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The Evolution of Dragline Initiation in Spiders: Multiple Transitions from Multi- to Single-Gland Usage

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Abstract: Despite the recognition of spider silk as a biological super-material and its dominant role in various aspects of a spider's life, knowledge on silk use and silk properties is incomplete. This is a major impediment for the general understanding of spider ecology, spider silk evolution and biomaterial prospecting. In particular, the biological role of different types of silk glands is largely unexplored. Here, I report the results from a comparative study of spinneret usage during silk anchor and dragline spinning. I found that the use of both anterior lateral spinnerets (ALS) and posterior median spinnerets (PMS) is the plesiomorphic state of silk anchor and dragline spinning in the Araneomorphae, with transitions to ALS-only use in the Araneoidea and some smaller lineages scattered across the spider tree of life. Opposing the reduction to using a single spinneret pair, few taxa have switched to using all ALS, PMS and the posterior lateral spinnerets (PLS) for silk anchor and dragline formation. Silk fibres from the used spinnerets (major ampullate, minor ampullate and aciniform silk) were generally bundled in draglines after the completion of silk anchor spinning. Araneoid spiders were highly distinct from most other spiders in their draglines, being composed of major ampullate silk only. This indicates that major ampullate silk properties reported from comparative measurements of draglines should be handled with care. These observations call for a closer investigation of the function of different silk glands in spiders.

Keywords: spider silk; spinneret; major ampullate silk; minor ampullate silk; aciniform silk; piriform silk

1. Introduction

Spider silk has been praised as a biological super-material combining extreme strength and extensibility at a low density, biodegradability and biocompatibility [1]. Its biotechnological manufacturing has become the 'holy grail' in biomimetic material research with a multitude of promising applications on the horizon [2,3]. The increased interest in spider silk has led to a boost in spider research; however, most silk studies focus on the pure material properties, macro-molecular synthesis and assembly in a few model species, with the biology and variation of spider silk being largely neglected. This dismisses the potential of broader comparative studies both for bioprospecting and a comprehensive understanding of the ecology and evolutionary history of spiders. For example, it is unclear why predatory traps are so rare outside of spiders, and it has been hypothesized that biomechanical constraints strictly limit the 'evolvability' of silken constructs [4].

Unlike silk spinning insects, spiders possess a number of different types of silk glands that produce fibres with different physical properties and different biological functions [5,6]. The gland's ducts lead to nozzle-like openings, the spigots, that are scattered across usually three pairs of moveable spinnerets: the anterior lateral spinnerets (ALS), the posterior median spinnerets (PMS) and the posterior lateral spinnerets (PLS). In addition, some spiders possess an anterior spinning plate, the cribellum.

Among the major clade of spiders, the Araneomorphae, several general types of silk glands have been described and homologised [7], with their main functions being characterised in orb web spiders (Araneidae) [6]. The most common types are:

(1) The major ampullate glands are present in all araneomorphs. They produce the major component of the dragline and are the main subject of applied spider silk research. There are usually one to a few large major ampullate glands and their ducts lead to spigots on the inner margin of the ALS [8].
(2) The minor ampullate glands lead to spigots on the PMS [8]. In orb weavers, their product is used for auxiliary lines that play a role during web construction and locomotion (i.e., bridging lines and auxiliary spiral lines) [9,10].
(3) The aciniform glands produce very fine and tough silk [5,11]. In orb weavers, it is used to wrap prey [8,12], to produce web decorations or to be added to other silk products such as egg sacs [13] or bridging lines [9]. These glands are small and grouped on the PMS and the PLS [7].
(4) The piriform (sometimes spelled 'pyriform') glands produce glue-coated, fine fibres used to affix silk to substrates [14] or for thread joints during web building. There are usually a large number of small piriform glands, which are clustered on the ALS adjacent to the major ampullate spigots [7,8].

There are further types of silk glands that only occur in certain clades, such as the flagelliform glands (capture spiral silk) and aggregate glands (produce viscid glue) in members of the Araneoidea, or that only occur in the female sex, such as the tubiliform glands used in egg sac construction. These glands are not considered in this study and hence not discussed in further detail.

