The Unique Ribbon Morphology of the Major Ampullate Silk of Spiders from the Genus *Loxosceles* (Recluse Spiders)

Jonathan A. Coddington,[†] Henri D. Chanzy,[‡,§] Catheryn L. Jackson,[*,‡] Gail Raty,[||] and KennCorwin H. Gardner[||]

Department of Entomology, National Museum Natural History, Smithsonian Institution, Washington, D.C. 20560, National Institute of Standards and Technology (NIST), 100 Bureau Drive, Stop 8541, Gaithersburg, Maryland 20899, Centre de Recherches sur les Macromolécules Végétales, CNRS-CERMAV, affiliated with the Joseph Fourier University of Grenoble, BP 53, 38041 Grenoble Cedex 9 France, and DuPont Central Research & Development Department, Experimental Station, P.O. Box 80302, Wilmington, Delaware 19880-0302

Received June 25, 2001; Revised Manuscript Received October 29, 2001

The morphology of silk produced by recluse spiders (*Loxosceles arizonica*) was investigated by scanning electron microscopy, atomic force microscopy, and transmission electron microscopy. This silk consisted entirely of very long, thin ribbons of width 2−4 μm and thicknesses of no more than 40 nm. The correspondence in shape and dimension between the silk ribbons and the elongated aperture of the major ampullate spigot indicated that these ribbons were major ampullate silk. Selected area electron diffraction patterns from single ribbons were indexed with an orthorhombic unit cell (a = 9.43(2) Å, b = 8.96(3) Å, c = 6.96(1) Å). This unit cell is in good agreement with that previously reported for synthetic poly(L-alanylglycine). Thus it is likely that the crystalline regions of the major ampullate silk of *L. arizonica* consist of an alternating glycine−L-alanine motif that has adopted a β-sheet structure. The amino acid composition achieved with the silk of *L. arizonica* as well as that of *L. laeta* confirmed that the major amino acid constituents of this silk were glycine and L-alanine in nearly equal amounts. As it was noticed that the dry ribbons were highly electrostatic, it is suggested that the electrostatic interaction plays an important role in prey capture for *Loxoseles*.

Introduction

In recent years the structure and properties of spider silks[1−6] have attracted much scientific attention because of their outstanding mechanical properties.[7] Nearly all of this work has been focused on the silks of a single lineage of orb-weaving spiders, the Araneoidea, and especially on the tetragnathid genus *Nephila*[8] and common araneid orbweaving genera such as *Araneus*.[9] Araneoid silk biology is fairly uniform in comparison with the great mechanical and structural diversity of spider silks in general. Confirming the work of experimentalists of the 1960s, the ordered component in the major ampullate silk of *Nephila* and *Araneus* consists of runs of the amino acid alanine.[10]

In contrast with the wealth of data on the Araneoid silk, little attention has been given to the silk of non-orb-weaving spiders. Although the sicariid genus *Loxosceles* is infamous because of their extraordinarily poisonous bites, very little is known of their unusual silk morphology and silk-producing apparatus.[11] In this report we discuss observations on the web of *Loxosceles arizonica* Gertsch & Mulaik (Arizona recluse),[12] 1 of the 12 *Loxosceles* species native or naturalized in the United States. Some data are also presented on the silk of *L. laeta* Nivolet, the Chilean recluse.

Experimental Section

Spiders and Their Silk. A female of *L. arizonica* was kept in a terrarium with lateral dimensions 10 × 15 cm. After 102 days, the web spanned the entire terrarium. Community webs, i.e., a web built by a number of spiders kept in the same container, for *L. arizonica* were also examined and used for the determination of amino acid composition. A community web of *L. laeta* was also collected for the amino acid composition of the corresponding silk.

Microscopy. Scanning electron microscopy (SEM) specimens were prepared by placing a SEM stub with a double sticky carbon tab in place in contact with the top surface of the web of *L. arizonica*. The samples were sputtered with gold/palladium and examined with a Hitachi S-800 field emission (FE-SEM) instrument[13] operated in secondary electron mode. For atomic force microscopy (AFM) and transmission electron microscopy (TEM) analysis, *L. arizonica* silk ribbons were captured by passing a glass slide above a community web. The silk ribbons, which adhered strongly to the glass, were observed with their glass substrate

* To whom correspondence should be addressed. E-mail: catheryn.jackson@att.net. Present address: 1731 Supplee Rd., Lansdale, PA 19446.

