

A comparison of the composition of silk proteins produced by spiders and insects

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Abstract

Proteins that are highly expressed and composed of amino acids that are costly to synthesize are likely to place a greater drain on an organism's energy resources than proteins that are composed of ingested amino acids or ones that are metabolically simple to produce. Silks are highly expressed proteins produced by all spiders and many insects. We compared the metabolic costs of silks spun by arthropods by calculating the amount of ATP required to produce their component amino acids. Although a definitive conclusion requires detailed information on the dietary pools of amino acids available to arthropods, on the basis of the central metabolic pathways, silks spun by herbivorous, Lepidoptera larvae require significantly less ATP to synthesize than the dragline silks spun by predatory spiders. While not enough data are available to draw a statistically based conclusion, comparison of homologous silks across ancestral and derived taxa of the Araneioidea seems to suggest an evolutionary trend towards reduced silk costs. However, comparison of the synthetic costs of dragline silks across all araneomorph spiders suggests a complicated evolutionary pattern that cannot be attributed to phylogenetic position alone. We propose that the diverse silk-producing systems of the araneoid spiders (including three types of protein glues and three types of silk fibroin), evolved through intra-organ competition and that taxon-specific differences in the composition of silks drawn from homologous glands may reflect limited or fluctuating amino acid availability. The different functional properties of spider silks may be a secondary result of selection acting on different polypeptide templates. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Molecular sequence and gene regulation are studied intensively to elucidate the evolution of proteins. Less frequently considered, and more difficult to quantify, is how the resources available to organisms can bias gene expression and hence protein evolution. The baseline cost of any protein is the synthetic cost of each of its amino acid components. Proteins that are highly expressed and composed of amino acids that the organism must synthesize are likely to place a greater drain

on an organism's energy resources than proteins that are composed of ingested amino acids or that are otherwise metabolically inexpensive to produce. This would seem to suggest that, in the absence of other functional constraints, selection will favor lower costs for secreted proteins.

One class of highly expressed proteins are silks produced by insects and spiders. Silks are used for several purposes, including providing shelter, protection for eggs or tools for prey capture [1]. Some investigators have proposed that the diverse amino acid composition of silks may be the result of relaxed selection on proteins used outside of a cellular environment [2]. Alternatively, given the diverse demands placed on silks produced by spiders, in particular those silks used for

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prey capture, it has also been argued that amino acid composition of silks evolved in response to selection for specific functional properties [1,3–8]. Moreover, if the metabolic cost of silk synthesis has been important in the evolution of spiders and insects, then their amino acid composition may reflect the dietary pool of available amino acids in addition to any functional constraints that limit protein configuration.

1.1. Defining cost and cost selection

The sum of the allocation of resources to different metabolic functions is often referred to as an organism's energy budget and includes a measure of the cost of metabolite synthesis as well as a measure of how much of the metabolite is used. For example, if a protein is highly expressed, it may represent a large component of the organism's energy budget and hence restrict the amount of energy that can be diverted to other purposes such as reproduction. However, if the amino acids that make up a protein are abundantly available, then their incorporation may not be metabolically costly [9]. The metabolic cost of protein synthesis discussed here focuses on defining a baseline cost of amino acid synthesis alone. We do not include a usage function that would account for the total amount of silk produced or the availability of amino acids in the organisms environment because these data are not available. Furthermore, while silk recycling is a plesiomorphic (ancestral) character of the Orbiculareae [10], it has not been observed in other spider taxa or arthropods. Therefore, its impact cannot be evaluated without information on the pool of amino acids available to insects and spiders. However, even in the absence of the data outlined above, it is interesting to compare the composition of silks produced by a broad range of insects and spiders and to estimate the inherent metabolic costs of amino acids diverted from the central metabolic pathways.