The current knowledge of the biology of spider silk is almost entirely built on work done on some orb weavers (Araneidae) and cob-web spiders (Theridiidae). Many representatives of these families build extensive webs and can produce large quantities of silk. Comparative studies on the mechanical properties of draglines have shown that araneids spin some of the toughest silks [15]. For this reason, araneids have been in the focus of bioprospecting and biotechnology [2].

However, insights from araneids and theridiids might not simply be transferable to the majority of spider taxa. It is likely that the biological function and, accordingly, material properties of homologous gland products might have evolved in very different directions in distinct lineages of spiders.

For example, ampullate glands differ considerably in their shape, size and relative proportion of distinct secretory tissues between different species, and in some spiders, the minor ampullate glands are relatively larger than the major ampullate glands [16,17]. Another example is piriform silk, that is widely used to attach and combine silk lines, but in daddy long-leg spiders (Pholcidae) and ground spiders (Gnaphosidae), the piriform glands are highly enlarged and are also used to immobilise dangerous prey [18,19].

An evolutionary perspective of the biological function of different silk gland products will lead to a better understanding of spider ecology and facilitate the directed search for biomimetic models to develop a new generation of tailored, high-performance biopolymers.

Here, I comparatively studied the spinneret use during silk anchor and dragline formation in representative species sampled across the phylogenetic tree of araneomorph spiders. The aim of this study was to gain an evolutionary perspective on the spinneret and silk gland usage in silk anchor and dragline spinning.

2. Material and Methods

2.1. Spider Collection

Spiders were collected in Eastern Australia (New South Wales, Queensland, Victoria and Tasmania), New Zealand (North Island) and Germany, or obtained from lab stocks (3 species), and kept in the lab in plastic jars or boxes with slightly moistened tissue (complete list of species and the origin and deposition of the material in Supplementary Table S1). I aimed for three individuals per species, though for some species, only one or two individuals were found (see Table 1 and Supplementary Table S1 for sample sizes).

Table 1. List of species studied (ordered per family, with taxonomy following the World Spider Catalog V.19.5), number of replica (f, female; m, male; juv, juvenile) and common usage of spinnerets during attachment disc and dragline formation (PMS, posterior median spinneret; PLS, posterior lateral spinneret). For full details of specimen origin, voucher location and additional voucher material used for species identification, please see Supplemental Table S1.