[†] Smithsonian Institution.

[‡] National Institute of Standards and Technology.

[§] Centre de Recherches sur les Macromolécules Végétales, CNRS-CERMAV, affiliated with the Joseph Fourier University of Grenoble.

[||] DuPont Central Research & Development Department.

10.1021/bm010108m CCC: $22.00
Published on Web 00/00/0000

Table 1. Approximate Amino Acid Composition of the Web Silks of Mature Female *Loxosceles arizonica* and *L. laeta*

	L. arizonica	*L. laeta*
CYS-acid	*a*	*a*
CMCYS	*a*	*a*
ASX	4.6	2.9
THR	2.1	1.6
SER	4.7	6.1
GLX	6.5	5.0
PRO	1.7	1.2
GLY	30.2	30.0
ALA	32.6	40.5
VAL	2.4	1.6
MET	0.5	0.2
ILEU	1.8	1.3
LEU	3.7	3.2
TYR	2.8	2.4
PHE	2.2	1.8
HIS	1.4	0.7
TRP	*a*	*a*
LYS	1.2	1.7
ARG	1.6	1.7

[a] Not determined.

in a noncontact mode, using a Topometrix Accurex/Explorer AFM instrument.[13] Typical AFM images were recorded on 10 μm^2.

For TEM, the silk ribbons were floated off on water after briefly immersing the glass slide in very dilute HF.[14] The ribbons were then mounted on carbon-coated 3 mm electron microscopy grids. TEM was performed with a Philips 400T. TEM samples for imaging were either shadowed with tungsten/tantalum (W/Ta) and observed under normal exposure conditions at 80 kV or unshadowed and examined under low dose illumination conditions at 120 kV. The electron diffraction experiments were achieved at 120 kV under low dose conditions. Some electron diffraction patterns were recorded without rotation and others were recorded after rotating by $\pm 60°$ about the silk ribbon axes. Electron diffraction patterns were recorded both at room temperature and at liquid nitrogen temperature, employing a cryo sample holder.

Amino Acid Composition. The molar ratio of the amino acids was determined from samples of community webs using a standard method.[15] The analysis was done in triplicate and the mean values are reported in Table 1.

Results and Discussion

An adult female *L. arizonica* is shown in Figure 1A. This spider is typical of the genus, with the characteristic six eyes and violin-shaped mark on the cephalothorax. Spiders of the genus *Loxosceles* are slow web spinners and spin only a few centimeters of silk in a 24-h period. Figure 1B shows the female *L. arizonica* during the silk spinning process. The web spun by this spider over a period of 102 days is shown in Figure 1C. The web is highly electrostatic and consists of a disordered sheet containing "clumps" of silk dispersed on a network of what appears to be a fibrous component.

An SEM of a "clump" region, Figure 2A, shows that the web consists of disorganized clumps of silk ribbons sitting

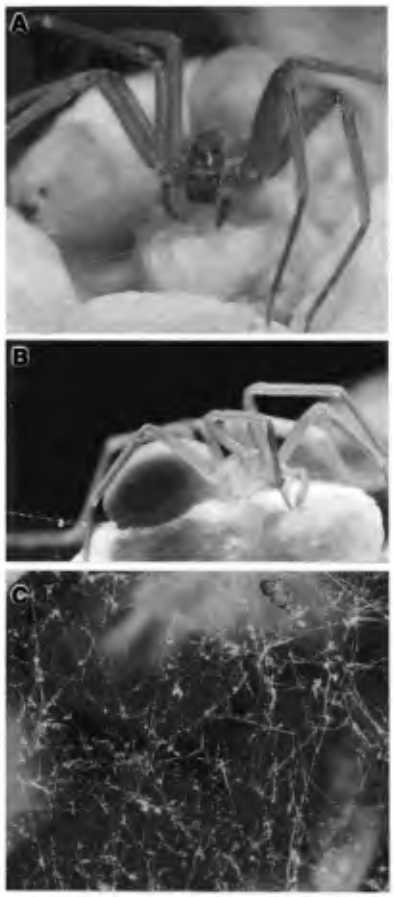

Figure 1. Recluse spider and web: (A) Adult female *Loxosceles arizonica*. (B) *L. arizonica* spinning major ampullate lines. Note clumped appearance on lines. (C) Dorsal view of typical web, consisting of clumps of major ampullate silk on a meshlike substrate of major ampullate silk.