The starting metabolites of amino acids are derived from the central metabolic pathways that are common to all eukaryotes. Therefore, the amount of ATP devoted to amino acid synthesis can be calculated as the net energy that is sacrificed when a metabolite has been diverted from the breakdown of glucose plus the energy invested in its particular synthetic pathway [11]. With the exception of a unique pathway by which glycine is synthesized by Lepidoptera, the synthetic pathways by which amino acids are produced are thought to be similar for insects and spiders [12]. Therefore, the amount of ATP invested in the production of amino acids represents a common currency through which a relative investment in proteins, and hence silks, can be compared across taxa.

2. Methods

2.1. Silk analysis

Available data on the amino acid composition of silks were compiled from the literature for those produced by Lepidoptera in the Psychidae, Galleriinae, Lasiocampidae, Bombycoidea, and Noctuoidea; by Hymenoptera in the Argidae and Tenthredinidae (sawflies, [2]), and in the Apidae, Vespidae and Sphecidae (bees and wasps [1,13–15]); by the Coleoptera, *Hypera postica* [16]; by the Mantoidea, *Mantis* sp. [17,18]; by the Neuroptera, *Chrysoptera flava* [16]; by the Diptera, *Chironomous tentans* [19]; by the Trichoptera, *Pycnopsyche guttifer* [19] and by spiders in the genera *Araneus* and *Nephila* [2]. We supplement these data with an analysis of silks collected from two different genera of larval Lepidoptera, one species of Embiidina, and 17 species of spiders. Amino acid analyses of the silks were carried out on a Waters–Pico-tag system. All fibers were hydrolyzed in HCl, neutralized, and derivatized with phenylisothiocyanate. The derivatized amino acids were separated and quantified by reverse-phase HPLC.

2.2. Cost estimates

The central metabolic pathways are those through which all ingested material is processed and the energy (ATP) organisms use for growth, maintenance and reproduction is generated. These include the Embden–Meyerhof pathway, which converts glucose 6-phosphate to pyruvate; the tricarboxylic acid cycle (TCA), which oxidizes acetyl CoA to CO₂; and the pentose phosphate pathway, which oxidizes glucose 6-phosphate to CO₂. The intermediate metabolites of these pathways also provide starting molecules from which all nonessential amino acids are produced by insects and spiders. The total cost of any amino acid can therefore be equated to the energy (in the form of ATP) that is lost whenever a metabolite is diverted from the oxidation of glucose, plus the net energy invested into its specific synthetic pathway. Table 1 lists the estimated ATP costs of the input metabolites, the electron carriers, recycled by-products and direct ATP investments that result when insects and spiders synthesize the ten nonessential amino acids found in the proteins they express.

2.3. Comparative analyses

Although almost all insect taxa produce fibrous proteins, silks are derived from different glands (colleterial, salivary, dermal glands, Malpighian tubules) and produced in both adult insects and larvae, whose foraging ecology might be expected to differ. Some of these

Table 1
Estimated bioenergetic costs of amino acids produced by spiders, Lepidoptera and other insects

Amino acids	Metabolites ^a	By-products (energy recycled)	ATP ^b	NADH 1.5 ATP	NADPH 2.5 ATP	Energy sacrificed (ATP)
Alanine (A)	pyr, α kg	α kg			1	15.0
Aspartate (Asx)	oaa, glu	α kg				16.0
Cysteine (T)	M, S	α kb	3			-1.5
Glutamate (Glx)	α kg				1	23.5
Glycine ^c (G)	glyx, A	pyr				14.5
Proline (P)	glu		1		1	27.0
Serine (S)	gly-3p, glu	α kg		-1		14.5
Tyrosine (Y)	F				1	2.5

^a Metabolite data and costs extracted from [31,32].

