2001. The Journal of Arachnology 29:82–94

VARIATION IN THE CHEMICAL COMPOSITION OF ORB WEBS BUILT BY THE SPIDER *NEPHILA CLAVIPES* (ARANEAE, TETRAGNATHIDAE)

Linden E. Higgins: Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003 USA

Mark A. Townley and **Edward K. Tillinghast:** Department of Zoology, University of New Hampshire, Durham, New Hampshire 03824 USA

Mary Ann Rankin: Department of Organismal Biology, University of Texas at Austin, Austin, Texas 78712 USA

ABSTRACT. The adhesive droplets in the orb webs of araneoid spiders contain, among other constituents, an aqueous solution of organic low-molecular-weight compounds. The chemical composition of this solution has been investigated for pooled web collections from several species, but little is known about how the composition might vary among individuals or among environments. To begin addressing these questions, we analyzed serial collections of orb webs spun by individual juvenile *Nephila clavipes* from three different populations held first under field conditions and then under laboratory conditions.

Our results indicate that the composition of the organic low-molecular-weight solution is not fixed. We found significant differences in the droplet composition among individuals, among populations, and with the transfer of spiders to laboratory conditions. The possible origins and consequences of these differences are discussed.

Keywords: Orb web chemistry, interpopulational variation, intrapopulational variation, compatible solutes, adhesive spiral

Ecribellate orb-weaving spiders invest physiologically important compounds in the construction of their webs, including some that are nutritionally essential (i.e., not synthesized by the spider in sufficient quantity to meet its needs). This is particularly true for the adhesive spiral of the orb. Not only is the majority of the web's desiccated weight typically contributed by the adhesive spiral, but presumed essential amino acids make up a relatively large molar percentage of the proteins of the adhesive spiral (Tillinghast & Townley 1994). Also, at least one component of the aqueous solution on the adhesive spiral, choline, is nutritionally essential in insects (Dadd 1985) and evidence to date indicates that this is also true for araneoid orb-weavers, including *Nephila clavipes* (Linnaeus 1767) (Araneae, Tetragnathidae) (Tillinghast & Townley 1994; Higgins & Rankin 1999). Thus, the factors and mechanisms controlling the allocation of physiologically important compounds to adhesive spiral construction are by no means inconsequential to the spider's fitness, as they have a direct impact on both orb web function and the spider's physiological state.

The adhesive spirals of ecribellate orb webs are composed of a pair of core fibers of flagelliform gland origin upon which an aqueous, adhesive coating of aggregate gland origin is deposited (Sekiguchi 1952; Peters 1955). Components of this adhesive coating include, but are not necessarily limited to, inorganic ions (Fischer & Brander 1960; Schildknecht et al. 1972), at least one large phosphorylated glycoprotein (Tillinghast 1981; Dreesbach et al. 1983; Vollrath & Tillinghast 1991; Tillinghast et al. 1993), lipids (Peters 1995, Schulz 1997), and organic low-molecular-weight compounds (LMW) (Fischer & Brander 1960). Collectively, the organic LMW are present in high concentration (Vollrath et al. 1990) and typically account for 30% or more of the desiccated weight of the orb

web (Fischer & Brander 1960; Tillinghast 1984; Tillinghast & Christenson 1984; Townley et al. 1991). Several of these are identical or closely related to compounds employed as osmolytes in various osmotically-stressed organisms of wide taxonomic distribution (see Discussion).

Within the last decade, nuclear magnetic resonance spectroscopy (NMR) has been applied to the study of the organic LMW of the adhesive spiral, both as a means for identifying compounds and for estimating their relative molar proportions in the sticky coating (Vollrath et al. 1990; Townley et al. 1991). These analyses used laboratory-built, pooled web samples from multiple individuals and were designed neither to examine compositional variation among individuals, nor to examine potential factors influencing organic LMW composition. As a first step along these lines of inquiry, we have used proton NMR to compare webs collected in the field and in the laboratory from individual *N. clavipes* from three disjunct populations. In this way, we examined variation among individuals within a population and among populations, and the sensitivity of organic LMW composition to changes in environment and diet, such as occur when spiders are brought into the laboratory.

Previously, organic LMW components of the adhesive coating in webs of *N. clavipes* have been shown to include 4-aminobutyramide (GABamide), glycine, and a compound yielding taurine upon acid hydrolysis (Tillinghast & Christenson 1984), now known to be *N*-acetyltaurine (Vollrath et al. 1990). Here we report that choline and glycine betaine, earlier identified in the webs of four araneid species (Vollrath et al. 1990), are also present in webs of *N. clavipes,* as are two compounds not previously reported in orb web adhesive spiral coatings, putrescine and alanine. In comparing field- and laboratory-built webs, we have focused our attention on quantitative analysis of these seven compounds. Our results indicate that organic LMW composition changes significantly when spiders are moved to the laboratory, that there is significant variation among individuals in the same environment, and that there are significant differences among populations in the wild.

METHODS

Study species.—*Nephila clavipes* is a large orb-weaving spider distributed from the southeastern United States to Misiones, Argentina. Males mature after 4–5 juvenile instars, females mature after 7–10 juvenile instars. Penultimate instar males can be distinguished from juvenile females by swollen pedipalps. Juveniles of 0.5 cm leg I tibia + patella length (fifth instar) that did not have swollen pedipalps were assumed to be juvenile females. Voucher specimens from all three study populations have been deposited at the Smithsonian Institution.

Handling spiders.—Fifteen 4–7th instar *N. clavipes* were collected in each of three sites (Los Tuxtlas, Mexico; Chamela, Mexico; and Brazos Bend, Texas, USA; Table 1) the evening before starting the experiment and placed into redwood boxes (26 cm × 24 cm × 8 cm) that had screen on the four narrow sides and sliding acrylic plastic sheeting (''Plexiglass''™) doors front and back. In all sites, the boxes were put along an edge between open and wooded habitats. At dawn, the Plexiglass doors were removed, allowing the spiders to capture prey in a normal fashion. However, if a spider had not built, or if it was premolt (as indicated by abdomen volume and web condition; Higgins 1990), the box was left closed. At dusk, each web with a vertical radius length greater than 10 cm was collected (see below), and the Plexiglass doors were replaced. In Mexico (Los Tuxtlas and Chamela), the boxes were moved at night and during heavy rainstorms (nearly every afternoon) to a sheltered area to protect the webs from rainfall damage. They were moved back out again after rainfall. Although we moved the boxes during the afternoon storms, we left them open to allow prey capture.