on top of, or entangled with, a network of extended ribbons, not fibers. This ribbon morphology is, as far as we know, unique among spiders and, indeed, among silk-producing organisms. All of the ribbons in the web spun by this adult female have the same width, suggesting that the web consists entirely of this single type of silk ribbon structure. An enlargement of two crossing ribbons is shown in Figure 2B. The ribbons have a width of approximately 4.2 μm and show a tendency, especially when put under tension, to roll into a cylinder. The origin of the unusual ribbonlike silk strands is apparent when the single major ampullate spigot (anterior spinneret) for *L. arizonica* is considered (Figure 2C, apical view and Figure 2D, lateral view). The spigot has a transversely ridged base and a longitudinally ridged top. The longitudinally ridged region tapers from a circular cross section to an elongated cross section.[11] The spigot aperture is a slit approximately 4.2 μm long and has a width of approximately 150 nm. The slit length roughly corresponds to the width of the silk ribbons within the web. This correspondence demonstrates that the web consists entirely of major ampullate silk. The spigot geometry and ribbon morphology of the silk produced by the *Loxosceles* species *arizonica* is unique. For other spiders, including orb-weaving spiders, the major ampullate spigots have approximately circular apertures resulting in cylindrical fibers of much larger dimensions.

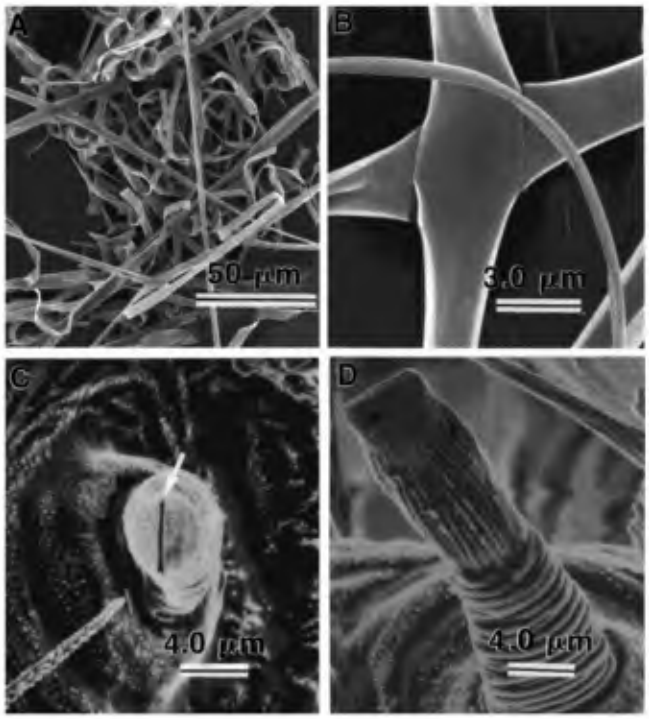

Figure 2. SEM of web and spigot morphology: (A) Silk "clump" from web in Figure 1C showing network of extended ribbons. (B) Higher magnification SEM showing two silk ribbons crossing and the tendency for ribbons to roll into cylinders. (C) Apical view of major ampullate spigot, showing slit-shaped aperture surmounting the round spigot tip. (D) Lateral view of same spigot showing basal transversely rigid section, medial longitudinally ridged section, and a terminally thickened and laterally flattened section of spigot shaft.

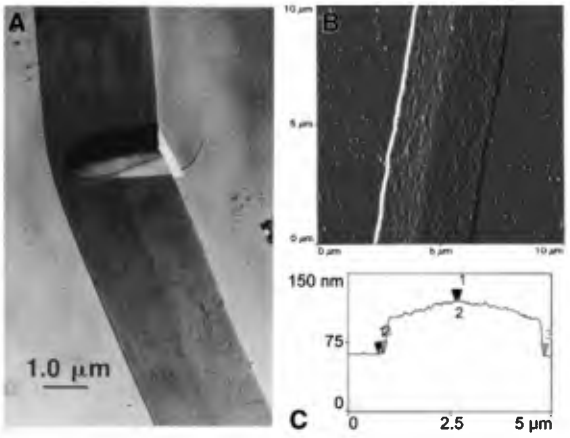

Figure 3. Morphology of individual *L. arizonica* silk ribbons from a community web: (A) Bright-field TEM of individual ribbon on carbon substrate shadowed with W/Ta, 2.5 μm wide. (B) Noncontact AFM image of ribbon on glass substrate, uncoated. (C) Line scan of AFM image in Figure 3B to measure approximate ribbon thickness (40–50 nm); see text.

