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Physicochemical Property Variation in Spider Silk: Ecology, Evolution, and Synthetic Production

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Annu. Rev. Entomol. 2017. 62:443–60

First published online as a Review in Advance on December 7, 2016

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi:
10.1146/annurev-ento-031616-035615

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Keywords

biomaterial, chemical properties, hierarchical structure, MaSp model, molecular arrangement, physical properties, spinning processes, supercontraction, tensile properties

Abstract

The unique combination of great stiffness, strength, and extensibility makes spider major ampullate (MA) silk desirable for various biomimetic and synthetic applications. Intensive research on the genetics, biochemistry, and biomechanics of this material has facilitated a thorough understanding of its properties at various levels. Nevertheless, methods such as cloning, recombination, and electrospinning have not successfully produced materials with properties as impressive as those of spider silk. It is nevertheless becoming clear that silk properties are a consequence of whole-organism interactions with the environment in addition to genetic expression, gland biochemistry, and spinning processes. Here we assimilate the research done and assess the techniques used to determine distinct forms of spider silk chemical and physical property variability. We suggest that more research should focus on testing hypotheses that explain spider silk property variations in ecological and evolutionary contexts.

Spidroins: the fibrous proteins from which all spider silks are made. The name is abbreviated from spider silk fibroins

Dope: silk proteins in a liquid crystalline form dissolved in aqueous solution. The dope feedstock for spider major ampullate silk synthesis contains spidroins in aqueous media

Mechanical

properties: suite of properties, including ultimate strength, extensibility, toughness, and Young's modulus, pertaining to the tensile performance of the silk

INTRODUCTION

Silk is a proteinaceous fiber that is naturally produced by many invertebrates. The most well-known silk producers include larvae of the domesticated silk moth, *Bombyx mori*, and other silk moths of the families Bombycidae and Saturniidae. Other silk producers include web spinners (Embioptera), trichopteran larvae, some Hymenoptera, and all spiders (Araneae) (29, 31). Of the spiders, the web builders (superfamily Araneoidea, suborder Araneomorphae) have the most impressive silk toolkits (9). Most silks, but especially spider silks (47, 48, 72), are exceptionally tough. The toughness of silk is considered to be primarily a consequence of their amino acid sequence and spinning processes (17, 27, 101), the mechanisms of which are described herein. The amino acid sequences of specific silk proteins, or spidroins (see definition), are well described and appear to be conserved within individual spiders and species. Differential spidroin expression, nonetheless, may occur in certain circumstances in particular silks (18, 33, 54). Silk proteins flow through the gland as a concentrated aqueous solution, or dope, before being drawn from a spinneret. The proteins aggregate and form a fiber during the final stages of spinning. Many factors, including the rate of drawing and other physiological factors, influence the chemical and physical properties of a given silk type (39, 80, 122, 135).

The immense toughness of spider silks and the fact that they are naturally synthesized in water rather than caustic chemicals (30, 101) make the commercial production of synthetic spider silks desirable (38, 142). Nevertheless, the commercial synthesis of materials with spider silk-like properties has not been achieved (34, 136, 138). There are many reasons why silks produced using recombinant technologies have not mimicked natural spider silks (73). One reason is that none of the recombinant proteins used were based on full-length spidroin-encoding sequences (7, 8, 28, 60, 120). A more significant reason for the failure to produce synthetic silks with attributes of natural spider silks is an incomplete understanding of how secretion and spinning induce the cloned proteins and/or the spun threads to vary in properties across environments (27, 136).

There are now several good reviews detailing the natural and synthetic production of spider silk (e.g., 31, 43, 82, 132, 137). However, an overview of the mechanisms by which spidroin synthesis and spinning induce variation in spider silk across environments and the implications of this process for producing synthetic silk is currently lacking.

Spider Silks: Types, Properties, and Uses

Spiders of the large and diverse superfamily Araneoidea produce seven distinct types of silks, each of which is secreted by different silk glands (**Figure 1**). Major ampullate (MA) silk is used as a safety line by most spiders and as web frame and radii by orb web spiders. It has the most impressive mechanical properties of all spider silks, as it combines high strength with high extensibility. Minor ampullate (MiA) silk is used as a temporary capture spiral by orb web builders and for prey wrapping by cobweb builders (77, 107, 134). Flagelliform silk is used in the axial threads of the capture spirals of orb webs (109). It has less than half the strength of MA silk, but it is about seven times more extensible (125, 127). In orb webs, the flagelliform silk is coated with a viscous, gluey, aggregate silk that facilitates the retention of captured prey (109, 134). Pyriform silk cements the dragline and web frame to substrates and glues silk threads together during web construction (98, 102). Aciniform silk is similar in strength to MA silk and is considerably stiffer (9). It is used to wrap prey, form the outer lining of egg sacs, and decorate some orb webs (9, 130, 144). Finally, tubuliform (or cylindriform) silk forms the outer coating of the egg sacs (49, 65, 66). Of these silks, the vast majority of published information focuses on property variations in MA silk (44, 82, 123, 132), so we focus on this silk here unless otherwise stated.