^b α kg, α -ketoglutarate (21 ATP); α kb, α -ketobutyrate (19 ATP); glu, glutamate (23.5 ATP); glycerate-3-phosphate, gly-3p (14.5 ATP); glyx, glyoxylate (12 ATP); iso, isocitrate (12 ATP); oaa, oxaloacetate (13.5 ATP); pyr, pyruvate (12.5 ATP); F, phenylalanine (0 ATP); M, methionine (0 ATP); S, serine (14.5 ATP); suc, succinate (6 ATP);

^c spiders and insects other than Lepidoptera. As in other insect and spider taxa, alanine, rather than glutamate, serves as the chief amino-group donor in glycine synthesis; however, in the silk glands of Lepidoptera, alanine is an intermediate metabolite that results from the transamination between glutamate and pyruvate [16]. Therefore, the total cost to synthesize glycine in the silk glands of Lepidoptera is estimated to equal 8.5 ATP and results from 1 GTP plus 2 NADH required to produce glyoxylate via conversion of isocitrate to succinate, and 1 NADPH (2.5 ATP) required for the transamination between glutamate and pyruvate.

silks are spun into fibroins and are the sole material used to produce cocoons or egg cases. Others are not spun, but are deposited into fibrous sheets or used as glues [1]. We plotted the amino acid distributions of silks produced by insects in the different orders for which data were available using Kristensen's hypothesis of the phylogeny of hexapods (Fig. 1; 20). More detailed distributions were plotted on independently derived phylogenies for the Hymenoptera, Lepidoptera and spider clades to determine which taxa might represent independent phylogenetic contrasts (Fig. 2a–c). We have included some taxa in the phylogenies to clarify ancestral-descendent relationships but for which we do not have silk data. We limited our cross-taxa comparisons to silks that are derived from similar glands, that are produced during comparable stages of arthropod development and that are used for similar purposes [1]. For example, we compared the composition and cost of silks produced in the colleterial glands by female mantids (*Mantis* sp.: Dictyoptera:Mantoidea [16,17] to the average cost of silks produced in colleterial glands of adults in the order Coleoptera (*H. postica*: Curculionidae [15]) and Neuroptera (*Chrysopa flava*: Chrysopidae [2]). All three groups of insects use silks to protect their eggs and produce them in similar glands during similar stages of their lives [2,18,21].

With the exception of a few taxa (i.e. Psocoptera), the only insects that produce labial gland silks are those whose development is holometabolous [1]. These include larval Hymenoptera, Diptera, Siphonaptera, Trichoptera and Lepidoptera. The estimated costs and amino acid distributions of silks spun by the Hymenoptera are mapped on a phylogeny (Fig. 2a) drawn from recent, independently derived molecular phylogenies [22–24]. Even though both ancestral and derived

Hymenoptera larvae produce silks [18], among the Symphyta, silks are issued from labial glands and spun by larvae, while among the Apocrita they are produced in Malpighian tubules of adult bees and wasps [18,25] (with the exception of the colleterial gland silks of the Colletidae (Apoidea) and the labial gland silks of larval weaver ants). Because these glands may not be homologous, within-order comparisons were not made.

Substantial data are available only on silks produced by Lepidoptera and spiders and therefore these groups are the focus of our analysis. The Lepidoptera phylogeny illustrated in Fig. 2b is redrawn from a phylogenetic estimate proposed by Minet that is based on morphology. It does not include all of the taxa that Minet analyzed [26] and serves to only indicate the relative evolutionary relationships among the Ditrysia for which amino acid data were available. Furthermore, cross-taxa comparisons were only made among groups for which we could estimate mean costs and mean standard errors. For example, because we have only one sample of silk produced by larval Galleriinae, we compared the mean cost of silks produced by the Psychiidae with the mean costs of silks produced by Lepidoptera in the more distant taxa Lasiocampoidea. An additional comparison was made between the Bombycoidea (including Bombycidae and Saturniidae) and the Papilionidae.