Family	Species	Individuals Studied	Number of Observations	PMS Used	PLS Used
Agelenidae	*Tegenaria ferruginea* (Panzer, 1804)	3 juv	26	–	–
Amaurobiidae	*Amaurobius fenestralis* (Ström, 1768)	2 f, 1 juv	15	+	+
(to Cycloctenidae)	*Storenosoma* sp.	1 juv	6	+	+
(to Cycloctenidae)	*Storenosoma terraneum* Davies, 1986	1 juv	4	+	+
Anyphaenidae	*Amaurobioides litoralis* Hickman, 1949	3 juv	34	+	–
	Anyphaena accentuata (Walckenaer, 1802)	2 juv	16	+	–
Araneidae	*Argiope keyserlingi* Karsch, 1878	2 f, 1 juv	21	–	–
	Cyrtophora hirta L. Koch, 1872	2 f, 1 juv	24	–	–
	Eriophora sp.	1 f, 3 juv	24	–	–
	Nephila plumipes (Latreille, 1804)	3 f, 1 juv	15	–	–
	Phonognatha graeffei (Keyserling, 1865)	3 f	28	–	–
Arkyidae	*Arkys cornutus* L. Koch, 1872	1 f	9	–	–
	Arkys furcatus (Balogh, 1978)	1 m	7	–	–
Austrochilidae	*Hickmania troglodytes* (Higgins & Petterd, 1883)	2 f, 1 m	31	+	+
Clubionidae	*Clubiona* sp. 1	1 m	10	+	–
	Clubiona sp. 2	1 f	5	+	–
	Clubiona sp. 3	1 f	8	+	–
Corinnidae	*Leichhardteus albofasciatus* Baehr & Raven, 2013	1 f, 1 juv	18	+	–
	Nyssus coloripes Walckenaer, 1805	1 f, 1 m	15	+	–
	Nyssus albopunctatus (Hogg, 1896)	1 f, 1 juv	19	+	–
Cycloctenidae	*Cycloctenus cf. crytophilus* Hickman, 1981	1 f	7	–	–
Deinopidae	*Deinopis subrufa* L. Koch, 1879	2 f, 1 m	34	–	–
Desidae	*Austmusia wilsoni* Gray, 1983	3 juv	15	+	–
	Badumna insignis (L. Koch, 1872)	2 f, 2 juv	33	+	+
	Cambridgea foliata (L. Koch, 1872)	2 f, 1 juv	22	–	–
	Paramatachia sp. 1	1 f	7	+	+
	Paramatachia sp. 2	1 f	10	+	+
	spec. *Namandia* group	1 f, 1 m, 1 juv	21	–	–
(to Stiphidiidae)	*Taurongia* sp.	1 f	7	+	–
Dictynidae	*Brigittea civica* (Lucas, 1850)	3 f	27	–	–
	Paradictyna rufoflava (Chamberlain, 1946)	3 f	23	–	–
Eresidae	*Stygodyphus dumicola* Pocock, 1898	1 f, 3 juv	37	–	–
Eutichuridae	*Calamoneta* sp.	3 juv	32	+	–
Filistatidae	*Kulkulcania hibernalis* (Hentz, 1842)	3 juv	24	+	+
	Wandella orana Gray, 1994	3 f	24	–	–
Gradungulidae	*Kaiya terama* Gray, 1987	3 juv	18	+	+
Hersiliidae	*Tamopsis brisbanensis* Baehr & Baehr, 1987	1 f	8	+	–
	Tamopsis sp. 2	2 juv	20	+	–
	Tamopsis sp. 3	1 juv	8	+	–