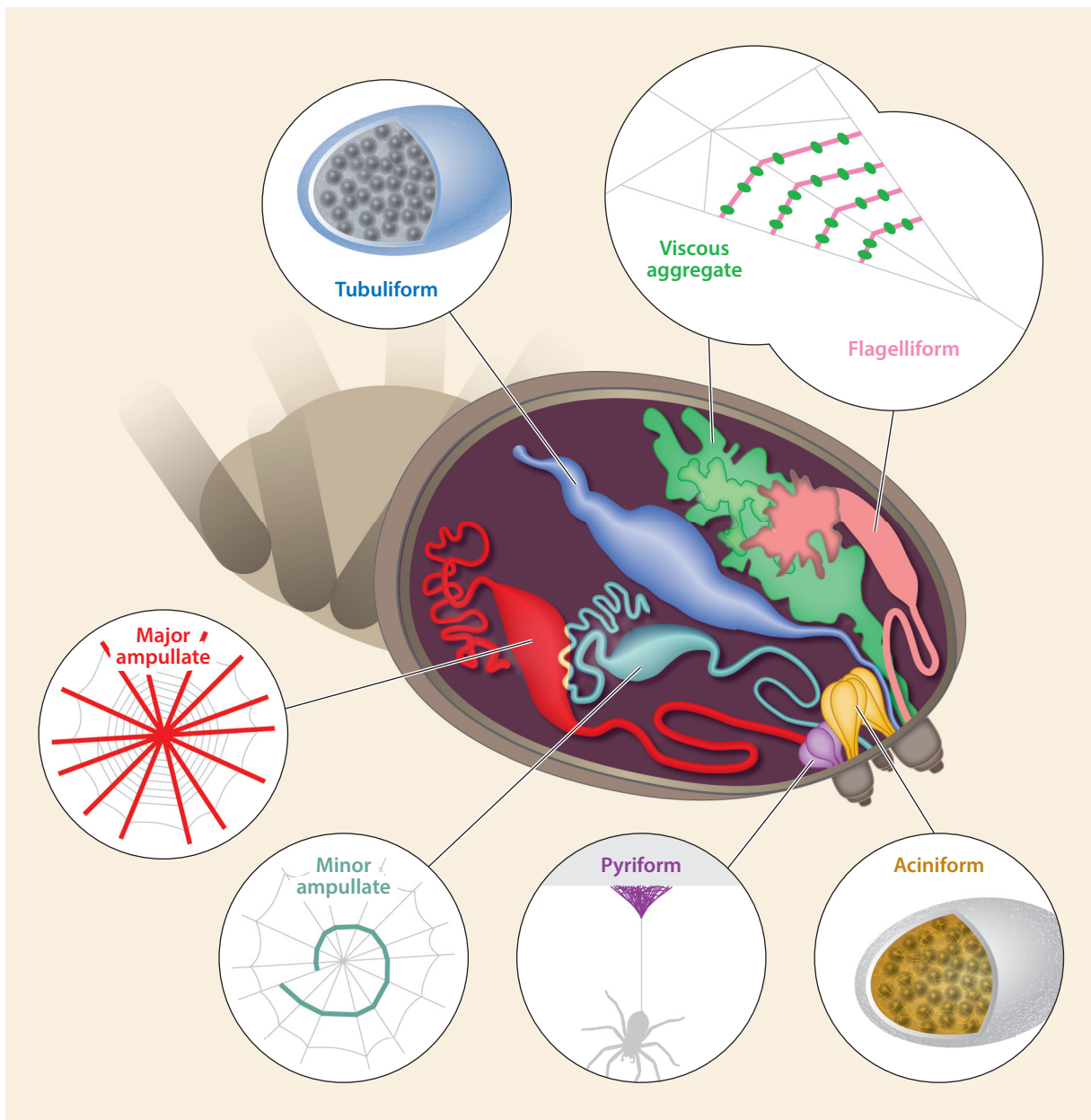


Figure 1

The glands of origin, proposed ecological function, and mechanical properties of the seven different types of spider silk: major ampullate silk (*red*), minor ampullate silk (*slate*), flagelliform silk (*pink*), viscous aggregate silk (*green*), pyriform silk (*purple*), aciniform silk (*yellow*), and tubuliform silk (*blue*).