When at least five webs had been collected from each spider, all were moved in their boxes into the laboratory. In Mexico, the spiders were moved to the Institute of Ecology of the National Autonomous University (UNAM) in Mexico City, where they were held in an unheated, uncooled indoor room with windows admitting natural light. In Texas, the spiders were moved to the University of Texas at Austin, where they were kept in a climate controlled chamber (14:10 L:D, 25 °C). Each day in the laboratory, they were offered water

Table 1.—Characteristics of the study sites. TX: Austin, Texas; MX: Mexico City, Mexico. (Data from: Garcia 1973; S. H. Bullock personal communication; Texas Department of Parks personal communication).

Site	Coordinates	Annual rainfall (mm)	Laboratory diet	Laboratory conditions
Brazos Bend	29°25′N 95°35′W	1120	crickets	growth chamber (TX)
Los Tuxtlas	18°30′N 95°W	4400	crickets	normal room (MX)
Chamela	19°30′N 105°W	700	flies	room (MX), chamber (TX)

from a syringe and fed a monotypic diet (one cricket per day for animals from Texas and Los Tuxtlas, one housefly per day for animals from Chamela as crickets were not available). For the first wk under these conditions, spiders were allowed to recycle their webs. Subsequently, while maintaining the same feeding and watering regimen, webs were collected each day until at least five had been collected from each animal (but note the following exception).

Due to unforeseen circumstances, the laboratory treatment of Chamela spiders was interrupted before all animals had spun five orb webs. Therefore, these spiders were transported to Austin. Some of these spiders spun five or more orbs in both Texas and Mexico, allowing us to compare Mexico laboratory- and Austin laboratory-spun orb webs from the same individuals.

Handling orb webs.—The orb webs were collected each evening onto a clean glass rod (6.35 mm × 30.5 cm; one rod per spider per treatment—laboratory or field). The orbs were collected by cutting radii with a clean scalpel (wiped with 50% ethanol between samples), collapsing the orb, then winding it upon a section of the rod not already occupied by a previously collected web. Rods were stored suspended inside transparent PVC pipes, with a cork at each end having a hollow place for the rod to rest.

Orb web extraction.—After all webs for a given treatment had been collected, each web was scraped off of the rod with a clean razor blade and was placed in its own microfuge tube. The orb webs were washed twice in 50 μL distilled, deionized water (first wash 6 h, second wash 16 h; without agitation at room temperature). The web washes from a given treatment from a given spider were then combined and taken to dryness in a Savant Speed Vac concentrator. These specimens were shipped to the University of New Hampshire for analysis by ^{1}H NMR.

^{1}H NMR analysis and LMW identification.—Each pooled web wash sample was dissolved in 0.5 mL 99.96% D_2O (Cambridge Isotope Laboratories) and analyzed by ^{1}H NMR using a Bruker AM-360 spectrometer with a 5 mm proton selective probe operating at a frequency of 360.135 MHz and a temperature of 300 K. An internal standard of 2-methyl-2-propanol, with a chemical shift of δ1.2200(ppm), was added to each sample just prior to NMR analysis. At a spectral width of 5000 Hz, 64K data points were acquired and an additional 64K data points with zero amplitude were appended to these (i.e., zero filled to 128K) prior to Fourier transformation to improve digital resolution in the frequency spectrum. Pulse width was 4.3 μsec (*ca.* 53°), acquisition time was 6.55 sec and pulse repetition time (τ) was 8.28 sec. The number of transients accumulated varied depending on sample size, ranging from about 300–8500, with about 1000 typical. Integrated peak areas in the frequency spectra were used to calculate the molar percentages of seven organic LMW in the web washes (*N*-acetyltaurine, 4-aminobutyramide (GABamide), glycine, choline, putrescine, glycine betaine, alanine).

Five of the LMW quantitatively studied have previously been reported in aggregate gland secretions of other araneoid species (Fischer & Brander 1960; Tillinghast & Christenson 1984; Vollrath et al. 1990). Identification of alanine resulted from a screening of various amino acids by ^{1}H NMR and was confirmed by analyzing web washes before and after the addition of alanine. Proline, a minor constituent detected in some web washes, was identified in the same way. Putrescine has been previously identified in web washes from the colonial araneid *Metepeira incrassata* via

partial purification and NMR analysis (Townley & Tillinghast pers. obs.).

Data analysis.—^{1}H NMR analysis provided the molar percentage of each of the seven LMW measured quantitatively (not necessarily totalling 100%, as these seven LMW were not the only identified organic LMW in web washes; see Qualitative variation section in Results). LMW composition was determined for no more than 11 individuals under both treatments from each population because of predation and natural mortality, together with failure to spin large enough webs (only webs with longest radius $>$ 10 cm were collected). Laboratory conditions varied among the three populations studied (Table 1) and it is not possible to do a single statistical analysis testing for differences between the field and laboratory collected webs among all populations. Therefore, separate comparisons were made, three testing for an effect of environment (field *vs.* laboratory) within each population and one testing for differences among the field-collected webs of the three populations. The data were analyzed using multiple analysis of variance (MANOVA) with the GLM module of SYSTAT (Wilkinson 1992). Because percentages are not normally distributed, all data were arcsin (squareroot) transformed prior to analysis. Transformed molar percentages of the seven LMW were the dependent variables and either location (field *vs.* laboratory) or population was the independent variable. Similarly, MANOVA was used to compare the LMW composition of webs spun by Chamela spiders in the laboratory in Mexico City with those spun in the laboratory in Austin.

There are indications that juvenile males often build webs that are chemically distinct from webs of females. However, there were too few males from any one site (Los Tuxtlas, 2♂; Chamela, 2♂; Brazos Bend, 3♂) and sex was not included in the analysis as an independent variable.

