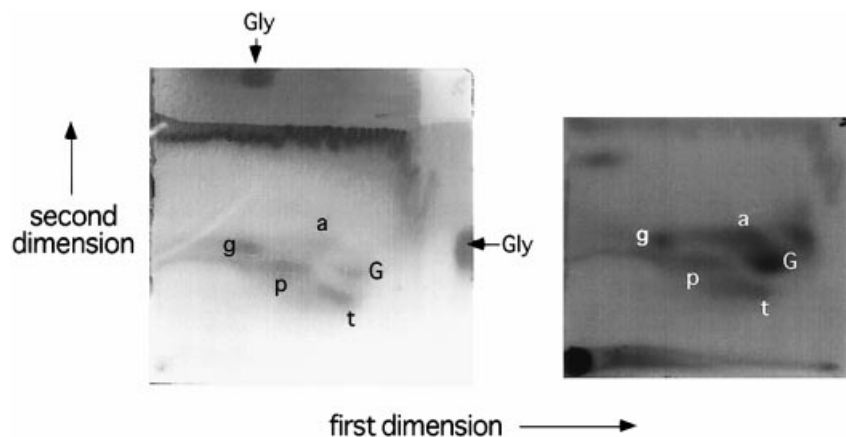


Fig. 1. Two-dimensional miniature thin-layer chromatogram (with glycine (Gly) standards) and autoradiogram of the same plate. g: glycine; t: N-acetyltaurine; G: GABamide; a: alanine; p: putrescine.

Table 1. Proportion of 25 juveniles from Brazos Bend that spun one or more webs with each of five amine components and three most common unknown amine components present and radioactively labelled (1994).

Compound	Detected (stained)	Radioactively labelled
Glycine	0.92	0.92
Alanine	0.88	0.80
Putrescine	0.92	0.64
N-acetyl taurine	0.92	0.52
GABamide	0.96	0.88
Unknown 1	0.48	0.24
Unknown 2	0.28	0.04
Unknown 3	0.16	0.08

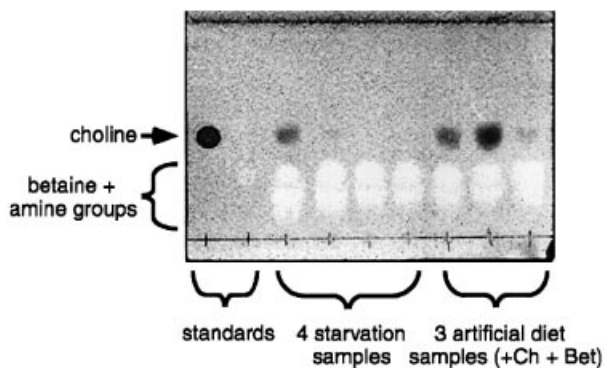


Fig. 2. One-dimensional miniature thin-layer chromatogram of serial web collection from a spider starved then fed a diet containing choline and glycine betaine.

Table 2. Presence and absence of choline on the web of spiders fed an artificial diet with or lacking choline. Presence on any web while on diet is taken as a positive reading.

Diet composition	n	Orb web composition	
		Choline present	Choline absent
+ Choline	9	9	0
-Choline	5	1	4

