Sequential origin in the high performance properties of orb spider dragline silk

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The major ampullate (MA) silk utilized by orbicularian spiders in the construction of their iconic orb webs is the toughest biological material known1. Coupled with the silk’s production at ambient temperatures in living tissue and its biocompatibility2, orb spider MA silk is therefore an important model for biomimicry. While the ecological function of MA silk as the energy-absorbing backbone of aerial orb webs likely acted as a critical agent of natural selection for its remarkable toughness3, MA silk evolved much earlier in the history of spider diversification. Ancestrally, MA silk was utilized primarily in terrestrial webs that simply entangled pedestrian insects or acted as lifelines4. The innovative behavioral shift of utilizing MA silk to construct discrete aerial frameworks for the webs of orb-weaving spiders4 was accompanied by significant changes in the genes encoding MA silk proteins5. Phylogenetic comparison of the mechanical performance of major ampullate silk suggests that at least some aspects of its impressive mechanical performance predate the origin of the orb web by hundreds of millions of years6,7, providing substantial opportunity to understand how the impressive material properties of orb spider MA silk arose. However, MA silk properties are poorly investigated outside of orb web spiders. Moreover, some spiders exert substantial control over structure as silk is spun resulting in large intra-specific and even intra-individual variability in silk performance8, severely obscuring relationships between the evolution of spider silk genes and silk performance.

We overcome this limitation by exposing MA silk fibers to water to supercontract them to their “ground state”, where mechanical performance is independent of previous loading history9,10, to understand the sequence of evolution of orb spider’s MA silk toughness over its ~376 million year history. Spiders control the properties of their MA silk by applying shear force to silk as it emerges from their spinnerets11. This shear force helps to determine the overall alignment of silk proteins, which is then “frozen” into place by hydrogen bonding12,13. Silk processing allows spiders to tailor MA silk performance to ecological function, but also obscures inherent variability determined by protein composition per se. During supercontraction13, water infiltrates silk and disrupts hydrogen bonding, allowing the fiber to return to its “ground state”. This allows comparison of the intrinsic properties of silk performance9,14,15 and is particularly important because supercontraction itself varies substantially among spider taxa16.

Results
The material properties of MA silk are shown in Figure 1 for representatives of all major groups of spiders. We use the aciniform silk spun by Mygalomorphae (tarantulas and their relatives), the sister taxa to Araneomorphae, as
Among orb spiders, MA silk is a composite of two types of proteins (major ampullate spidroins: MaSps) dominated by four types of highly repetitive amino acid sequences called motifs. Tarantula silk contains low frequencies of two of these motifs – glycine-alanine (GA) and poly-alanine (poly-A) – which in MA silk form β-sheets that stack together into nanocrystals, interlocking silk molecules. The existence of nanocrystals in tarantula silk is untested, but GA and poly-A motifs form nanocrystals in the MA silk of all studied species. However, the amino acid sequences of mygalomorph silk proteins are highly heterogeneous, therefore containing only few GA and poly-A motifs. This heterogeneity of amino acid sequences correlates with relatively poor performance of their silk. Elastic modulus ($E=2.5\pm0.6$ GPa), tensile strength ($\sigma_u=140\pm10$ MPa) and strain at breaking ($\varepsilon_u=0.17\pm0.08$) are lower than other spider taxa, leading to approximately ten fold lower work to fracture ($W_f=16\pm9$ MJ/m$^3$), compared to MA silks. Tarantula silk also does not supercontract (Fig. S1). Thus, ancestral MA silk likely was inferior in performance and spinning plasticity to all modern araneomorph spiders.
MA silk performance improved rapidly among basal lineages of araneomorph spiders that diverged from one another ~376MYA, correlating with three key changes in silk proteins. Haplogyne spiders are among the most basal taxa to produce MA silk, but their amino acid sequences lack many key features found in orb spider MA silks. Both poly-A and GA motifs are present, and appear more uniform compared with those observed in Mygalomorphae, but the overall amino acid sequences of haplogyne MA silk is still quite heterogeneous (Fig. S1). Haplogyne MA silk cDNAs notably contain a few isolated glycine-glycine-X motifs (GGX; where X is a small subset of amino acids), which are hypothesized to be plasticized by water during supercontraction15,16. Glycine favors ordered backbone turns that are stabilized by hydrogen bonding among amino acid residues when dry, but promotes mobility and disorder of protein backbones when water disrupts those hydrogen bonds22. Thus, hydration of glycine–rich residues relaxes MA silk through the glass transition and accounts for one component of the supercontraction response of orb spider MA silk23. However, full mobilization of the amorphous fraction of MA silk is only achieved by an interaction between glycine and proline residues22. Because haplogyne silks contain relatively few glycine residues, and almost no proline, they show modest supercontraction of approximately 10% or less (Fig. S1), and reach an elastic modulus of E=5.5 to 8.6 GPa (for Sycytodes and Kukulcania respectively), which is higher than the ground state in evolutionarily derived lineages. Tensile strength and strain at breaking (maximum values, Sycytodes, $\sigma_{\text{a}}=640\pm10$ MPa, Kukulcania $\sigma_{\text{a}}=0.27\pm0.06$) are still much lower than derived taxa, but work to fracture is higher than that of Kevlar or ultra-high-molecular-weight-polyethylene (Sycytodes, $W_f=90\pm40$ MJ/m², Kevlar, $W_f=50$ MJ/m²). Thus, remarkably high performance silk fibers evolved early in the history of spiders, at least 130MY before the origin of aerial orb webs (Figures S2–S3).