Protein secondary structures: regions within proteins organized into regular structures; includes 3_{10} helices, α -helices, spirals, β -spirals, β -turns, and β -sheets in silk proteins

MA silk is made up of a lipid-rich layer and a glycoprotein-rich skin covering a fibrous outer and inner core (61, 122, 123). The core is composed of two types of proteins, or spidroins, called MaSp1 (derived from major ampullate spidroin 1) and MaSp2 (major ampullate spidroin 2) (122). These proteins contain ordered crystalline and disordered noncrystalline regions (the latter is often called the amorphous region, although it is not technically amorphous). The crystalline regions contain stacked pleated β -sheets, whereas the amorphous region arranges as a matrix of 3_{10} helices, β -turns or β -spirals, and other protein secondary structures depending on the amino acid composition (68, 69, 76, 122) (Figure 2).

MaSp1 and MaSp2 are encoded by two or more homologous genes (59, 62, 141). These genes have been sequenced for the cobweb-building western black widow spider (*Latrodectus hesperus*) and some orb web-building spiders, such as *Nephila clavipes* (7, 62, 141). The gene sequences for

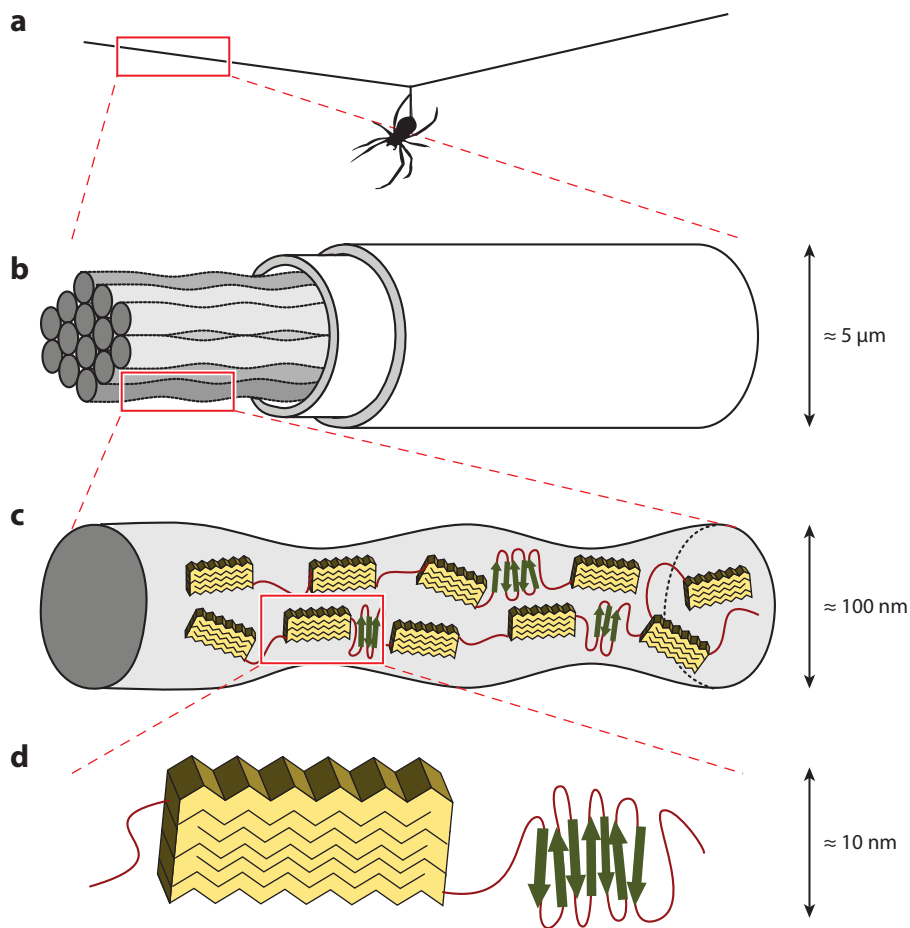


Figure 2

The hierarchical structure of spider silk. (a) A single thread is depicted, composed of (b) skin-covered fibrils, which are (c) conglomerates of proteins (spidroins) that are arranged as (d) crystalline β -sheets separated by protein chains of variable structure representing the so-called amorphous region. The scale arrows indicate the approximate sizes of the structures.

the MA silk of these species are thus used as models from which MaSp1 and MaSp2 expression is estimated from amino acid composition in other spiders.

Because a *MaSp2*-like gene sequence has been identified only in orb web spiders, MaSp2 is thought to have arisen from an ancestral version of MaSp1 in this group of spiders (8). Both MaSp1 and MaSp2 contain a region of highly repetitive amino acid sequences (50, 127) and nonrepetitive but conserved C- and N-terminal domains (44, 121, 139). These terminal domains are important for the formation of protein secondary structures (49, 57, 124).