Linyphiidae	*Linyphia triangularis* (Clerck, 1757)	1 f, 1 m, 1 juv	22	–	–
Megadictynidae	*Megadictyna thilenii* Dahl, 1906	1 f, 1 m, 2 juv	29	+	+
Mimetidae	*Australomimetus* sp.	3 f	26	–	–
Miturgidae	*Argoctenus* sp.	1 m	6	+	–
	Mituliodon tarantulinus (L. Koch, 1873)	1 f, 1 m, 1 juv	25	+	–
	Nuliodon sp.	3 f	18	+	–
Nicodamidae	*Dimidamus dimidiatus* (Simon, 1897)	1 f, 1 m	18	–	–
	Litodamus olga Harvey, 1995	1 f	7	–	–
	Oncodamus bidens (Karsch, 1878)	2 f, 1 juv	33	–	–
Oecobiidae	*Oecobius navus* Blackwall, 1859	4 f	30	+	+
Orsolobidae	*Cornifalx insignis* Hickman, 1979	1 f	6	+	–
Oxyopidae	*Oxyopes molarius* L. Koch, 1878	2 f, 1 m	21	+	–
Philodromidae	*Philodromus aureolus* (Clerck, 1757)	3 f	25	+	–
	Tibellus tenellus (L. Koch, 1876)	1 f	9	+	–

Table 1. *Cont.*

Family	Species	Individuals Studied	Number of Observations	PMS Used	PLS Used
Pholcidae	*Pholcus phalangioides* (Fuesslin, 1775)	3 f	27	+	−
Pisauridae	*Dolomedes wollemi* Raven & Hebron, 2018	3 juv	20	+	−
	Dendrolycosa icadia (L. Koch, 1876)	2 m, 1 juv	23	+	−
Salticidae	*Sandalodes superbus* (Karsch, 1878)	2 f, 1 juv	30	+	−
Scytodidae	*Scytodes thoracica* (Latreille, 1802)	1 f	10	+	−
Segestriidae	*Ariadna* sp. 1	1 juv	8	+	−
	Ariadna sp. 2	1 juv	10	+	−
	Gippsicola sp.	1 f	8	+	−
	Segestria florentina (Rossi, 1790)	3 f	27	+	−
Sparassidae	*Isopeda villosa* (L. Koch, 1875	1 f, 2 juv	23	+	−
Stiphidiidae	*Neolana dalmasi* (Marples, 1959)	2 f	21	+	−
	Stiphidion sp.	1 f	9	+	−
	Therlinya vexillum Gray & Smith, 2002	3 f	26	+	+
Tetragnathidae	*Leucauge dromedaria* (Thorell, 1881)	3 f	28	−	−
Thomisidae	*Australomisidia pilula* (L. Koch, 1867)	2 f, 1 juv	26	+	−
	Sidymella longipes (L. Koch, 1874)	1 f	8	+	+
	Stephanopis sp. 1	1 f	7	+	+
	Stephanopis sp. 2	1 juv	8	+	+
Theridiidae	*Cryptachaea gigantipes* (Keyserling, 1890)	3 f	22	−	−
Toxopidae	*Toxopsoides* sp.	3 m	25	−	−
	Toxopsoides macleayi Smith, 2013	2 m, 1 juv	20	−	−
Uloboridae	*Philoponella congregabilis* (Rainbow, 1916)	3 f	23	+	−
	Philoponella variabilis (Keyserling, 1887)	4 f, 1 juv	28	+	−
Zoropsidae	*Kilyana* cf. *hendersoni* Raven & Stumkat, 2005	1 f, 1 m, 1 juv	20	+	−