RESULTS

The chemical composition of the aqueous solution of the adhesive spiral varied among individuals both qualitatively, with differences in which compounds were found, and quantitatively, with differences in the relative amounts of the compounds. Comparisons between field and laboratory web chemistry are based upon analysis of 58 web collections from 29 spiders that spun at least five webs under both field and laboratory conditions. In addition, comparisons between webs spun in Mexico City and Austin laboratories are based upon analysis of webs from eight spiders from Chamela. Below, we present first a description of the qualitative differences found among individuals between treatments and among populations, then a description of the quantitative differences found when seven major organic components of the aqueous solution are considered.

Qualitative variation.—Most of the individuals in all three populations spun webs containing all seven of the organic LMW that we examined quantitatively (*N*-acetyltaurine, GABamide, glycine, choline, putrescine, glycine betaine, alanine). *N*-acetyltaurine, choline and glycine betaine were invariably detected in web washes. Occasionally, one or more of the other four compounds was not detected by ^{1}H NMR (Table 2). Most notably, GABamide, typically a major constituent, was not detected in nine web collections built by six spiders. Putrescine, glycine and alanine each went undetected in at least one web collection. A disproportionately high percentage of such compound-deficient webs were obtained from juvenile males (Table 2).

While the seven measured LMW constitute a large percentage of the organic LMW (we estimate about 80–90% typically), they are not the only organic LMW in the viscid coating of *N. clavipes* adhesive spirals. Two compounds observed in some web washes, taurine and 4-aminobutyric acid (GABA) (Table 2), are presumed precursors of *N*-acetyltaurine and GABamide, respectively. Taurine was present in sizable quantity (9–14 mole %) only in laboratory-collected webs from two male Chamela spiders. These webs were also characterized by relatively low or undetectable levels of GABamide and glycine. Detectable amounts of GABA (2–15 mole %) were observed in 9 web collections, all but one from Brazos Bend, Texas.

A compound indistinguishable by ^{1}H NMR from acetate occurred in several Chamela web washes in the range of 3–17 mole % (Table 2). All of these web washes contained little or no detectable GABamide. Several other web washes of spiders from Chamela and Los Tuxtlas also appeared to contain small amounts of

Table 2.—Qualitative variation in the composition of *Nephila clavipes* web washes examined in this study. The identification numbers of the individual spiders exhibiting a given web composition feature are given in parentheses below non-zero values. [1] Field and laboratory web collections were obtained from 6 females and 3 males from Brazos Bend. Following collection of webs in the laboratory, the spiders were killed en masse by freezing before the sex of each numbered individual was determined. Thus, we do not know the sex of each individual. [2] F = field-collected; LM = laboratory-collected in Mexico City; LA = laboratory-collected in Austin. [3] An unidentified compound producing a singlet at 4.30 ppm in ^{1}H NMR spectra of some web washes. See Qualitative variation section of Results.

Population:	Chamela, Mexico						Los Tuxtlas, Mexico				Brazos Bend, Texas	
Sex:	male			female			male		female		male & female[1]	
Location: [2]	F	LM	LA	F	LM	LA	F	LM	F	LM	F	LA
# of web washes analyzed:	2	2	2	9	6	11	2	2	7	7	9	9
# lacking GABamide	1 (C14)	2 (C10, 14)	1 (C14)	1 (C12)	0	0	2 (T3, 6)	1 (T6)	0	0	0	1 (B15)
# lacking putrescine	0	0	0	0	0	0	2 (T3, 6)	0	1 (T13)	0	0	0
# lacking glycine	0	0	1 (C14)	0	0	0	0	0	0	0	0	0
# lacking alanine	0	0	1 (C14)	0	0	0	0	0	0	0	1 (B15)	0
# with taurine (≥9 mole %)	0	1 (C14)	2 (C10, 14)	0	0	0	0	0	0	0	0	0
# with GABA (≥2 mole %)	0	0	0	1 (C16)	0	0	0	0	0	0	4 (B1, 6, 8, 10)	4 (B, 1, 6, 10, 15)
# with acetate (≥3 mole %)	2 (C10, 14)	1 (C10)	0	1 (C12)	0	0	0	0	0	0	0	0
# with 4.30 ppm compound[3]	0	0	0	0	0	0	0	0	0	0	8 (B1, 3, 4, 5, 6, 8, 10, 11)	1 (B4)

Table 3.—Pearson correlation matrices for each population, including both field and laboratory collected webs. The molar percentage of each compound was arcsin (squareroot) transformed prior to analysis. Abbreviations: gly = glycine; N-tau = N-acetyltaurine; GABam = GABamide; put: putrescine; cho = choline; bet = glycine betaine. Bonferroni-corrected *P*-values: * $P \leq 0.05$, ** $P \leq 0.001$.

Los Tuxtlas

	gly	N-tau	GABam	put	cho	bet
N-acetyltaurine	−0.941**					
GABamide	0.735*	−0.833**				
putrescine	0.508	−0.678*	0.341			
choline	−0.221	0.186	−0.484	0.259		
glycine betaine	−0.528	0.656	−0.712*	−0.490	0.230	
alanine	0.538	−0.512	0.399	0.111	−0.149	0.067

Chamela

N-acetyltaurine	−0.847**					
GABamide	0.451	−0.529				
putrescine	−0.214	0.001	−0.401			
choline	−0.375	0.320	−0.846**	0.496		
glycine betaine	−0.304	0.449	−0.889**	0.338	0.797**	
alanine	0.795**	−0.804**	0.530	−0.024	−0.425	−0.360

Brazos Bend

	gly	N-tau	GABam	GABA	put	cho	bet
N-acetyltaurine	−0.736*						
GABamide	0.133	−0.562					
GABA	0.227	0.070	−0.626				
putrescine	−0.703*	0.724*	−0.597	0.012			
choline	−0.656	0.855**	−0.683*	0.047	0.843**		
glycine betaine	−0.191	0.398	−0.428	0.129	0.058	0.416	
alanine	0.362	−0.444	0.178	−0.249	−0.135	−0.252	−0.495

acetate (< 1 mole %). Proline was detected in web washes from individuals of all three populations. Those web washes containing sufficient proline to allow certain identification were from webs built by females in the laboratory. Proline accounted for no more than 3 mole % of the organic LMW.