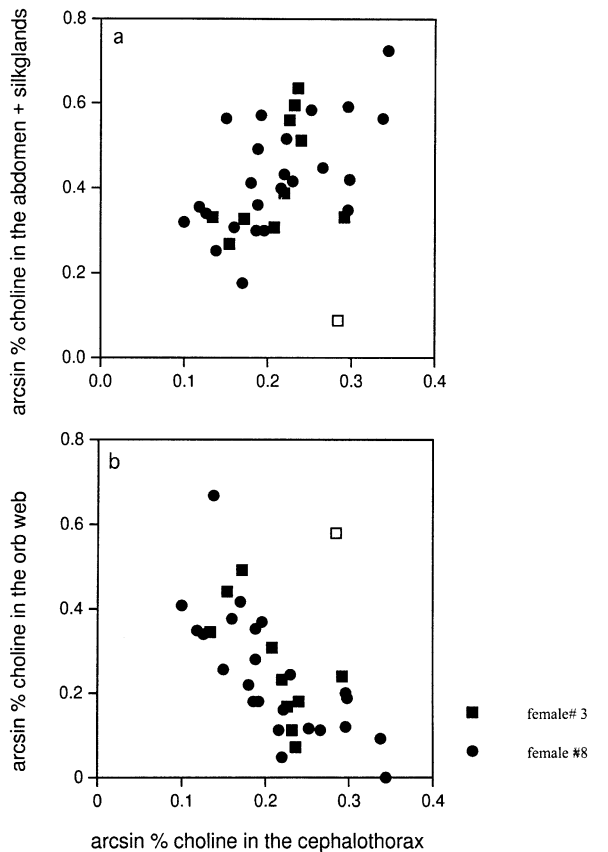


Fig. 3. (a) Positive correlation of choline allocated to the cephalothorax and to the abdomen (arcsin transform of percent radioactivity; $y = 0.098 + 0.267x$; $R^2 = 0.31$). ■ and ●: the offspring of the two different females. There was no difference between the two groups ($T = -0.001$, $P = 0.99$). □: the data from the outlier, not included in the final statistical analysis. (b) Negative correlation of choline allocated to the cephalothorax and to the first orb-web built (arcsin transform of percent radioactivity; $y = 0.29 - 0.31x$; $R^2 = 0.51$). Excluding the outlier, there was no difference between the two groups ($T = -0.34$, $P = 0.74$). Symbols as in (a).

putrescine and N-acetyltaurine less commonly so. The three adult females from Brazos Bend all produced webs with labelled glycine, alanine and GABAamide. The five spiders from Los Tuxtlas all spun webs with labelled glycine, and four of the five spun webs with labelled alanine and GABAamide.

Radioactive N-acetyltaurine was not detected on the webs from one adult and one Los Tuxtlas juvenile. Radioactive putrescine was not detected on the webs of two adults and two Los Tuxtlas juveniles.

Spiders occasionally appeared to put glucose or acetate directly onto the webs, as detected from the autoradiographs. In addition to the seven known compounds, several unknown compounds occasionally appeared on the plates. All unknowns were occasionally labelled, indicating that they may be synthesized by the spider from glucose or acetate. Running known amino acids and checking for congruence with the locations of the unknowns suggests that the following trace compounds may occasionally be present along with the regular seven: unknown 2: leucine, threonine or tyrosine; unknown 3: glutamic acid; unknown 5: aspartic acid; unknown 7: glutamine or histidine. No amino acids tested corresponded with unknowns 1, 4, or 6. Further research is required to identify these rare compounds.

Artificial diet

Choline was easily distinguished from the other six water-soluble orb-web components (Fig. 2). Glycine betaine was more difficult to detect, as it co-migrated with some of the amine-containing groups found on the web that also stained with Dragendorff's stain. Plates where the identification of glycine betaine was ambiguous were stained with Dragendorff's stain, then subsequently sprayed with ninhydrin to better distinguish glycine betaine from the amine-containing water-soluble web components that reacted with Dragendorff's stain. However, in many cases this did not resolve the ambiguity, and herein we report only the presence or absence of choline.

The miniature TLC plates stained with Dragendorff's reagent allow detection of very small quantities of choline (to 5×10^{-11} moles). Therefore, we are confident that, when absent from the plate, choline was indeed absent from the orb-web. Choline was present on the webs if present in the diet (Table 2). Only one spider had choline on the web when absent from the diet, and this individual had choline only in the first web sample after the diet was started (first two webs).

Choline allocation

The first two experiments provide strong evidence that *N. clavipes* juveniles cannot synthesize choline. The question remains whether choline is ever sufficiently limiting to force the individual to make allocation decisions. Evidence for trade-off decisions would include a negative correlation between two expenditures. To examine the allocation of choline among body parts and between the body and the orb-web, we fed a tracer of 1–2 C^{14} choline chloride to spiders reared on quantitatively different diets. We compared the relative amounts of radioactive choline in the cephalothorax, abdomen and first orb-web.