2.2. Observation of Spinning Behaviour

Silk spinning was observed using the methodology described in Wolff et al. [20]. Spiders were attached to wooden sticks with a droplet of dental wax (President Light body, Coltène/Whaledent AG, Altstätten, Switzerland) or dental paint (Shade modification Tint Blue, SDI Ltd., Bayswater, VIC, Australia) on their upper carapace, and brought manually in contact with a glass slide. Leg contact with the glass slide often triggered silk attachment behaviour, i.e., the abdomen was bent down towards the slide and an attachment disc with a consecutive dragline was produced to gain a secure substrate attachment. Spinning events were filmed from underneath the glass slide, using a Basler Ace 640 × 480 pix USB 3.0 high-speed video camera (Basler AG, Ahrensburg, Germany), equipped with a Navitar Precise Eye extension tube including a 1.33× magnification lens (Navitar, Inc., Rochester, NY, USA). A 0.25× accessory lens was used for larger spiders (body length > 10 mm). The resulting field of view was 1.3 × 1.0 mm at a pixel size of 2.1 µm for the basic configuration, and 5.3 × 4.0 mm at a pixel size of 8.3 µm for the configuration with the 0.25× lens. This resolution was sufficient to observe the emergence of silk fibres from distinct spigots. Videos were recorded with 500 frames per second, using the TroublePix software (NorPix, Inc., Montreal, QC, Canada) with continuous looping and post event trigger.

After each spinning event, trials were continued if the spider was still active, or stopped if the spider was inactive or had consecutively spun five anchors. After stopping, spiders were given about 15–30 min to rest, before another trial was initiated.

For each individual spider, I aimed for at least five and up to 15 video recordings of spinning events with at least one ALS, PMS and PLS in frame and focus (see Table 1 for information on species-wise and Supplementary Table S1 for individual replica number). If less than five spinning events were recorded in one run, trials were repeated another day, with repeats on up to three different days, after which no further trials were attempted if the number of spinning observations still remained under five.

Videos were analysed for the action of different spinnerets. The contribution of a spinneret to attachment disc and dragline formation was defined as positive if (a) there was a stereotypic movement of the spinneret during attachment disc spinning and (b) there was a distinct emergence of silk fibres from the spinneret.

2.3. Phylogenetic Comparative Methods

For the comparative analysis, an ultrametric summary tree was calculated from a sample of phylogenomic trees taken from Wolff et al. [21] using the TreeAnnotator programme of the BEAST2 software package [22].

Subsequent analyses were performed in *R* v3.5.1 [23]. The tree was trimmed to match the species list (i.e., dropping of species without data) using the *match_dataphy* command in *sensiPhy* [24]. PMS and PLS usage were binary coded and the evolution of the character state was analysed separately for each trait in *phytools* [25]. Character evolution was modelled by stochastic character mapping [26] with 100 iterations. To choose the best model of character evolution, corrected Akaike information criterion (AICc) values for equal rates (ER) and all rates different (ARD) models were calculated. ER models had lower AICc values and hence, results from calculations using this model are shown in the following.