Some additional organic LMW have not been identified. Most notable is a compound producing a sometimes prominent singlet (at most, peak area comparable to that of *N*-acetyltaurine's singlet) at 4.30 ppm in ^{1}H NMR spectra, observed in all but one of the field-built web collections from the Brazos Bend population. Again with a single exception, this compound was absent from the laboratory-built web collections from this population and in the one exception, it was present in lower relative quantity than was observed in the field-collected webs. It was not observed at all in the two Mexican populations studied (Table 2).

Quantitative variation.—There were some strong correlations among the seven LMW analyzed in this study (*N*-acetyltaurine, GABamide, glycine, choline, putrescine, glycine betaine and alanine; Table 3). Among all three populations, the amount of *N*-acetyltaurine was negatively correlated with the amount of glycine. There was a tendency for a negative correlation of GABamide with glycine betaine (not significantly for Brazos Bend), choline (not significantly for Los Tuxtlas) and *N*-acetyltaurine (significant only for Los Tuxtlas). Between pairs of chemically similar compounds, the amounts of choline and glycine betaine and the amounts of glycine and alanine tended to be positively associated, although these relationships were significant only for webs spun by Chamela spiders. No significant correlation was found between glycine and glycine betaine. There was a non-significant trend toward a negative correlation of the amount of GABA (common only in webs of spiders from Brazos Bend) with the amount of its derivative, GABamide.

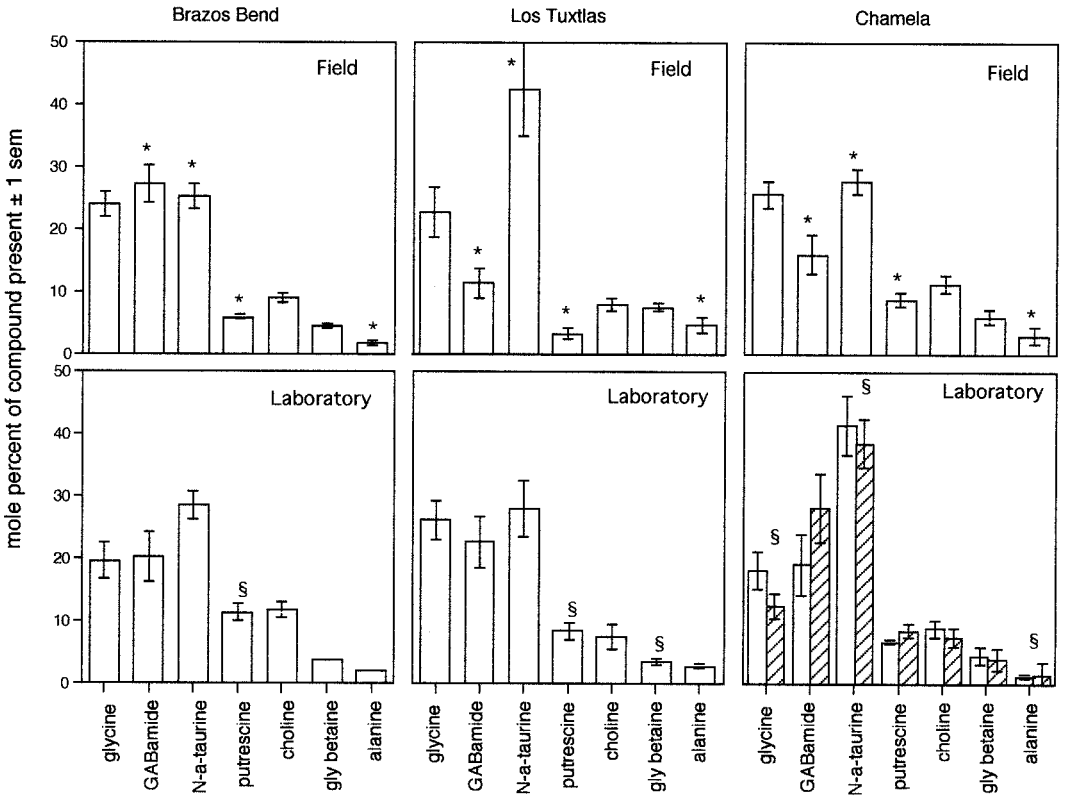

Figure 1.—The average molar percentage of each of the seven studied low-molecular weight organic compounds (± SEM) for each population under field and laboratory conditions. Data from Chamela include observations made in the laboratory in Mexico City (open bars) and in the laboratory in Austin (hatched bars). *N*-a-taurine: *N*-acetyltaurine; gly betaine: glycine betaine. ⋆ = Significant difference ($P \leq 0.05$) among populations; § = significant difference ($P \leq 0.05$) between field and laboratory conditions within a population (Bonferroni -corrected *P* values).

Testing for significant patterns of variation in these components among populations and between field and laboratory conditions involved multivariate analysis of variance of arcsin (square root) transformed relative quantities of the seven primary compounds (mole %; Fig. 1). Separate tests were done to examine patterns of variation among webs from different sites and, within a population, between field- and laboratory-spun webs.

There were significant quantitative differences in LMW composition among webs collected from different field sites (Fig. 1, Table 4). Examination of the univariate F tests for the individual components shows that the molar percentages of *N*-acetyltaurine and alanine were significantly higher and putrescine was lower in webs spun at Los Tuxtlas compared to the other two sites (*N*-acetyltaurine: $F_{(2, 26)} = 4.274$, $P = 0.025$; alanine: $F_{(2, 26)} = 3.898$, $P = 0.033$; putrescine: $F_{(2, 26)} = 9.129$, $P = 0.001$). The webs spun at Brazos Bend had higher relative amounts of GABamide than those from the other two sites ($F_{(2, 26)} = 4.878$, $P = 0.016$). Variation in the molar percentages of choline and glycine betaine was nearly significant ($P \leq 0.06$).

The relative quantities of these components of the LMW solution changed when spiders were moved from the field to the laboratory (Fig. 1). Analyzing the data for each population separately (Table 4), the spiders from Chamela and Los Tuxtlas significantly altered the composition of the LMW, and the spiders from Chamela further altered the web chemistry when they were moved from the laboratory in Mexico City to Austin. The spiders from Brazos Bend showed non-significant

Table 4.—Multiple analysis of variance: Differences among field-spun webs from spiders in three populations, and differences between field- and laboratory-spun webs from three populations. The two entries for Chamela field to laboratory comparisons reflect comparisons between field and the laboratory in Mexico (F/LM) and between field and the laboratory in Austin (F/LA).