VARIABILITY IN SILK PROPERTIES

Chemical Properties

Techniques such as cloning and peptide mapping (43, 54), high performance liquid chromatography (18, 55), nuclear magnetic resonance (NMR) (6, 35, 119), differential scanning calorimetry (52, 133), fluorimetry, Raman and polarized Fourier transform infrared spectroscopy (81, 117), circular dichroism (23, 60), and wide- and small-angle X-ray scattering (14, 19, 104, 106) have identified that amino acid sequences (which are interpreted to indicate the ratio of spidroins expressed), protein secondary structure (e.g., proportional composition of β -sheets, 3_{10} helices, β -turns, or β -spirals), and crystalline orientation, alignment, and density can vary extensively between and within spider species. These variations may induce subsequent variations in silk mechanical properties.

Mechanical Properties

The tensile properties of MA silk fibers are the properties of greatest interest, as understanding them enables the generation of materials that serve a particular function. They are usually determined by mounting the fibers onto cardboard, plastic, or aluminum foil frames; placing the frames within the grips of a tensile testing machine; cutting away the sides of the frame; and stretching the silk until rupture to calculate the engineering stress (σ):

$$\sigma = \frac{F}{A_0},$$

where F is the force applied to the specimen and A_0 is the cross-sectional area of the thread calculated from diameter assuming constant thread volume (55), and strain (ε)

$$\varepsilon = \log_e \frac{L}{L_0},$$

where L is the instantaneous length of the fiber at a given extension value and L_0 is the original gauge length of the fiber (10).

Alternatively, researchers may calculate true stress as

$$\sigma = \frac{F}{A_i},$$

where A_i is the instantaneous cross-sectional area of the fiber at any given strain value (55, 85).

From plots of stress versus strain, ultimate strength (the stress at rupture); extensibility (strain at rupture); toughness or work of extension (the area under the stress strain curve); and Young's modulus, or mechanical stiffness (the slope of the curve during the initial elastic phase) are calculated (**Figure 3**). These properties are of interest to many researchers because their values can be normalized to different sample dimensions, thus facilitating property comparisons between and within spider species (11, 16, 125). Wide variations in mechanical properties among species have

Mechanical stiffness: the extent to which a material resists deformation, indicated by Young's (elastic) modulus

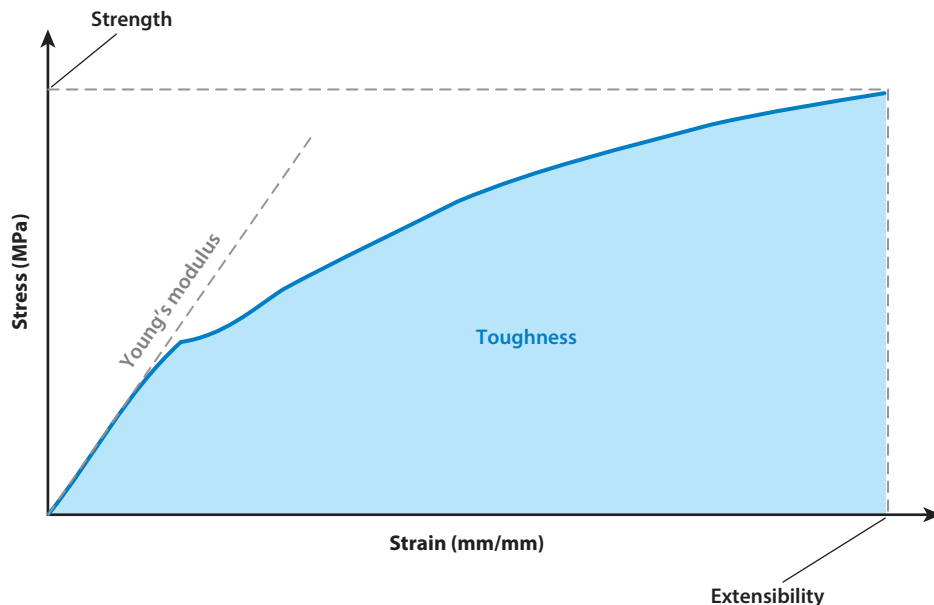


Figure 3

Components of the stress-strain curve, showing how ultimate strength (the stress at rupture); extensibility (strain at rupture); toughness (the total work of extension, calculated as the area under the stress strain curve); and Young's modulus, or stiffness (the slope of the curve during the initial elastic phase for each specimen), are determined.

been reported in the literature. For instance, *N. senegalensis* MA silk declines by half in strength and doubles in elasticity when relative humidity is raised from 25% to 80% (131). Different research groups, nevertheless, favor different measures of stress over others, and some measure the mechanics of silk after supercontraction in water whereas others measure dry silks. Such methodological inconsistencies lead to confusion when comparing properties between studies.