The evolution of silk glands and silk synthesis in spiders is even more complex than the evolution of silks in insects because individual spiders produce silks in multiple glands throughout their lives. We estimated the evolutionary relationships among taxa for which we had data on silk amino acid composition using recent phylogenies of the araneomorph spiders [27] and the Orbicularia [28] (Fig. 2c). All of the silks represented

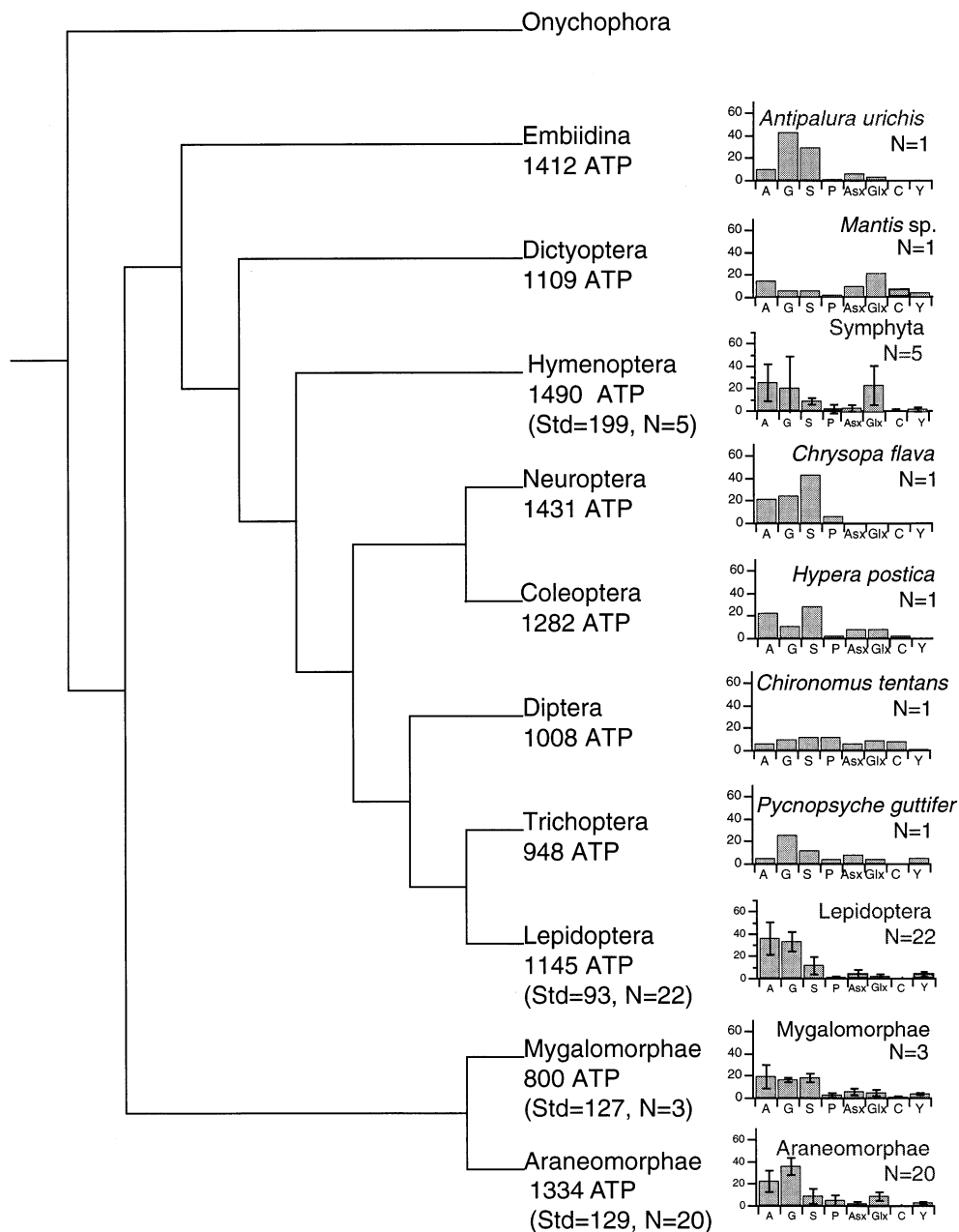


Fig. 1. Phylogenetic relationships among silk-producing orders of insects for which amino acid data are available. Nonparametric comparison of these distributions shows no statistical difference in distribution location. However, visual inspection of the data suggests that some silks are composed largely of alanine, glycine and serine (i.e. Embiidina, Lepidoptera and dragline silks of spiders), while other silks are more varied (i.e. Mantoidea, Coleoptera, Trichoptera).

by the Orbiculariae are drawn from the ampullate gland (dragline silks) and are, therefore, homologous. The location of the distributions of amino acids spun by both Lepidoptera and spider taxa were compared, as were the estimated costs of the silks they spin. The phylogenetic relationship among *Nephila* and spiders in the 'argiopine' clade, however, remains controversial (see [29]) as does the phylogenetic position of the Orbiculariae (Griswold, personal communication).