	Wilk's lambda	F-statistic	Degrees of freedom	*P* value
Among field-spun webs				
	0.190	3.700	14, 40	0.001
Between field- and laboratory-spun webs				
Brazos Bend	0.335	2.835	7, 10	0.066
Los Tuxtlas	0.130	9.578	7, 10	0.001
Chamela F/LM	0.051	10.73	7, 14	0.018
Chamela F/LA	0.085	21.54	7, 14	<0.001
Between laboratory settings				
Chamela	0.193	4.767	7, 8	0.022

shifts in the relative amounts of the seven compounds.

The changes in LMW composition accompanying the move from field to laboratory differed among the three populations. Spiders from Los Tuxtlas increased putrescine and decreased glycine betaine (putrescine: $F_{(1, 16)} = 10.36$, $P = 0.005$; glycine betaine: $F_{(1, 16)} = 22.58$, $P < 0.001$). The spiders from Chamela decreased free alanine when moved from the field into the laboratory in Mexico City and this change persisted when the spiders were moved to Austin (field *vs.* lab in Mexico: $F_{(1, 10)} = 8.92$, $P = 0.014$; field *vs.* lab in Austin: $F_{(1, 20)} = 10.645$, $P = 0.004$). Comparison of the field webs with the webs spun in Austin also showed a decline in the percentage of glycine and an increase in *N*-acetyltaurine (glycine: $F_{(1,20)} = 19.22$, $P < 0.001$; *N*-acetyltaurine: $F_{(1, 20)} = 10.417$, $P = 0.004$). The significant change in composition between the webs spun by the Chamela spiders in the laboratory in Mexico City and in the laboratory in Austin reflects overall trends; no single component changed significantly. In the case of the Brazos Bend population, although the multivariate statistic was nonsignificant, there was a significant increase in the molar percentage of putrescine when the spiders were moved from the field into the laboratory ($F_{(1, 16)} = 14.705$, $P = 0.001$).

In addition to the statistically significant changes, three trends are of interest because a majority of individuals from Los Tuxtlas or Chamela exhibited them. Relocation of Los Tuxtlas and Chamela females to the laboratory tended to result in decreased percentages of choline (7 of 7 from Los Tuxtlas, 12 of 13 from Chamela) and increased GABamide (7 of 7 field/laboratory comparisons from Los Tuxtlas, 12 of 13 from Chamela). Males from these populations (albeit a small sample size) did not exhibit these trends: among males, choline concentration tended to increase and GABamide tended to decrease with relocation to the laboratory. *N*-acetyltaurine percentages changed in opposite directions in the webs of individuals from these populations: Los Tuxtlas animals, male and female, tended to decrease the percentage of this compound (8 of 9) whereas, as mentioned above, the percentage increased significantly on webs built by male and female Chamela spiders in the laboratory relative to webs built in the field (17 of 17).

^{1}H NMR spectral data.—Data for GABamide, *N*-acetyltaurine, glycine, choline, glycine betaine, and taurine have been published (Townley et al. 1991). The additional LMW identified in this study yielded the following ^{1}H NMR spectral data in D_2O (chemical shifts in ppm, with the methyl hydrogens of the internal standard, 2-methyl-2-propanol, assigned a chemical shift of δ1.2200): acetate, singlet at δ1.88; alanine, quartet at δ3.75 (J = 7.3 Hz), doublet at δ1.45 (J = 7.3 Hz); GABA, triplets at δ2.99 (J = 7.5 Hz), δ2.27 (J = 7.3 Hz), quintet at δ1.87 (J = 7.4 Hz);

proline, multiplets at δ4.10, δ3.35 (δ3.40, δ3.30), δ2.31, δ2.01; putrescine, multiplets at δ3.02, δ1.73.

DISCUSSION

The current study extends previous reports on the chemical composition of the organic LMW solution found on ecribellate adhesive spirals by documenting variation in web chemistry within and among populations. Furthermore, we observed significant quantitative shifts in LMW composition correlated with changes in environment: the spiders from the two Mexican populations significantly altered relative amounts of some LMW on their webs when moved from the field into the laboratory. Examination of webs spun by individuals also revealed patterns of individual qualitative variation in the composition of the LMW solution. Some spiders, particularly juvenile males, spun webs in which compounds characteristic of *N. clavipes* webs were undetected and/or novel compounds were found. Following a discussion of the extrinsic and intrinsic factors that may singly or in combination result in spiders spinning webs with different LMW composition, we discuss the possible influence of LMW composition on web function.

There are four possible factors that may influence the chemistry of the LMW portion of the web: physical environment, diet, web recycling, and ontogenetic changes in web chemistry. First, if physical properties of the adhesive spiral (e.g., hygroscopicity, droplet viscosity, extensibility) are influenced by LMW composition, it seems unlikely that a single LMW composition would prove ideal in all environments inhabited by individuals of one species. Thus, there is the possibility that among-population differences in LMW composition and the shift in composition when individuals are moved from one environment to another may reflect individual spiders' adjustments to the conditions of the physical environment. Among-population differences may also reflect genetic differences among populations, as selection favors different chemical and physical properties in different physical environments. Second is the possibility that qualitative differences in diet affected LMW composition as spiders were shifted from the field to the laboratory. These spiders eat a variety of prey in the field (Higgins & Buskirk 1992), but were kept on a monotypic diet in the laboratory. As prey types vary among these three populations in the field (Higgins pers. obs.), qualitative dietary differences may contribute to among-population differences as well. Qualitative changes in diet have been found to alter amino acid composition of spider major ampullate silk (Craig et al. in press). Third, we now have evidence that web recycling influences LMW composition (Townley & Tillinghast pers. obs.) and recycling was an uncontrolled variable between the field and laboratory portions of the study. Spiders were collected from intact orb webs and the first web built in the field portion of the study, also the first web collected, presumably included little recycled material. In contrast, the spiders recycled the orb prior to collection of the first web in the laboratory. Last, there is the possibility of ontogenetic changes in LMW composition, independent of diet and environmental conditions. Ontogenetic changes in structural features of orb webs (e.g., number of radii, mesh size, shape) have been documented (Witt et al. 1972; Ramousse 1973; Eberhard 1985 and references therein; Eberhard 1986; Edmunds 1993) and it is possible that changes during development may extend to facets of web composition as well. Indeed, Osaki (1989) has reported changes in the color of major ampullate silk, presumably due to changes in chemical composition, with the approach of maturity in female *Nephila clavata*.