The amounts of radioactive choline in the abdomen and the cephalothorax were positively correlated, and the amounts of radioactive choline in the water-soluble component of the web and the cephalothorax were negatively correlated (Fig. 3). These correlations are significant for the offspring of female No. 8 but not for the offspring of female No. 3. Observations from one spider, daughter of female No. 3, are responsible for this difference between these two groups. This spider allocated much less choline to the abdomen and much more to the carapace and the orb than all other spiders (□ in Fig. 3), and it was an outlier in many other respects: it passed through the intermoult at nearly half the time, and spun much smaller orb webs, than all other spiders on the same diet. Excluding this individual, the preliminary analyses of covariance, with choline allocation to the cephalothorax as the dependent variable, showed no significant difference in slope between the two groups of spiders (no female \times dependent variable interaction). Therefore, this interaction term was dropped from the analyses. Final analyses, also excluding the data from the outlier, showed no significant difference in choline allocation between the offspring of these two females. The negative correlation between choline in the cephalothorax and in the orb-web is evidence of trade-offs in allocation. This suggests that under these conditions, choline is limiting.

Discussion

Observations from the field and laboratory indicate that variation in resource allocation to the orb web can alter growth rates in juvenile *N. clavipes*, and that variation in juvenile growth is a major factor altering size and age at sexual maturity (Higgins, 1992, 1993; Higgins & Rankin, 1996). Size at sexual maturity and the timing of maturation, in turn, are important determinants of the fitness of the individual (Higgins, 1999). Shifts in resource allocation, measurable as the allocation of choline between the orb and the body, may therefore have strong fitness consequences. In this paper, we have tracked the allocation of choline between the orb-web (foraging effort) and the body of this spider. These results suggest that this is a useful and possibly a model system for the study of the ecology and the evolution of resource allocation.

Choline is recognized as an essential nutrient in a variety of vertebrate organisms (e.g. humans, dogs, rats, fish; Griffin *et al.*, 1994; Zeisel & Blusztajn, 1994; Craig & Gatlin, 1996; Garner, 1995). Some mammals have the capacity to synthesize small quantities of choline, but experimental exposure of various mammals to choline-free diets has shown that this is insufficient to meet physiological demands (Zeisel & Blusztajn, 1994). However, choline is rarely limiting under natural dietary conditions because it is almost ubiquitous in food as either free choline or phosphotidylcholine; the latter is an important component of most cellular membranes (Zeisel, 1993). Choline is synthesized by plants and some microorganisms from serine (Hanson & Hitz, 1982). Because of the ready availability of choline in the diet, there are no descriptions of naturally occurring deficiency syndromes (Zeisel, 1993). However, animals fed experimental diets without choline

develop serious physiological disorders, including the spontaneous development of liver cancer (Griffin *et al.*, 1994; Zeisel & Blusztajn, 1994; Craig & Gatlin, 1996).

The ability to synthesize choline has been tested in relatively few arthropods. Dadd (1985) reports choline to be a dietary requirement for most insects, suspecting that experimental observations of good growth on choline-free diets probably reflects extrinsic contamination or intrinsic production by associated microorganisms. As reported for mammals (Zeisel & Blusztajn, 1994), Bridges (1973) concludes that although some insects may have the ability to synthesize choline, endogenous synthesis is probably inadequate to meet all the physiological needs for choline. In all cases tested thus far, insects reared on a choline-free diet failed to reach sexual maturity (Bridges, 1972, 1973).