The next innovation in MA silk genes occurred at the base of Entelegynae, prior to the divergence of orb spiders and RTA clade. No new amino acid motifs appear in their common ancestor (Fig. S1), but the GGX motif is significantly more frequent and repetitious compared to basal taxa. This increases the elastomeric properties of the proteins24. MA silk continues to increase in extensibility ($\varepsilon_{\text{e}}=0.46$) within Entelegynae (Fig. S3) and reaches a maximum tensile strength that is similar between RTA clade and Orbuliculariae ($\sigma_{\text{a}}=980\pm20$ and $1140\pm30$ MPa respectively) (Fig. S2). This combination yields a large work to fracture ($W_f=149$ MJ/m²) at the base of the Entelegynae at least 230MYA (Fig. S3). Within Entelegynae, the RTA clade silk still lacks some key features of orb spider silk. Moreover, RTA clade MA silks show remarkably similar tensile properties among species, despite evolutionary divergence for over 150MYA. Phidippus silk is notably stiffer, which may correlate with the unusual hunting ecology of the family Salticidae.

**Discussion**

The unrivaled toughness of orb spider MA silk coincides with the appearance of a novel MaSp2 protein, at the base of Orbuliculariae. MaSp2 contains a high content of larger side chain amino acids and, in particular proline, which is incorporated into a novel glycine-proline-glycine-glycine-X motif (where X is a small subset of amino acids; GPGGX). Proline generally destabilizes secondary structures in proteins, favoring amorphous protein networks25. The GPGGX motif kinks the backbone of the peptide into an extensible β-spiral24, which explains in part the up to five fold greater extensibility of the MA silk of orb spiders compared to RTA clade ($\varepsilon_{\text{e}}=0.8\pm0.2$ vs 0.39±0.09). This high extensibility explains why work to fracture is ~100% greater in orb spiders compared to RTA clade ($W_f=290\pm90$ vs 160±50 MJ/m²). The combination of high toughness and extensibility in the MA silk of orb spiders plays a critical role in how webs resist breaking under the impact of flying prey10,26.

Proline-rich MaSp2 imparted MA silk with a new flexibility at two very different levels. First, Orbuliculariae MA silk extensibility in its ground state varies almost three-fold among taxa ($\varepsilon_{\text{e}}=0.51$ to 1.3). The proline content of MA silk in orb spiders correlates closely with interspecific variation in compliance and extensibility23,27. In contrast, the tensile behavior of haplogyne and RTA clade silks are homogeneous within each clade. This suggests that shifts in the expression of MaSp1 versus MaSp2 provide a highly evolvable mechanism tailoring the functional properties of MA silk in different species of orb spiders. This is particularly evidenced by Arigope. This genus is ~30MY old, yet the performance of MA silk varies to almost the same degree as exhibited by the rest of the 210MY Old Orbuliculariae (Fig. 1). This high evolvability results in the MA silk of orb spiders occupying three quarters of the total performance space delimited by all other spider species.