Differences in LMW composition could affect various physical properties of the adhesive spiral and, thereby, affect the web's prey-catching ability. Therefore, the possible functional consequences of qualitative and quantitative differences in adhesive spiral composition merit further examination. For example, some of the LMW are hygroscopic (Vollrath et al. 1990; Townley et al. 1991) and the overall hygroscopicity of the LMW mixture presumably varies with LMW composition. Differences in hygroscopicity may have an impact on web function because adsorption and retention of water by the adhesive spiral is essential to its adhesiveness, elasticity, and extensibility. Water's involvement in adhesive spiral functioning may be a combination of direct effects, due to interactions between water and adhesive spiral components, and indirect effects, due to interactions between

LMW and adhesive spiral macromolecules that require an aqueous medium (Richter 1956; Schildknecht et al. 1972; Vollrath & Edmonds 1989; Bonthrone et al. 1992; Edmonds & Vollrath 1992; Gosline et al. 1994, 1995; Hayashi & Lewis 1998).

Beyond the possible consequences for adhesive spiral hygroscopicity, LMW compositional differences may also influence the effectiveness of the adhesive spiral by affecting its macromolecular structure more directly. Here we briefly discuss three hypothetical ways in which the organic LMW may accomplish this: as compatible solutes, through direct interaction with macromolecules, and as counteracting solute systems.

A wide variety of procaryotic and eucaryotic cells subject to osmotic stress employ certain organic osmolytes to adjust intracellular osmolarity. These osmolytes are sometimes referred to as compatible solutes (Brown & Simpson 1972) because, unlike inorganic ions in most organisms, they can occur at high concentrations without perturbing, and even while stabilizing, protein structure (Yancey et al. 1982; Le Rudulier et al. 1984; Somero 1986, 1992; Csonka & Hanson 1991; Kinne 1993; Galinski 1993, 1995). Protein stabilization by compatible solutes has been attributed to the tendency of these solutes to be excluded from the immediate vicinity of protein surfaces (so increasing the non-uniform distribution of solute) and to exhibit low specific binding to proteins (Arakawa & Timasheff 1985; Timasheff 1992). Thus, these solutes promote processes of protein folding and subunit aggregation that minimize protein surface area and typically favor protein stability. Although the web is external, certain of the adhesive spiral's organic LMW (glycine, glycine betaine, alanine, proline, taurine) are identical to known compatible solutes (Townley et al. 1991). Thus, these compounds may, by the same mechanism, help stabilize the native conformation of adhesive spiral proteins.

One important distinction between compatible solutes and some of the LMW compounds on the adhesive spiral concerns molecular charge. Compatible solutes are usually uncharged or net neutral molecules, whereas several of the adhesive spiral LMW components (*N*-acetyltaurine, GABamide, choline, putrescine) carry a net charge and might be expected to show a greater tendency to interact with proteins. Such direct interactions between organic LMW and other components of the spiral strand, e.g., the adhesive glycoprotein(s) or core fiber proteins, may be vital for the proper functioning of these adhesive spiral strand macromolecules (Townley et al. 1991; Gosline et al. 1995).

The combination of solutes in the aqueous solution on the web's adhesive spiral may also function as a counteracting solute system, wherein the perturbing influence to native macromolecular structure by one or more destabilizing solutes is offset by the presence of other, stabilizing, i.e., compatible, solutes (Somero 1986; Timasheff 1992). The best studied example of such a system is the urea (destabilizer)/methylamine (stabilizer) system of marine cartilaginous fish, the coelacanth, and mammalian kidneys (Yancey et al. 1982; Somero 1986, 1992; Garcia-Perez & Burg 1991; Yancey 1992). On the web, the perturbing influence of inorganic ions and/or one or more of the organic LMW (especially net charged organic LMW) could be countered by other, stabilizing LMW. Optimal performance of adhesive spiral macromolecules in such a solute system may depend on the various LMW occurring at fairly specific concentration ratios to one another (Yancey et al. 1982; Somero 1986, 1992; Yancey 1992).

In all three of these chemical models, differences in LMW composition, such as those documented herein, may directly translate into differences in macromolecular structure, with consequences for the adhesive spiral's trapping ability. However, we must emphasize that at this time the ability of the web's organic LMW to affect macromolecular structure by any of the aforementioned mechanisms is speculative. Whether the observed differences in web chemistry reflect adaptive responses to the environment or simply nonadaptive plasticity (Via 1993), these changes in LMW composition could be important both for web function and for physiological function. Precursors or derivatives of the organic LMW, if not the LMW themselves, play important physiological roles (e.g., as neurotransmitters and in cell membrane phospholipids). However, orb-weaving spiders must invest LMW and other essential and non-essential compounds in the synthesis of the orb web because they are completely dependent upon the web for capturing prey. Although

web recycling allows the spider to recoup a portion of this investment (Breed et al. 1964; Peakall 1971; Townley & Tillinghast 1988), some loss of material is inevitable. Thus, with each web-building event, allocation ''decisions'' must be made; and there is experimental evidence for trade-offs in allocation of limited resources between foraging (the orb) and physiological demands (Higgins & Rankin 1999). Because orb-weaving spiders depend entirely on the web for capturing prey and because the synthesis of the orb web requires an investment of physiologically important compounds, this group of spiders could become a model system for investigating resource allocation (Benforado and Kistler 1973; Higgins 1990, 1992, 1995; Higgins & Buskirk 1992; Sherman 1994, Blackledge 1998; Herberstein et al. 2000).