3. Results

3.1. Amino acid compositions and silk costs

The distributions of amino acids that make up the composition of silks produced by arthropods are summarized across phylogenetic groups and illustrated in Figs. 1 and 2. Sorting silks on the basis of their amino acid composition reveals three groups: (1) silks with

60% or more of their composition consisting of two of the three amino acids: alanine, glycine and serine, (2) silks with 60% of their composition consisting of a combination of two of the amino acids: alanine, glycine and serine plus either proline or glutamine and (3) silks with no two amino acids whose sum equals 60% or more of their composition. The small sample sizes available for most of these data prohibit statistical comparison of the distributions. However, visual inspection of the graphs suggests that almost of the silks produced by Lepidoptera larvae fall into group 1. Silks produced by Embiidina, larval Symphyta and spiders in the Orbiculariae characterize group 2. Silks produced by Diptera, Trichoptera, and Coleoptera larvae, adult mantids, adult Hymenoptera and the mygalomorph spiders fall into group 3 (Fig. 1). Comparison of the locations of the amino acid distribution of silks spun by Lepidoptera and dragline silks spun by spiders (Fig. 1), two groups for which adequate sample sizes exist, show that they differ significantly (Mann–Whitney, $N = 16$, $P < 0.05$).

Baseline costs and compositions of silks vary between 1649 ATP and 96% nonessential amino acids (cocoon silks produced by *Pachylota audouini*, sawfly larvae, Hymenoptera) and 798 ATP and 59% nonessential amino acids (underwater nets produced by *P. guttifer*, caddisfly larvae, Trichoptera; Fig. 3). If all the synthesized amino acids were equally costly to produce, then the metabolic cost of a protein would vary directly with the proportion of nonessential amino acids they contain. While the data plotted in Fig. 3 show this relationship ($P \ll 0.001$, $r^2 = 0.32$), the scatter in the data reveals that variation in amino acid composition can affect total silk cost by as much as 22%. For example, 87% of the composition of dragline silk produced by the spider, *Tegenaria astica*, consists of alanine (16 ATP) and serine (12.5 ATP) yielding a total estimated cost of 1360 ATP (S.D. = 39). In contrast, 87% of the silk produced by the sawfly larvae *P. audouini* (total cost equaling 1662 ATP) is made up of nonessential amino acids, but only 50% of these are alanine, serine and glycine. Glutamine, costing 24 ATP per amino acid, makes up the remaining 36% percent of the protein and as a result, the metabolic cost of silks produced by *P. audouinii* is estimated to be 22% higher (over 300 ATP) than the metabolic cost of silks produced by *T. astica*.

Comparison of mean costs shows that dragline silks spun by spiders are significantly more costly to synthesize than silks produced by larval Lepidoptera (for glycine = 8.5 ATP for Lepidoptera, glycine = 14.5 ATP for spiders; ANOVA, $P < 0.0001$, $N = 42$). Fig. 3 suggests that the estimated cost of silks produced by larval Lepidoptera is slightly less than the estimated cost of a random assemblage of amino acids while the estimated cost of dragline silks produced by spiders is similar to