Second, MaSp2 facilitates performance plasticity within individual orb spiders28. Supercontraction occurs in part as the GPGGX motif interacts strongly with water24, mobilizing the amorphous fraction of the silk. By stretching MA fibers in water, the amorphous fraction is increasingly aligned and this structure can be held in place by hydrogen bonds when the silk dries. This stiffens the silk and allows any particular stress-strain curve in the range of accessible properties to be reached in a predictable and reproducible way. This ‘wet stretching’ can therefore make the MA silk from a single orb spider occupy almost any region of the performance space in Fig. 1 from its ground state to the left of the performance space. During natural spinning, shear forces in the duct of the MA gland align silk molecules in still wet fibers, pulling them out of their ground state. Thus, spiders whose silk supercontracts strongly can access a greater range of performance for MA silk24,25.

Our results draw a coherent picture of spider dragline silk evolution, relating major innovations in MaSps to changes in silk functional properties. The origin of the orb web is a singular event in the evolutionary history of spiders that played a dominant role in the evolution of silk, but we reveal that the history of MA silk is significantly more complex. MA silk performance is characterized by stability within lineages, punctuated by evolutionary changes that correlate with innovations in molecular composition. Basal MA silk shows poor mechanical properties compared to derived taxa. Increased homogenization and repetitiveness of GA and poly-A motifs, as well as the origin of a new GGX motif, in MA silk proteins correlate with increases in strength and stiffness that pre-date the origin of orb webs. However, the extreme toughness of orb spider MA silk is only reached after the evolution of a novel protein, MaSp2 that greatly improved extensibility. In addition to facilitating the function of aerial orb webs, MaSp2 facilitates inter- and intra-specific variation in the mechanical performance of MA silk. The discovery of these evolutionary correlations between the molecular composition and tensile properties of MA silk should facilitate constructing bioinspired fibers that mimic the outstanding properties of natural orb spider dragline silk29.

**Methods**

**Taxon selection, phylogenetic relationships, and collection of silk.** Taxa span the phylogenetic diversity of MA silk producing spiders, with major lineages represented by species with diverse ecologies. Brief descriptions follow.

Mygalomorphae lack major ampullate glands but are sister to araneomorph spiders so that the undifferentiated sheets of silk that they spin provide the best available comparison to major ampullate silk. Mygalomorphae diverged from Araneomorphae at least 390MY ago. We tested silk from Aphonopelma seemanni purchased from the pet trade (Tarantulaspiders.com).

Araneomorphae “true spiders” consist of two large recent evolutionary radiations, RTA clade and Orbuliculariae, and several basal clades, here represented by Haplogynes. All produce MA silk from a pair of glands on their anterior lateral spinnerets.

Haplogynes are ecologically diverse and diverged from other araneomorphs at least 375MYA. Many use MA silk in the construction of terrestrial or aerial webs. The spitting spider Sycytodes is a wandering hunter that primarily trails MA silk as a dragline while the southern house spider Kukulcania hibernalis uses MA silk in the
Phylogenetic relationships were collected in various US and European localities. darwini level relationships were taken from Blackledge et al.4 to define the major clades of slacked and, under this condition, immersed in water39. After removing from the frame. The other sample was used for measuring the cross sectional area of the fiber. Each fiber. One sample was used for tensile testing and mounted on an aluminum foil Maximum supercontraction and testing of silk gland were collected. Silk was mounted across 15mm gaps on cardboard holders and stereomicroscope to ensure that only threads originating from the major ampullate sectioned in spinning gigantic orb webs across rivers in Madagascar using MA silk that is significantly tougher than other orb spiders. 1. Deinopis spinosa utilizes cribellate capture threads, representative of the earliest orbicularian spiders that evolved orb web spinning behaviors prior to the evolution of viscid glue (i.e. their capture spirals on three levels: interspecific, intraspecific and intraindividual. Int. J. Biol. Macromolecules 4, 301–306 (1999). Elices, M., Plaza, G. R., Pérez-Rigueiro, J. & Guine, G. V. The hidden link between supercontraction and mechanical behavior of spider silks. J. Mech. Behav. Biomater. 4, 658–669 (2011). Liu, Y., Spanner, A., Porter, D. & Vollrath, F. Proline and processing of spider silks. Biomacromolecules 9, 116–121 (2008).

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TAB, JPR, BP and AN conducted the research. TAB and JPR wrote the main text. All authors contributed to the data analysis and reviewed the manuscript.

Additional information

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