The full realization of this potential will be facilitated by further investigation of orb web synthesis, recycling, and composition, particularly of the adhesive spiral, which, even neglecting water content, often makes a considerably greater contribution to web weight than the non-adhesive web elements.

ACKNOWLEDGMENTS

The Instituto de Ecología, UNAM, provided logistical support and laboratory space for the portion of this study done in Mexico. Field work at Chamela and Los Tuxtlas was supported by the staff of the Instituto de Biología, UNAM, and field work in Texas was done by Jerry Drummond. Collecting permits were granted by the National Institute of Ecology in Mexico and Texas Parks and Wildlife. Drs. Craig Hieber and George Uetz kindly provided large samples of *Metepeira incrassata* orb webs from which putrescine was first identified. We are sincerely grateful to all these people and organizations. This work was supported by NSF grant #IBN-922094 to MAR and LEH and USDA HATCH grant #370 to EKT.

LITERATURE CITED

Arakawa, T. & S.N. Timasheff. 1985. The stabilization of proteins by osmolytes. Biophysical Journal 47:411–414.

Benforado, J. & K.H. Kistler. 1973. Growth of the orb weaver, *Araneus diadematus*, and correlation with web measurements. Psyche 80:90–100.

Blackledge, T.A. 1998. Stabilimentum variation and foraging success in *Argiope aurantia* and *Argiope trifasciata* (Araneae: Araneidae). Journal of Zoology, London 246:21–27.

Bonthrone, K.M., F. Vollrath, B.K. Hunter & J.K.M. Sanders. 1992. The elasticity of spiders' webs is due to water-induced mobility at a molecular level. Proceedings of the Royal Society of London, B, Biological Sciences 248:141–144.

Breed, A.L., V.D. Levine, D.B. Peakall & P.N. Witt. 1964. The fate of the intact orb web of the spider *Araneus diadematus* Cl. Behaviour 23:43–60.

Brown, A.D. & J.R. Simpson. 1972. Water relations of sugar-tolerant yeasts: The role of intracellular polyols. Journal of General Microbiology 72:589–591.

Craig, C.L., C. Riekel, M.E. Herberstein, R.S. Weber, D. Kaplan & N.E. Pierce. In press. Evidence for diet effects on the composition of silk proteins produced by spiders. Molecular Biology and Evolution.

Csonka, L.N. & A.D. Hanson. 1991. Prokaryotic osmoregulation: Genetics and physiology. Annual Review of Microbiology 45:569–606.

Dadd, R.H. 1985. Nutrition: Organisms. Pp. 313–390. *In* Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 4, Regulation: Digestion, Nutrition, Excretion. (G.A. Kerkut & L.I. Gilbert, eds.). Pergamon Press, Oxford, UK.

Dreesbach, K., G. Uhlenbruck & E.K. Tillinghast. 1983. Carbohydrates of the trypsin soluble fraction of the orb web of *Argiope trifasciata.* Insect Biochemistry 13:627–631.

Eberhard, W.G. 1985. The ''sawtoothed'' orb web of *Eustala* sp. (Araneae, Araneidae) with a discussion of ontogenetic changes in spiders' web-building behavior. Psyche 92:105–117.

Eberhard, W.G. 1986. Ontogenetic changes in the web of *Epeirotypus* sp. (Araneae, Theridiosomatidae). Journal of Arachnology 14:125–128.

Edmonds, D.T. & F. Vollrath. 1992. The contribution of atmospheric water vapour to the formation and efficiency of a spider's capture web. Proceedings of the Royal Society of London, B, Biological Sciences 248:145–148.

Edmunds, J. 1993. The development of the asymmetrical web of *Nephilengys cruentata* (Fabricius). Memoirs of the Queensland Museum 33: 503–506.

Fischer, F.G. & J. Brander. 1960. Eine Analyse der Gespinste der Kreuzspinne. Hoppe-Seyler's Zeitsschrift fuer Physiologische Chemie 320:92–102.

Galinski, E.A. 1993. Compatible solutes of halophilic eubacteria: Molecular principles, water-solute interaction, stress protection. Experientia 49:487–496.

Galinski, E.A. 1995. Osmoadaptation in bacteria. Advances in Microbial Physiology 37:273–328.

Garcia, E. 1973. Modificaciones al Systema de Classificación Climatica de Köppen. Universidad

Nacional Autonoma de Mexico Press, Mexico City.

Garcia-Perez, A. & M.B. Burg. 1991. Renal medullary organic osmolytes. Physiological Reviews 71:1081–1115.

Gosline, J., C. Nichols, P. Guerette, A. Cheng & S. Katz. 1995. The macromolecular design of spiders' silks. Pp. 237-261, *In* Biomimetics: Design and Processing of Materials. (M. Sarikaya & I.A. Aksay, eds.). American Institute of Physics Press, Woodbury, New York.

Gosline, J.M., C.C. Pollak, P.A. Guerette, A. Cheng, M.E. DeMont & M.W. Denny. 1994. Elastomeric network models for the frame and viscid silks from the orb web of the spider *Araneus diadematus*. Pp. 328–341. *In* Silk Polymers. Materials Science and Biotechnology. (D. Kaplan, W.W. Adams, B. Farmer, C. Viney, eds.). American Chemical Society, Washington, D.C.

Hayashi, C.Y. & R.V. Lewis. 1998. Evidence from flagelliform silk cDNA for the structural basis of elasticity and modular nature of spider silks. Journal of Molecular Biology 275:773–784.

Herberstein, M.E., C.L. Craig & M.A. Elgar. 2000. Foraging strategies and feeding regimes: Web decoration investment in *Argiope keyserlingi* Karsh (Araneae: Araneidae). Evolutionary Ecology Research 2:69–80.

Higgins, L.E. 1990. Variation in foraging investment during the intermolt interval and before egg-laying in the spider *Nephila clavipes* (Araneae: Araneidae). Journal of Insect Behavior 3: 773–783.

Higgins, L.E. 1992. Developmental changes in barrier web structure under different levels of predation risk in *Nephila clavipes* (Araneae: Tetragnathidae). Journal of Insect Behavior 5:635–655.

Higgins, L.E. 1995. Direct evidence for trade-offs between foraging and growth in a juvenile spider. Journal of Arachnology 23:37–43.