3. Results

Spinneret use patterns were consistent between juveniles and adults, and females and males of the same species. However, there was a high interspecific variability. I found that in two thirds of all studied species, silk from the PMS was included in attachment discs and draglines. Of these, a quarter added silk emerging from the PLS too (equals one sixth of all species studied). Of 36 species that added silk from the PMS but not PLS, five were cribellar and three were ecribellar web builders (e.g., Figure 1c), the remainder were free hunting spiders (mostly belonging to Dionychia, e.g., Figure 1f,g). Eight of the twelve species that added silk from both PMS and PLS were cribellar web builders (e.g., Figure 1a,b,d,e), and the remainder were ecribellar hunting spiders. Spiders that used silk from ALS only comprised some Filistatidae, members of the marronoid clade (‘Amaurobioidea’) and all tested members of the Araneoidea (e.g., Figure 1h), Nicodamidae and Eresidae, with 75% being web builders (including five cribellates). There was a rare instance of silk adding from the PMS in an araneid (*Phonognatha graffei*, Figure 1i). As this was not a regular occurrence and not observed in other individuals of this species nor other species of Araneidae, PMS spinning was still coded as absent in this species.

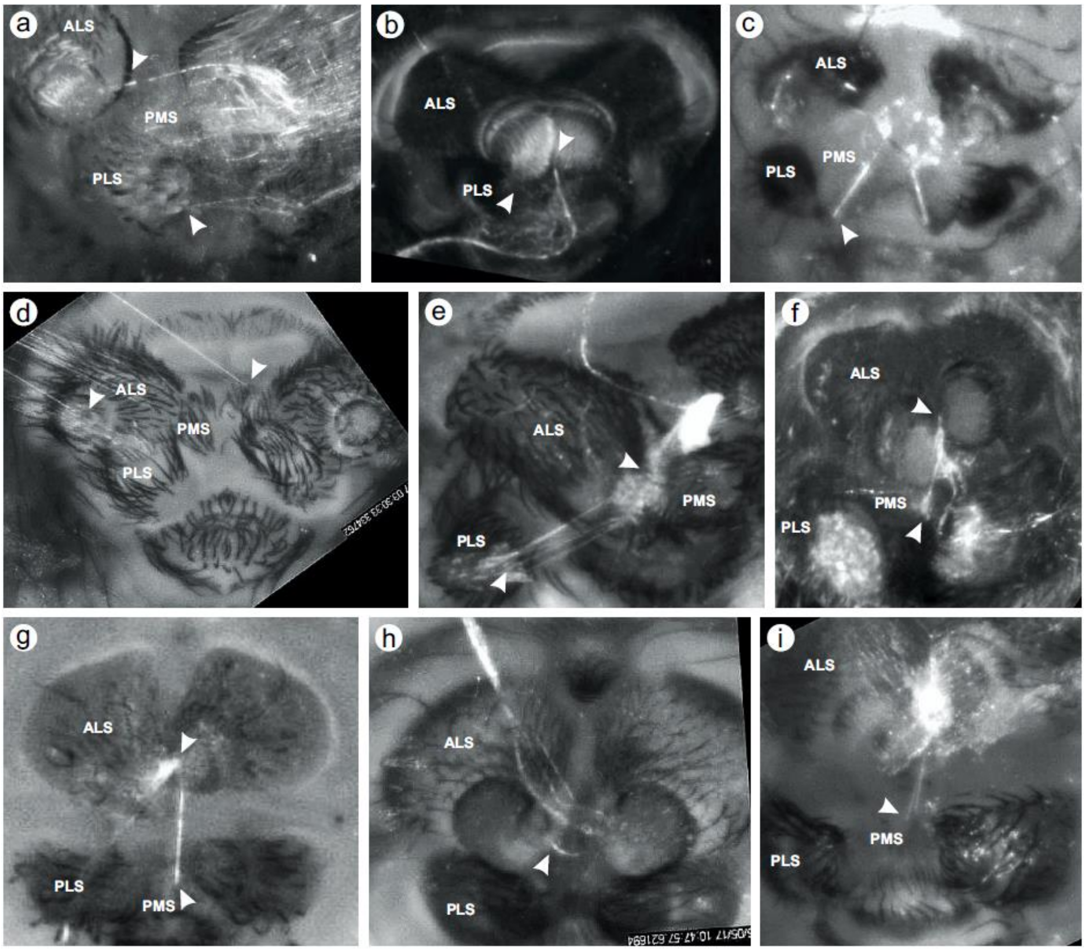

Figure 1. Video stills from comparative micro-high-speed video recordings of attachment disc and dragline formation. The spinning apparatus is seen from below through a glass slide, onto which the spider is attaching its silk. Images are orientated with the anterior side of the spinning apparatus on top. Arrowheads point to distinct silk fibres emerging from spigots on the anterior lateral spinnerets (i.e., major ampullate silk), the posterior median spinnerets (i.e., minor ampullate silk) and posterior lateral spinnerets (presumably aciniform silk), that contribute to the dragline thread. Abbreviations of labels: ALS, anterior lateral spinneret; PLS, posterior lateral spinneret; PMS, posterior median spinneret. (**a**) *Kukulcania hibernalis* (Filistatidae). (**b**) *Hickmania troglodytes* (Austrochilidae). (**c**) *Pholcus phalangioides* (Pholcidae). (**d**) *Amaurobius fenestralis* (Amaurobiidae). (**e**) *Paramatachia* sp. (Desidae). (**f**) *Isopeda villosa* (Sparassidae). (**g**) *Australomisidia pilula* (Thomisidae). (**h**) *Cyrtophora hirsuta* (Araneidae). (**i**) *Phonognatha graffei* (Araneidae).

Stochastic character mapping supported an ALS + PMS condition of spinneret use for attachment disc and dragline formation in the ancestor of araneomorph spiders, with multiple independent evolutionary changes to an either ALS only or an ALS + PMS + PLS condition (Figure 2). Among the clades studied, assuming equal rates of character loss and gain, there were nine reductions to the ALS only condition (at the base of Eresidae, Araneoidea+Nicodamoidea, Deinopidae, within Filistatidae and multiple times within the marronoid clade) and ten switches to the all spinnerets condition (at the base of Austrochiloidea, Megadictynidae, Oecobiidae, and within Filistatidae, Thomisidae and multiple times within the marronoid clade). If different rates are assumed for character gain and loss (i.e., losses occur at faster rates than gains), ALS + PMS remains an ancestral condition in Araneoidea + Nicodamoidea with independent reductions to the ALS only state at the bases of Nicodamidae and Araneoidea. For PLS use, model choice had no effect on the number of character changes.