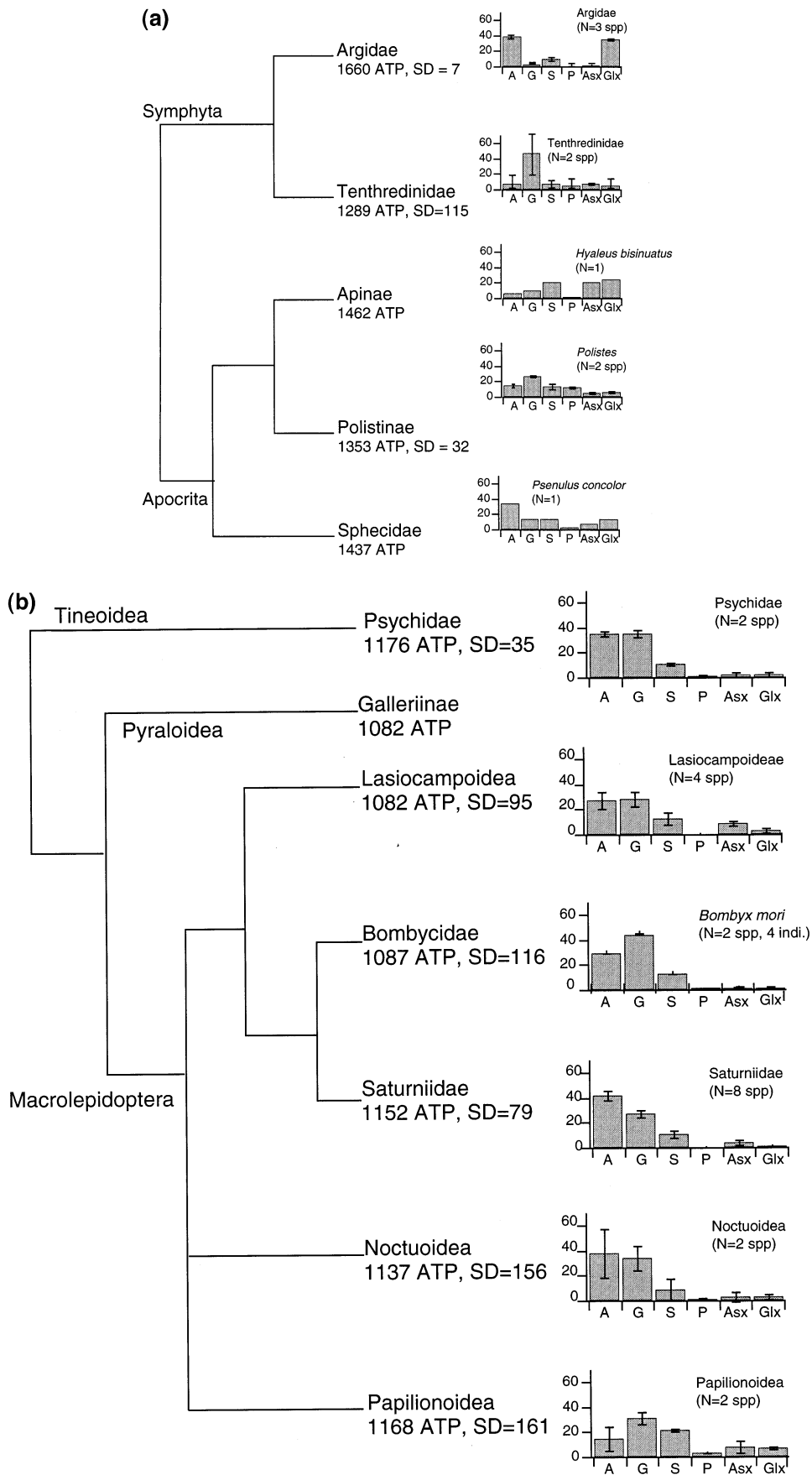
the estimated cost of polyalanine, the putative ancestral polypeptide of spider silks.