Supercontraction:

silk with high elasticity and low stiffness induced by exposure to water or high humidity. In the supercontracted state of spider silk, the amorphous region proteins are misaligned. The state is considered a ground state

Phylogenetic

variation: variation in the chemical or mechanical properties of a specific silk found among different spider species

TYPES OF PROPERTY VARIATION

Phylogenetic Variation

We refer to phylogenetic variation in silk properties as variation in the chemical or physical properties of one type (e.g., MA silk) of silk across different species (11, 17, 35, 125). It may not be surprising that there is considerable phylogenetic variation in silk properties given that silk use has evolved in spiders over approximately 400 million years (31).

The design principle of ordered crystalline regions interspersed among disordered noncrystalline regions is consistent across most spider groups (123). However, the MA silk of spiders in the Araneioidea has greater elasticity, and hence toughness, than that of spiders from other groups (114, 125). Silk elasticity thus seems to have coevolved with web-building behavior and may have been facilitated by the evolution of *MaSp2* (11, 35, 114). The highly extensible *MaSp2* spidroin accordingly may have been essential for the origin of the two-dimensional orb web (11, 18). Strong silk seems to have predated web building (11), however, so silk strength was likely imparted early in the evolution of web-building spiders and elasticity imparted later (11, 35). The innovation

of using extensible flagelliform and viscous aggregate silks in capture spirals instead of dry, stiff cribellate silk may have further facilitated a reduction in the production costs of orb webs (91, 92).

The uncertainty about the phylogenetic history of some spider groups makes it difficult to make generalizations about silk property evolution. For instance, the cribellate web-building spider clade Deinopoidea were moved in 2014 to become sister to the non-web-building retrolateral-tibial apophysis (RTA) clade (20), suggesting that the use of flagelliform and aggregate silks were not initially used to reduce the cost of producing orb webs. Molecular evidence suggests that two-dimensional orb webs were repeatedly and significantly modified through evolutionary time (12, 20, 64). How changes in silk properties have driven web evolutionary trajectories is nevertheless unresolved, but evidence suggests that there were significant benefits associated with investing in silks with different levels of biological plasticity (see term definitions for distinction from engineering plasticity) (11).

Biological Plasticity

The distal mechanisms inducing MA silk biological plasticity have been described across a range of contexts. Ecologically, climatic factors (19, 44, 83) and diet (13–15, 18, 128) may induce silk plasticity via one or several processes, including (a) differential MaSp1 or MaSp2 expression, (b) glandular processes, and (c) forces acting during spinning, drawing, and postspinning inducing variations in protein structure or amino acid alignment.

PROCESSES INDUCING BIOLOGICAL PLASTICITY

Differential Spidroin Secretion

The amount of MaSp1 compared to MaSp2 secreted is thought to affect the mechanical properties of the silk fibers. MaSp1 consists of multiple (GA)_n, (GGX)_n, and (A)_n repeated amino acid sequences (where G = glycine, A = alanine, and X = other amino acids) (121, 142), and these sequences promote the formation of crystalline β -sheets in the spun fibers (26, 129). MaSp2 consists of (GPGXX)_n repeated sequences (where P = proline) in addition to the (GA)_n, (GGX)_n, and (A)_n sequences (62, 67, 143). NMR analyses have shown that the proline-containing (GPGXX)_n motif in MaSp2 promotes the formation of β -spirals and type-II β -turns (35, 67–69). The crystalline β -sheets of MaSp1 give the silk strength. By contrast, the β -spirals and β -turns promoted by MaSp2 give the silk extensibility (86, 110, 121). Genetic, spectroscopic, and mechanical analyses show that the high extensibility of flagelliform silk is likewise due to the presence of an extended (GPGGX)_n motif (79, 80).

The MA gland consists of a tail, sac, duct, and spigot, and the gland opens at the spigot via a valve (132). There is a pH gradient (from approximately pH 8 at the tail to approximately pH 5 at the spigot) along the length of the gland, which is critical for the formation of protein structure (3) (Figure 4). MaSp1 and MaSp2 are secreted in various amounts, depending on species and amino acid availability, by the epithelial cells in the tail (3, 4, 134, 137) and stored as highly concentrated (approximately 20–50% protein) dope in the posterior region of the sac (134). The pH of the sac is maintained at the isoelectric point for MaSp1 and MaSp2 (i.e., approximately 6.8–7.0) by a proton pump and the actions of carbonic anhydrase (3). Na⁺, K⁺, and Cl⁻ ions secreted by the sac epithelial tissue prevent spidroin aggregation within the sac (60, 80). The different chemical composition of the two spidroins means they will aggregate and fold differentially during spinning. The ratio of MaSp1 to MaSp2 in silk thus influences the chemical and physical properties of the spun silk fibers (18, 33, 53).