The detailed morphology of major ampullate silk taken from a community web is shown in Figure 3, in images obtained by TEM and AFM. In the community web, different ribbon widths were observed in the range of 2–4 μm; this is probably due to the variation in the size and maturity of the spiders in the community. The TEM of a shadowed ribbon (Figure 3A) shows that the ribbon is extremely thin, has a constant width of 2.5 μm, and has a fibrous texture parallel to the ribbon axis. The AFM image (Figure 3B, top) also shows a uniform ribbon morphology. This ribbon has a

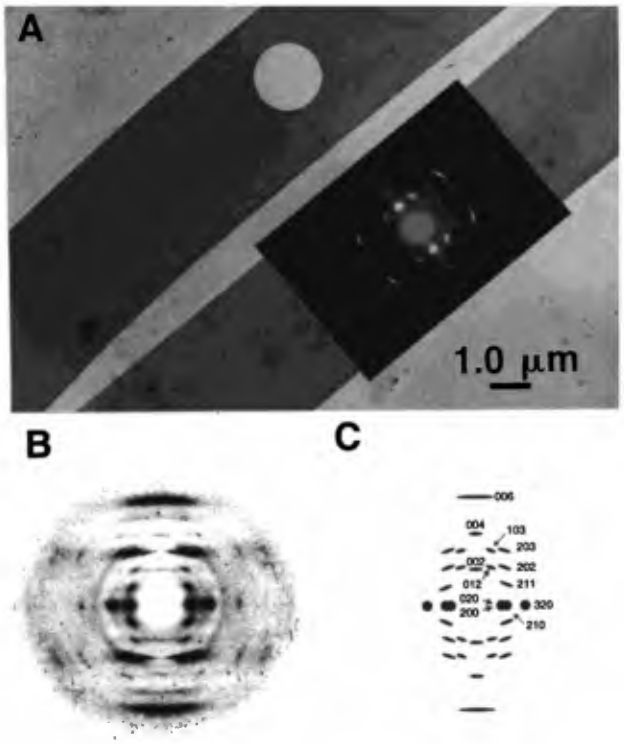

Figure 4. Low-dose TEM and electron diffraction of silk ribbon recorded at liquid nitrogen temperature: (A) Electron micrograph of two individual unstained and unshadowed ribbons of *L. arizonica* of width 4 μm. Insert: electron diffraction pattern recorded on the highlighted area of the upper ribbon. (B) As in panel A but after digitization, computer enhancement, and background removal. (C) Schematic diagram showing the indexation of the prominent reflections.

width of 4.2 μm, as measured in the line scan shown in the bottom of Figure 3C by the set of arrows labeled 3. The ribbon appears to have a tented structure and a measurement of the ribbon thickness varies from 39 nm at the ribbon edge to about 51 nm at the center ridge. The thickness of the ribbon at the center was measured by the sets of arrows labeled 1 and 2 in the line scan.

The ultrathin silk ribbons are electron beam transparent and electron diffraction allowed determination of the unit cell parameters and crystal morphology. A low-dose TEM image, recorded at liquid nitrogen temperature, of two major ampullate silk ribbons with electron diffraction patterns is shown in Figure 4. The ribbons were taken from the community web and are observed to be 4 μm wide, as shown in Figure 4A. Inserted in this figure is the selected area electron diffraction pattern recorded on the highlighted area of the upper ribbon. The low-temperature diagram is essentially the same but better resolved than similar diagrams recorded at room temperature, which shows fewer high-angle reflections and more inelastic scattering. Figure 4B corresponds to the diagram in Figure 4A, but after computer enhancement and background removal. The positions of 14 nonmeridional reflections were measured from the low-temperature diffraction pattern and indexed by a four-chain orthorhombic unit cell with dimensions $a = 9.43(2)$ Å, $b = 8.96(3)$ Å, and $c = 6.96(1)$ Å. A schematic diffraction pattern showing the prominent reflections is shown in Figure 4C. This unit cell is in good agreement with that reported for