3.2. Phylogenetic comparisons

Table 2 compares the estimated cost of silks produced in homologous glands of ancestral and derived arthropods. The data cannot be tested statistically, in part, due to small sample size, and in part due to the different phylogenetic scales over which the comparisons are made. For example, comparison of the costs of silk produced by sister taxa of the araneoid orb-spinning spiders, *Nephila* and *Araneus*, represents a much higher order evolutionary analysis than does a comparison between silks produced by *Hypochilus* (Paleocribellatae) and *Filistata* (Haplogynae). Furthermore, correct comparisons must use the ancestral species of each clade. Nevertheless, is interesting to compare these data in a phylogenetic context to identify possible pathways where more detailed analyses could be fruitful. For example, the mygalomorph spiders produce silks characterized by a mean estimated cost of 1062 ATP, S.D. = 60, and that are less costly than the silks produced by the Paleocribellatae, represented by *Hypochilus pococki*, costing approximately 1302 ATP. *Waitkera* (1235 ATP; Deinopoidea), sister outgroup to the Araneoidea, produce silks that are less costly than the average costs of silks produced by *Latrodectus* and *Nephila* (1421, S.D. = 110). Among the amauroboids, *Tegenaria* (1360, S.D. = 39) produce silks that are less costly than the average costs of silks produced by the more derived *Pisaura* (1463, S.D. = 39). Within the Araneoidea, however, the data seem to suggest a different trend, that more derived species produce silks characterized by reduced cost. This conclusion may change as the current phylogenetic relationships among spiders in the Orbiculariae become better defined (for example, compare [28]). Evolution towards reduced silk costs would be consistent with the direction of cost changes when ancestral-descendent comparisons are drawn between the Lepidoptera (Table 2).

4. Discussion

Herbivorous, silk-spinning Lepidoptera and predatory spiders differ fundamentally in the quantity and quality of resources available to them. The diet of herbivorous insects is energy rich but protein poor. Once an insect egg has hatched on an appropriate food plant, silk synthesis is limited only by the time it takes the larvae to consume and metabolize plant material. Furthermore, because food is likely to be predictable and abundant, larvae are able to produce all of the silk that they need in one kind of silk gland that grows to as much as 60% of the larvae's body weight [30]. At least