Biological plasticity: differing of chemical or mechanical properties of a type of silk within a single species as a direct result of the environment to which the spider is exposed. Contrast with engineering plasticity

Engineering plasticity: ability of a material to be repeatedly deformed without rupture

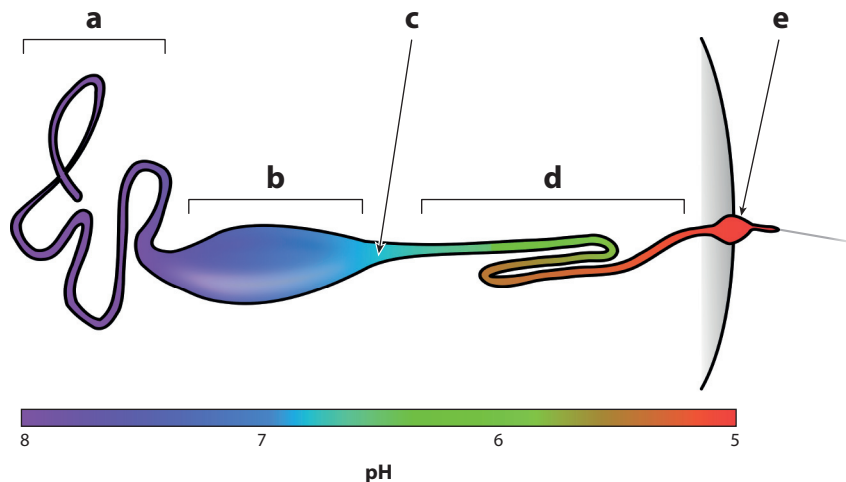


Figure 4

The major ampullate silk gland. Showing the location of the (a) tail, (b) sac, (c) entrance to duct, or ampulla, (d) duct, and (e) spigot. The pH gradient range from approximately pH 8 at the tail to approximately pH 5 at the spigot is indicated.

Influence of Diet

Spiders on different diets or consuming prey differing in nutritional value may produce silks differing in the amount of MaSp1 and MaSp2 expressed, which may be measurable as variation in amino acid composition (13, 14, 18, 33, 128). Spiders on a low-protein diet produce MA silk with lower proline and serine, as a consequence of MaSp2 downregulation, than spiders on high-protein diets (14, 18, 53). One hypothesis is that MaSp2 is synthesized at a higher metabolic cost than MaSp1 because it contains the chemically complex amino acids proline and serine (32, 53). This hypothesis predicts that the greater metabolic cost of expressing MaSp2 compared to MaSp1 forces some spiders to vary the ratio of the spidroins expressed when their protein or energy intake is low (18, 32, 53). Nevertheless, when measuring metabolic cost in terms of the ATP consumed to synthesize one mole of amino acid from one mole of glucose, serine and proline production are comparable to glycine and alanine and low compared to methionine and leucine (70). Moreover, variation in MaSp1 and MaSp2 expression alone does not always account for the diet-induced variations in silk chemical and mechanical properties (14). More research is accordingly required to discover the precise mechanisms involved.

Postsecretion Processes

We identify postsecretion processes as those inducing protein phase transitions as the dope flows through the duct. During these processes, the proteins arrange into different secondary structures (60, 61, 134) under the influence of changes in water content, salt concentration, pH, and shear stress (37, 39, 58, 78).

The sac of the MA gland is separated from the duct by the funnel-shaped ampulla (36, 82, 132) (**Figure 4e**), which varies in size and position as the spider ages (36). The lumen width decreases sharply at the ampulla so the dope flows faster and is drawn under shear stress to induce shear thinning (22). The length of the duct affects the amount of shear thinning; the longer the duct,

the greater the thinning (22, 36, 80). These forces may partly explain why adult spiders (who have longer spinning ducts than juvenile spiders have) spin tougher silks than juvenile spiders do (115).

In the duct, a reduction in salt concentration, in particular phosphate salts, results in phosphorylation of the dope and buildup of phosphoric acid, reducing luminal pH from ~ 6.8 to ~ 6.0 . This process destabilizes the N-terminal domains inducing protein dimerization (50, 58, 76, 112) and facilitating aggregation and assembly (60, 75, 132, 145). The C-terminal domain acts as a switch controlling the rate of assembly (57, 124). The dope is now a crystalline liquid amyloid (24, 26, 71, 134). Shear stress and a further reduction in pH induce the proteins to fold and form β -sheets and other secondary structures (3, 74, 134) at a rate depending on temperature (71, 135). The series of reactions within the duct thus appear to function to get the dope into a gel-like state to enable shear forces to act on it.