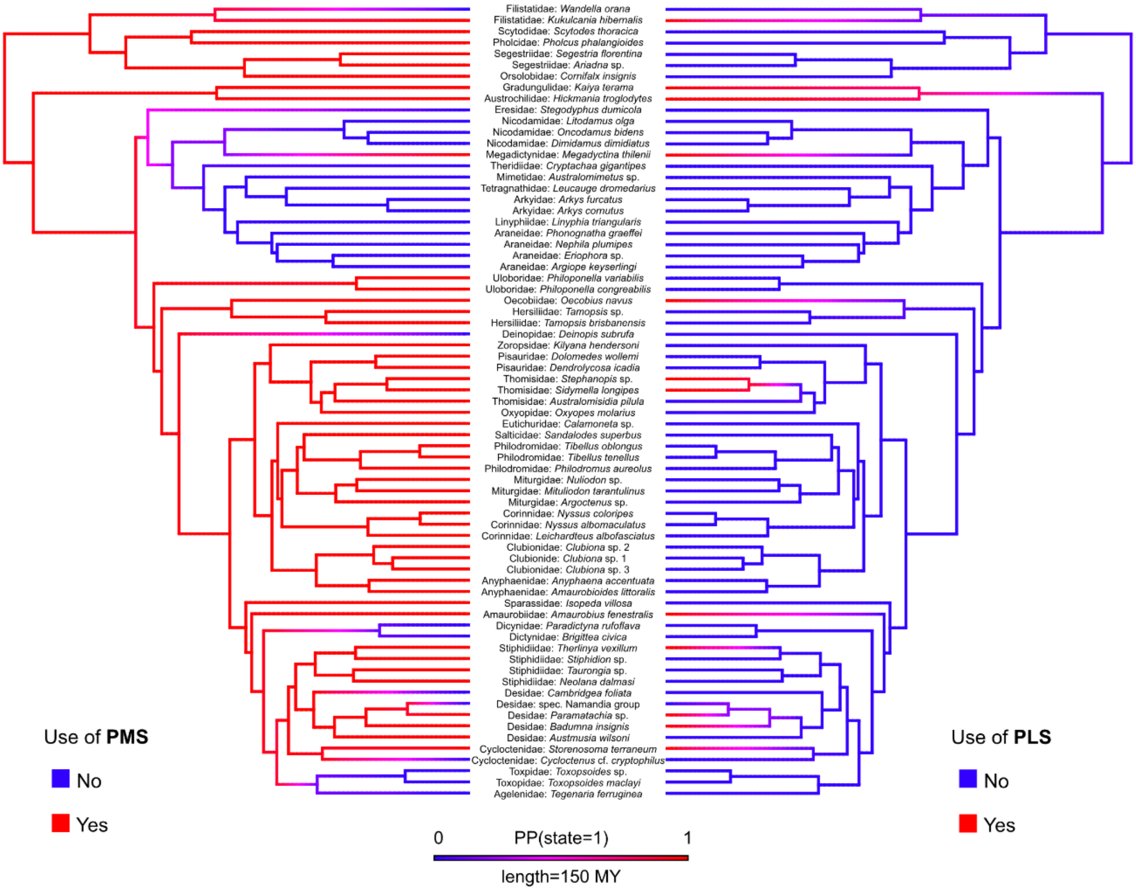

Figure 2. Ancestral character estimation (stochastic character mapping) of spinneret use in araneomorph spiders. Left tree: contribution of silk from posterior median spinnerets (PMS) in attachment disk and dragline formation; right tree: contribution of silk from posterior lateral spinnerets (PLS) in attachment disk and dragline formation. The colour hue represents the posterior probability of the character state in any specific point in the tree, and the length of the scale bar indicates the scale of the geological age represented by branch lengths. Note that for the evolution of PMS use (left tree) in Eresidae + Nicodamoidea + Araneoidea depending on the model used either independent losses of the trait at the origins of Eresidae, Nicodamidae and Araneoidea or a regain of the trait in Megadictynidae are favoured.

4. Discussion

4.1. Most Draglines Are Compound Products

Previous studies have demonstrated that silk lines are attached to substrates with piriform silk [14]. It has been proposed that the spatial arrangement of piriform and major ampullate spigots on the ALS facilitate the fast formation of strong anchorages [8]. This idea presumes that draglines are composed of major ampullate silk only, a widely believed perspective. However, my comparative analysis revealed that single-type draglines are the exception rather than the rule in spiders. The inclusion of minor ampullate silk (from the PMS) is wide-spread, as is the occasional addition of (presumably) aciniform silk from the PMS and, in cases, from the PLS. This has major implications for our understanding of the function of different silk glands in spiders and the evolution of silk properties.

Minor ampullate silk has generally been regarded as a light-weight 'helping line' with temporary character [5,6,9]. The results from this study overturn this image and show that minor ampullate silk is included in a variety of silk threads, some of which may have a long-term function (e.g., draglines as web support structures). The function of aciniform silk has been largely unclear, despite its demonstrated importance in prey wrapping and floating lines for locomotion [5,6,9]. These fine and strong fibres could be added as a means of reinforcement of the silk product or to generate a certain degree of thread stickiness.

While few studies noted the occasional presence of differently sized silk fibres in draglines [8,27–30] interspecific differences of dragline strength have largely been attributed to differences in the structure of proteins in major ampullate silk [31–33]. The major ampullate silk of araneids has been praised for its combination of strength and extensibility, which is outstanding and unachieved by man-made materials [1]. However, the view that araneid major ampullate silk has superior mechanical properties and is hence the best candidate for a biomimetic approach could be flawed, if different fibres in the dragline bundle contribute differently to the material performance of the dragline. For instance, one fibre type could substantially increase the dragline diameter while contributing relatively little load resistance, leading to underestimated strength values of the other fibre type. It follows that future comparative studies of silk properties should incorporate models of deformation and fracture behaviour of compound threads and the separate measurement of isolated fibres.