Choline is a precursor for a variety of other compounds with important functions, such as the osmoprotectant glycine betaine, the membrane components phosphotidylcholine and lysophosphotidylcholine (the latter a putative messenger in transmembrane signalling), and the neurotransmitter acetylcholine (Hanson & Hitz, 1982; Le Rudulier *et al.*, 1984; Zeisel & Blusztajn, 1994; Garner *et al.*, 1995). In insects, acetylcholine is the primary excitatory neurotransmitter in the central nervous system (Dadd, 1985). Although the same is probably true for spiders, no published work on this topic is available. Fabian & Seyfarth (1997) report that acetylcholine may be a neurotransmitter in mechanosensitive neurones in the spider *Cupiennius salei*. With the exception of some Diptera that have the ability to utilize other molecules as substitutes for choline (Bridges, 1973), most insects are believed to be wholly dependent upon dietary choline as a precursor for cell-membrane phospholipids.

Little is known about the nutritional requirements of spiders (Riechert & Harp, 1987). Dietary requirements have been inferred from the amino acid content of spiders (Greenstone, 1979) and from survivorship on different diets (Uetz *et al.*, 1992; Li & Jackson, 1997). To our knowledge, the work reported here is the first experimental determination of a specific required nutrient in a spider. Given the general reliance of most animals upon their diet to meet choline requirements, it is not very surprising that the spider *Nephila clavipes* cannot synthesize choline from glucose, acetate or a protein diet. It is important to note that methionine, a precursor of choline in mammals that synthesize choline through transmethylation, is present only in very small quantities in chicken egg vitellin (Clark, 1970). Therefore, we have not ruled out the existence of the methionine pathway for synthesis of small quantities of choline.

What may be unique to viscid-orb weaving spiders is that, even on normal diets, choline might be sufficiently limiting as to require trade-offs in allocation. Tracking choline use under different levels of food availability revealed that spiders can vary the allocation of available choline between their body and their orb-web.

Choline on the web may function as an osmoprotectant, as it does in microorganisms, plants and in the mammalian kidney (Yancey *et al.*, 1982; Vollrath *et al.*, 1990; Townley *et al.*, 1991). The glycoprotein component of the viscid droplets on

the orb web requires a humid or even aqueous molecular environment in order to maintain its proper conformation and function as a glue (E. Tillinghast, pers. comm.). In addition, the strength and elasticity of the silk of the web depends on the appropriate degree of hydration (Gosline *et al.*, 1984; Gosline *et al.*, 1986). The mixture of hydrophilic and hygroscopic low molecular weight compounds in the droplets may therefore attract and hold atmospheric water and prevent drying, thus improving the ability of the web to capture flying insects (Tillinghast & Townley, 1987; Townley *et al.*, 1991). We do not know whether other compounds were added to the mixture of low molecular weight water-soluble compounds on the web when choline was absent. We have determined (unpublished manuscript) that the composition of the low molecular weight mixture does change when the spiders experience different environments and different diets, and presumably such changes would also occur in response to reduced availability of one or more components. How such shifts in web chemistry might alter web function (elasticity and stickiness) remains to be determined.

Choline-deficient diets are reported to cause a variety of physiological disorders in mammals (Zeisel, 1993). These spiders were held on the artificial diets for short periods, and we did not observe any symptoms of choline deficiency. In fact, several spiders moulted during the study without any obvious problems. Following treatment with the artificial diets, the spiders were fed live prey; no signs of neurological disorders in spinning webs or handling prey appeared. However, studies of choline deficiency in vertebrate and invertebrate organisms lead us to believe that extended choline deprivation would cause neurological or physiological disorders (Bridges, 1972; Zeisel, 1993). Almost certainly, the spiders would be unable to reach sexual maturity: even the higher flies, which can utilize other phospholipids to synthesize cell membranes, fail to reach maturity when fed choline-free diets (Bridges, 1973).

This web-spinning spider presents unique opportunities for studying the allocation of resources necessary for physiological maintenance, growth and foraging effort. Because choline is required for both web construction and for physiological function, and because the web is used only for foraging, the allocation of resources between the body and foraging effort can be measured unambiguously. The response of the spiders to quantitative differences in diet, altering the relative amounts of choline allocated to the body and to foraging effort (web building) indicates that the allocation of choline is a flexible behaviour. The next step will be to determine exactly how choline allocation decisions are made, and how differences in choline allocation might alter important fitness parameters such as juvenile growth rates and the age and size at sexual maturity.

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