Silk secondary protein structure often varies with environmental parameters, such as wind speed, without variation in spidroin expression (19). Such factors should affect silk properties by altering water availability, pH, salt concentration, or other physiological parameters in the duct (132). Changes in the rates of MaSp1 and MaSp2 aggregation and folding in different conditions may also be a source of biological plasticity (122).

Spinning Processes

We identify spinning processes as those that induce the crystalline and amorphous protein chains to self-align (22, 80, 134) and become stiffer and less elastic (81, 87, 99). In the final stages of spinning, the dope becomes increasingly gel-like and eventually pulls away from the wall of the duct, a phenomenon called draw-down taper (74, 84). Under draw-down taper, the proteins self-align and the silk dehydrates and solidifies (36).

Models (e.g., 22, 24) predict that different frictional forces at the valve cause the crystalline and amorphous chains to align differently. Spiders thus can change the frictional force at the valve to alter the alignment of the silk proteins and tune the performance of the silk (40, 52). The silk spun by spiders in free fall are thicker and have greater mechanical compliance compared to that of spiders crawling on a horizontal surface, and these differences may be a consequence of the different frictional forces applied under different pulling speeds (45, 46, 88). Spiders walking on a horizontal surface draw silk at slower speeds than do spiders falling rapidly using their weight, and this variation in draw speed influences the frictional force applied at the valve (88). Comparisons of silks that have been forcibly fast pulled and slow pulled have found structural differences in alignment in the crystalline and amorphous regions, with fast-pulled silks showing the greater alignment (24, 27, 63, 108).

Anesthetization of spiders using CO₂ or N₂ during forcible silking may prevent the spiders from applying the so-called friction brake, but the anesthesia itself can cause variation in silk properties (96, 105). Silks obtained from anesthetized spiders are thinner and stiffer with a higher storage modulus and greater density of skin relative to core than silks obtained from unanaesthetized spiders (90, 93). The precise mechanisms by which anesthesia induces property variations are, nevertheless, largely unknown.

Postspin Variation

The media into which the silk is drawn influences the molecular structure and mechanical properties of spider silk (27, 84, 118). For instance, MA silks spun in air are stiffer and stronger than those spun in water under similar tension (84). All spiders, with the exception of the aquatic spinning spiders *Argyroneta aquatica*, naturally spin their silk in air most of the time. Exposure to dry air

Mechanical compliance: the material property of having low stiffness

dehydrates the silk fibers, enabling hydrogen bonds between the protein chains to reform, and the fiber to stiffen (118).

Studies (116–118) comparing the properties of MA silks spun in air, water, urea, and ethanol have found that silks spun in ethanol and urea have similar properties to those spun in water. This is because spinning silk into polar solvents such as water, urea, or ethanol disrupts hydrogen bonds between protein chains in the amorphous region, resulting in highly elastic and compliant silk (116).

Supercontraction

MA silk spun in water, or immersed in water or high humidity (>~70% relative humidity) post-spinning, shows an increase in elasticity and decrease in stiffness up to 200% (2, 41, 140). An increase in strength, with an increase in breaking energy and stiffness, and a decrease in breaking strain are characteristics of silks spun underwater (27, 84). These reversible changes in silk mechanical properties induced by water are called supercontraction (87, 111, 140).

Supercontraction occurs in MA silk because water molecules disrupt hydrogen bonds between crystalline blocks, mobilizing the proteins and causing changes in their alignment (2, 51, 116). The amorphous region consequently becomes mobile and realigns and reorientates (4, 98). The amount of shrinking experienced as a percentage of original length depends primarily on the amino acid composition, as certain amino acids, in particular proline, influence the molecular bonding and subsequently protein secondary structures (87, 103).

Because supercontraction in MA silk removes the influence of amorphous region alignment on its mechanical properties, the supercontracted state may be considered a ground state of MA silk. Supercontraction can thus be utilized to return MA silks from different spider species to a state that is independent of any previous loading (21, 40, 42). It is therefore useful for comparing phylogenetic variation in silk properties (11, 89). The supercontracted state may also be utilized to determine the amount and mechanisms of biological plasticity in MA silk (11, 17, 40, 42). For instance, it has been used in conjunction with X-ray diffraction analyses to assess the relative influence of crystalline and amorphous region alignment on diet-induced biological plasticity (17).

Minor ampullate silk shows amorphous region realignment, but it has no measurable supercontraction on exposure to water (25, 56). The interactions between water and silk proteins in the different types of silk are, accordingly, more complex than current models describe, with much detail remaining to be determined.