4.2. Evolutionary Trends

Although an all-spinneret use was found in species that are considered very basal in Araneomorphae (such as *Hickmania troglodytes*), the phylogenetic analysis did not support that this is an ancient character state. The ancestor of araneomorphs most likely used silk from both ALS and PMS, but not from PLS, for draglines. The hypothesis of the combined use of both pairs of spinnerets in ancestral lineages is further supported by the close relationship of the major ampullate gland leading to the ALS and the minor ampullate glands leading to the PMS. The PLS are generally more variable and often elongated in both mygalomorphs and araneomorphs and their silk is often used for special purposes, such as egg sac construction, prey wrapping or the spinning of silken sheets [6]. Why its involvement in attachment disc and dragline formation has repeatedly evolved can only be speculated. A possible explanation is that the addition of more aciniform silk reinforces the dragline and its anchorage. However, as this leads to higher costs of dragline production and complicates the spinning process, there may be a trade-off with the frequency of silk use. I observed that species with the all-spinnerets condition generally needed an exceptionally long time to complete an anchorage and generally made little use of draglines.

The reduction to the ALS-only condition could be associated with an evolutionary enhancement of major ampullate silk properties that made dragline reinforcement by adding minor ampullate or aciniform fibres unnecessary. This led to a simplification of the spinning process and presumably a reduction of material costs, enabling a more versatile and efficient silk use with potential implications for the evolution of different web shapes.

While patterns of spinneret use seem to be very conserved in most clades (e.g., Araneoidea, Dionychia), there are two clades that show an interestingly high dynamism of spinneret function evolution: the Filistatidae, a spider family that diverged early in the evolution of araneomorph spiders, and the marronoid clade, a large group comprised of both cribellar and ecribellar spiders and with notorious problems of its systematics due to the frequent lack of distinct diagnostic characters.

Presuming that the ancestor of filistatids showed the ALS + PMS spinning pattern, and given both studied species are representative for their sub-family, spinneret use went on a divergent evolutionary track in Filistatinae and Prithinae. Both lineages are cribellar web builders and while Prithinae are, on average, smaller there is no major difference in their ecology. However, Prithinae stick cribellar silk threads directly to the substrate without the aid of piriform silk [34], and hence, there may be a reduced need for strong attachment discs, which may be an explanation for the reduced spinneret use.

The marronoid clade is particularly interesting for its unusually frequent switches in spinning patterns. The lability of the trait in this clade is accompanied by frequent changes in the morphology of the spinning apparatus (i.e., repeated reduction or loss of the cribellum) and silk ecology (i.e., diversity of web shapes and high frequency of web reduction and loss). This is especially apparent in the family Desidae, in which all three types of spinneret use, both cribellar and ecribellar and both web builders and free hunters, occur simultaneously. Unfortunately, the Australian and Zealandian marronoids are systematically poorly resolved with the herein used phylogeny being in some part speculative (see Reference [20]). Clearly, the lability of spinning behaviour and silk biology in this group calls for a closer investigation of its evolutionary history. This could lead to major insights into the drivers and associated functions of cribellum loss, which remains an enigmatic key event in the evolution of spiders.

5. Conclusions and Outlook

I have shown that the assembly of draglines and their anchors is much more variable than widely presumed. Clearly, the combination of different silk materials into composite silk products is a topic that should not be neglected in the biomechanical study of spider webs, if we want to achieve a better understanding of silk and web function. These findings suggest that the diversification of silk glands have been a key innovation in spiders that enabled the versatile combination of different silk materials, which may have facilitated the evolution of predatory traps. The behavioural adjustments of silk properties by combining different silk materials may evolve faster than the silk properties based on amino acid sequences. Together with the known plasticity of silk production [35], spinning behaviour could thus play a key role in the evolvability of predatory traps.

To understand the biological role and ecological benefits of compound silk threads, data on the material properties of the different silk types and models of the mechanical behaviour of such compound threads is strongly needed. To date, there is no study that investigated the mechanical properties of all fibrous silk types in one species, and there is only a single study that performed tensile tests of all fibrous silks except piriform silk produced by the same species [5]. A phylogenetic perspective on the properties of different gland products can also guide bioprospecting to identify the best candidates for biomimicry to design novel bio-fibres with superior mechanical performance [2,36]. Bringing back biology into the recently very biotechnology-centred silk research and shifting away from a focus on araneid major ampullate silk could lead to a major advancement of silk biomimicry and overcome persistent problems, such as the fabrication of fibres from an artificial silk dope [2].

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/1/4/s1, Table S1: List of study material and sample sizes.

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Conflicts of Interest: The author declares no competing financial interests.

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