Higgins, L.E. & R.E. Buskirk. 1992. A trap-building predator exhibits different tactics for different aspects of foraging behaviour. Animal Behaviour 44:485–499.

Higgins, L.E. & M.A. Rankin. 1999. Nutritional requirements for web synthesis in the tetragnathid spider *Nephila clavipes*. Physiological Entomology 24:263–270.

Kinne, R.K.H. 1993. The role of organic osmolytes in osmoregulation: From bacteria to mammals. Journal of Experimental Zoology 265:346–355.

Le Rudulier, D., A.R. Strom, A.M. Dandekar, L.T. Smith & R.C. Valentine. 1984. Molecular biology of osmoregulation. Science 224:1064–1068.

Osaki, S. 1989. Seasonal change in color of spiders' silk. Acta Arachnologia 38:21–28.

Peakall, D.B. 1971. Conservation of web proteins in the spider, *Araneus diadematus*. Journal of Experimental Zoology 176:257–264.

Peters, H.M. 1955. Über den Spinnapparat von *Nephila madagascariensis* (Radnetzspinnen, Fam. Argiopidae). Zeitschrift fuer Naturforschung 10b:395–404.

Peters, H.M. 1995. Ultrastructure of orb spiders' gluey capture threads. Naturwissenschaften 82: 380–382.

Ramousse, R. 1973. Body, web-building and feeding characteristics of males of the spider *Araneus diadematus* (Araneae: Araneidae). Psyche 80:22–47.

Richter, G. 1956. Untersuchungen über Struktur und Funktion der Klebefäden in den Fanggeweben ecribellater Radnetzspinnen. Naturwissenschaften 43:23.

Schildknecht, H., P. Kunzelmann, D. Krauß & C. Kuhn. 1972. Über die Chemie der Spinnwebe, I: Arthropodenabwehrstoffe, LVII. Naturwissenschaften 59:98–99.

Schulz, S. 1997. The chemistry of spider toxins and spider silk. Angewandte Chemie International Edition in English 36:314–326.

Sekiguchi, K. 1952. On a new spinning gland found in geometric spiders and its functions. Annotationes Zoologicae Japonenses 25:394–399.

Sherman, P.M. 1994. The orb-web: An energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. Animal Behaviour 48:19–34.

Somero, G.N. 1986. Protons, osmolytes, and fitness of internal milieu for protein function. American Journal of Physiology 251:R197–R213.

Somero, G.N. 1992. Adapting to water stress: Convergence on common solutions. Pp. 3–18. *In* Water and Life: Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels. (G.N. Somero, C.B. Osmond & C.L. Bolis, eds.). Springer-Verlag, Berlin.

Tillinghast, E.K. 1981. Selective removal of glycoproteins from the adhesive spiral of the spiders orb web. Naturwissenschaften 68:526–527.

Tillinghast, E.K. 1984. The chemical fractionation of the orb web of *Argiope* spiders. Insect Biochemistry 14:115–120.

Tillinghast, E.K. & T. Christenson. 1984. Observations on the chemical composition of the web of *Nephila clavipes* (Araneae, Araneidae). Journal of Arachnology 12:69–74.

Tillinghast, E.K., M.A. Townley, T.N. Wight, G. Uhlenbruck & E. Janssen. 1993. The adhesive glycoprotein of the orb web of *Argiope aurantia* (Araneae, Araneidae). Pp. 9–23. *In* Biomolecular Materials. (C. Viney, S.T. Case & J.H. Waite, eds.). Materials Research Society Symposium Proceedings, Vol. 292. Materials Research Society, Pittsburgh, Pennsylvania.

Tillinghast, E.K. & M.A. Townley. 1994. Silk glands of araneid spiders: Selected morphological and physiological aspects. Pp. 29–44. *In* Silk Polymers: Materials Science and Biotechnology. (D. Kaplan, W.W. Adams, B. Farmer & C. Viney, eds.). American Chemistry Society Symposium, Series 544. American Chemical Society, Washington, D. C.

Timasheff, S.N. 1992. A physicochemical basis for the selection of osmolytes by nature. Pp. 70–84. *In* Water and Life: Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels. (G.N. Somero, C.B. Osmond & C.L. Bolis, eds.). Springer-Verlag, Berlin.

Townley, M.A. & E.K. Tillinghast. 1988. Orb web recycling in *Araneus cavaticus* (Araneae, Araneidae) with an emphasis on the adhesive spiral component, GABamide. Journal of Arachnology 16:303–319.

Townley, M.A., D.T. Bernstein, K.S. Gallagher & E.K. Tillinghast. 1991. Comparative study of orb web hygroscopicity and adhesive spiral composition in three araneid spiders. Journal of Experimental Zoology 259:154–165.

Via, S. 1993. Adaptive phenotypic plasticity: Target or by-product of selection in a variable environment? American Naturalist 142:352–365.

Vollrath, F. & D.T. Edmonds. 1989. Modulation of the mechanical properties of spider silk by coating with water. Nature 340:305–307.

Vollrath, F., W.J. Fairbrother, R.J.P. Williams, E.K. Tillinghast, D.T. Bernstein, K.S. Gallagher & M.A. Townley. 1990. Compounds in the droplets of the orb spider's viscid spiral. Nature 345: 526–528.

Vollrath, F. & E.K. Tillinghast. 1991. Glycoprotein glue beneath a spider web's aqueous coat. Naturwissenschaften 78:557–559.

Wilkinson, L. 1992. SYSTAT: Statistics, Version 5.2 Edition. Systat, Inc., Evanston, Illinois.

Witt, P.N., J.O. Rawlings & C.F. Reed. 1972. Ontogeny of web-building behavior in two orb-weaving spiders. American Zoologist 12:445–454.

Yancey, P.H. 1992. Compatible and counteracting aspects of organic osmolytes in mammalian kidney cells in vivo and in vitro. Pp. 19–32. *In* Water and Life: Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels. (G.N. Somero, C.B. Osmond & C.L. Bolis, eds.). Springer-Verlag, Berlin.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus & G.N. Somero. 1982. Living with water stress: Evolution of osmolyte systems. Science 217: 1214–1222.

Manuscript received 13 May 2000, revised 13 November 2000.