Aging

The positioning of the protein chains is completed in MA silk upon drawing of the fiber. Nevertheless, silk fibers left in dry environments and silk within spider webs or cocoons exposed to direct sunlight experience further short-term increases (1, 16, 100) or long-term decreases (95) in strength and elasticity. These changes are brought about as a consequence of molecular vibrations displacing the hydrogen bonds between proteins in the amorphous region (16, 72). The molecular vibration rate is variable and depends on ambient temperature, humidity, and the intensity of UV and other forms of radiation (94, 97, 100). Cracks in the skin induced by UV radiation are responsible for further postdraw changes in the mechanical properties of MA silk, but the critical sizes of the cracks have proven difficult to quantify (97).

IMPLICATIONS FOR SYNTHETIC PRODUCTION

The production of synthetic silks or spidroins generally involves incorporating cloned spidroin-encoding genes into a bacterial (e.g., *Escherichia coli*), yeast, plant (e.g., tobacco), or animal (e.g.,

goat) host (28, 73, 126), and the secreted proteins are assembled and spun into fibers using acid bath coagulation in a caustic environment before drawing through microfluidic or electrospinning devices (73, 113, 120, 138). Two studies (5, 30) nonetheless describe silk development using water as the spinning medium. The current understanding of the physiology and biochemistry of the natural spinning process is nevertheless lacking in detail. We contend here that a better understanding of the biochemical and physiological spinning mechanisms inducing property variability across environments will improve the quality of silks produced by recombinant technologies and artificial spinning. Future advancements in recombinant technology and refinements in artificial spinning techniques will in turn facilitate a better understanding of the mechanisms inducing biological plasticity as it will become possible to vary spinning processes while holding spidroin expression constant. Advances in modeling and analytical technologies, such as the development of greater computing power, more sensitive tensile testing machines, and new laser and synchrotron sources, will unquestionably provide for more precise silk structure and property measurements that will accompany the new spinning techniques.

CONCLUSIONS

Previous studies of spider silk have focused on the ultimate structure of spider MA silk, utilizing an array of advanced techniques, and have elucidated silk production in detail. There is nevertheless much that remains to be learned before the creation of synthetic silk analogs can be realized. Here we reviewed the research on silk chemical and mechanical property variability and documented how secretion, postsecretion, spinning, and postspinning mechanisms can facilitate silk property variation among and between spider species. We suggest that a more detailed understanding of these mechanisms in ecological and evolutionary contexts is critical for improving our understanding of how spiders synthesize fibers that combine extreme strength with elasticity.

SUMMARY POINTS

1. Web-building spiders produce up to seven different types of silk, each of which is secreted from different glands, has different functions, and has different chemical and physical properties.
2. A combination of high strength and extensibility makes spider major ampullate silk highly desirable for many commercial applications. Generally, silk products are produced using recombinant and various spinning technologies, but these methods have not been able to produce fibers with properties similar to natural spider silk.
3. Although amino acid sequences alone can influence the secondary structures and the consequent mechanics of spider MA silk, postsecretion physiological and biochemical mechanisms acting within the duct of the gland will induce further variations in the secondary structures of the proteins secreted into the tail.
4. Silks spun into air have different mechanical properties to silks spun into water or other polar solvents. Supercontraction is the term used to describe the properties induced upon exposure to water. In the supercontracted state, internal mobility of the proteins and a disruption of hydrogen bonds cause proteins within the amorphous region to misalign.

5. Silk biological plasticity is the variation in the silk of an individual spider across different environments. Biological plasticity may occur as a consequence of variations in the secretion, postsecretion, spinning, and postspinning environment. Studies attempting to understand the mechanisms inducing silk plasticity should examine all of the mitigating factors. Silk supercontraction may be utilized in such experiments as it provides a means for returning silk to its ground state.

FUTURE ISSUES

1. It is imperative to understand the natural processes that cause spider silk properties to vary among species (phylogenetic variability) and within species (silk plasticity).
2. Major ampullate silk properties in the supercontracted state may be considered a ground state because the influence of previous loading and amorphous region alignment is removed. Returning silk to a ground state may be useful for experiments examining the mechanism of silk biological plasticity.
3. We identified the processes that influence spider silk mechanical properties that act during secretion, postsecretion, spinning, and postspinning. A more thorough understanding of the influence of each process on silk mechanical properties is essential if the development of synthetic silk is to be realized.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We acknowledge research support from grants from the National Science Council, Taiwan (NSC-102-2311-B-029-001-MY3 and NSC-102-2811-B-029-001; I-M. T. and S.J.B.); Tunghai University Global Research and Education on Environment and Society (I-M. T.); Australian Research Council (DE140101281; S.J.B.); *Journal of Experimental Biology* (S.J.B.); and the Austen BioInnovation Institute, University of Akron (T.A.B.). We thank Nadia Ayoub, Chris Holland, Randolph Lewis, and Anna Rising for providing feedback on early drafts.

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