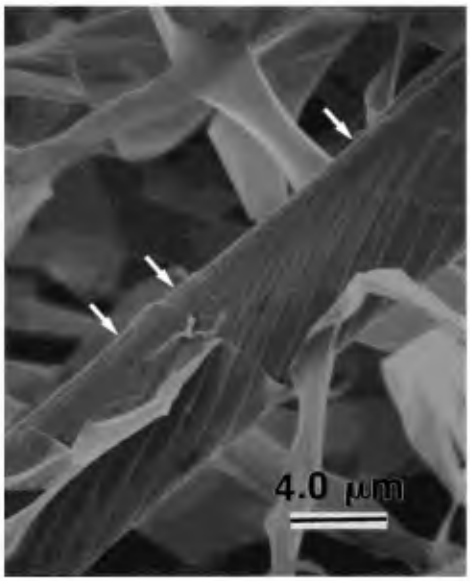

Figure 5. Major ampullate ribbons tightly wrapped around a cricket antenna, in a manner consistent with the proposed electrostatic capture mechanism. Silk ribbons are indicated by white arrows.

synthetic poly(L-alanylglycine) (a = 9.44 Å, b = 8.96 Å, and c = 6.94 Å)[16] and indicates that the crystalline regions of the major ampullate silk consist of an alternating glycine–alanine motif that has adopted a β-sheet structure. The molecular axis of the β-sheet lies parallel to the long axis of the silk ribbon. As the electron diffraction patterns did not present any modification during rotation about the silk ribbon axis, there was no direct evidence for preferred orientation of the hydrogen-bonded β-sheets with respect to the ribbon plane.

The web of *L. laeta* was also examined and found to consist exclusively of similar silk ribbons. The amino acid composition determined from community web samples (presumably major ampullate silk) of both *L. arizonica* and *L. laeta* is summarized in Table 1. Because of the small sample size, the values can be considered only approximate. Consistent with our unit cell determination, glycine and alanine are the two majority amino acid components. These silks lack the high concentration of glutamic acid found in the major ampullate silks of orb-weaving spiders. To the best of our knowledge, this is the first published report of the amino acid composition for spiders of the genus *Loxosceles*.

Spiders of the genus *Loxosceles* lack those silks known to be crucial to silk-mediated prey capture, i.e., aggregate or cribellate silk,[17] even though the web has been reported to be quite sticky.[18] The web of *Loxosceles* may alert the spider via vibrations to the presence of prey, but it is unclear whether or how the web may also function to retain prey. As noted above, the *Loxosceles* web is highly electrostatic. Parts of the web, presumably the ribbons contained in the disordered clusters, were observed to move toward and adhere to objects, e.g., tweezers, glass slide or a finger, brought near the web. In nature this electrostatic interaction between the web and prey may facilitate prey capture for *Loxosceles,* particularly in freshly built or maintained webs. In Figure 5, major ampullate ribbons are observed to be tightly wrapped around a cricket antenna (community web), in a manner consistent with the proposed electrostatic capture mechanism. Electrostatic charge has also been proposed to play a role in cribellate capture-silk function.[19] Experiments on aging in cribellate capture threads could not account for the reduction in cribellate silk stickiness with age on purely structural criteria,[20] and electrostatic charge has been identified as a potential contributing factor to thread stickiness in cribellate spiders. Although *Loxosceles* threads are quite unlike cribellate silk, they both have relatively large surface areas, which could play a role in the effectiveness of electrostatic charge. It seems possible that spiders of the genus *Loxosceles* may use the curiously modified major ampullate silk as both structural and capture components in the web.

Acknowledgment. We thank P. Y. Webber for determining the amino acid compositions and G. Watson for taking the photographs used in Figure 1. B. J. Drake is acknowledged for assistance with the AFM measurements. H. D. Chanzy acknowledges NIST for financial support as a guest scientist for a 9-month sabbatical from his host institution of CERMAV-CNRS in Grenoble France.

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(13) All commercial equipment, instruments, or materials are identified in the article in order to adequately specify the experimental procedure, such identification does not imply recommendation by the NIST, nor does it imply that materials or equipment identified are necessarily the best available for the purpose.
(14) Specimens were also mounted on carbon-coated glass and floated off on water. In this case, the silk was never in contact with water but gave the same electron diffraction results. Supercontraction is observed when the major ampullate silk of orb-weaving spiders comes in contact with water. We do not see any evidence of supercontraction in this silk. Electron diffraction patterns without the carbon substrate are presented because they are cleaner and lack the background scattering produced by the carbon film.
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BM010108M