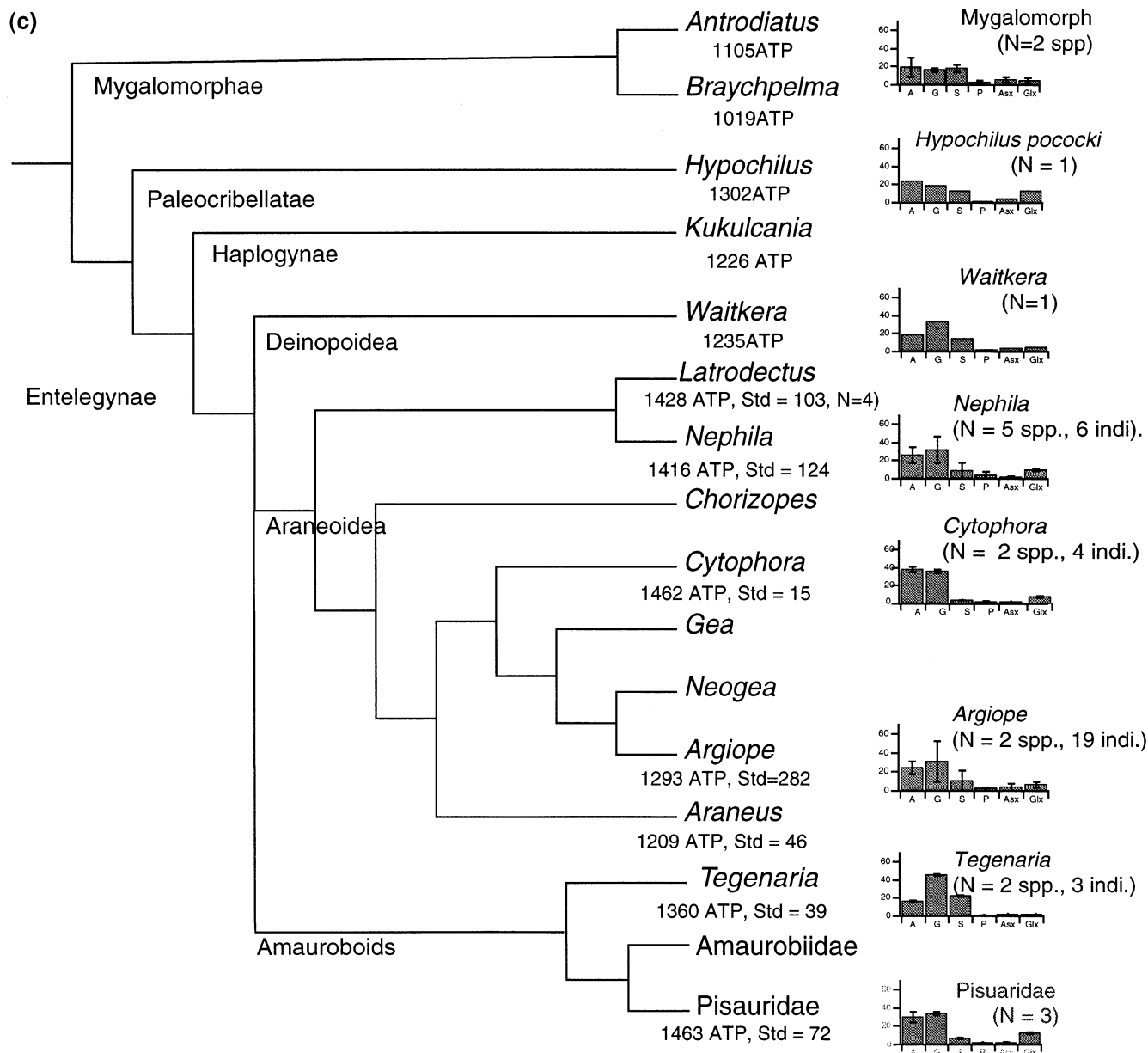


Fig. 2. (a) Phylogenetic relationships among silk-producing Hymenoptera, Lepidoptera and spiders for which amino acid data are available. Proposed phylogenetic relationships among the Hymenoptera are redrawn from recent, independently derived molecular phylogenies [23–25]. Hymenoptera species include *P. audouinii*, *Digelansinus diversipes*, *Arge ustulata*, (Argidae); *Nematus ribesii*, *Phymatocera aterrima* (Tenthredinidae); *Psenulus concolor* (Sphecidae); *Hyaleus bisinuatus* (Apoideae). The location of the distribution of synthesized amino acids and estimated metabolic costs of silks produced by the ancestral (Symphyta) and derived (Apocita) Hymenoptera were not statistically different. (b) The proposed phylogenetic relationships of the Lepidoptera are redrawn from the independently derived phylogenies of Minet [26] and are based on morphological characters. The distribution of amino acids for silks produced by the Ditrysia show no statistical differences, nor do they differ statistically in estimated cost of silk synthesis. Lepidoptera species include: *Clania* sp., *Canephora asiatica* (Psychidae); *Galleria mellonella* (Galleriinae); *Lasiocampa quercus*, *Dictyoploca japonica*, *Pachypasa otus*, *Braura truncata*, *Malacosoma neustria* (Lasiocampoideae); *Antheraea assamensis*, *A. pernyi*, *A. mylitta*, *A. yamamai*, *Attacus pryeri*, *Cricula andrei*, *Eriogyna pyretorum*, *Loepa katinka* (Saturniidae); *Bombyx mori*, *B. mandalina* (Bombycidae); *Bena prasinana* (Agrotidae), *Arctia caja*, (Arctiidae), both Noctuoidea; *Jalmenus evagorus*. (Lycaenidae), *Thisbe irnea* (Riodiniidae) both Papilionoidea (c). Phylogenetic relationships are estimated from independently derived phylogenies of spiders [29,29] and are based on morphological data. Comparison among all taxa show that the locations of the distribution of amino acids do not differ significantly. However, the data suggest that the metabolic costs of silks produced by the Mygalomorphae are less costly to synthesize than the metabolic costs of silks produced by the Araneomorphae. The most striking difference among silks produced by the Araneoidae and other spider taxa is the high degree of variation in silk composition between species within some spider genera, as well as between individuals within some species. Spider species represented on the cladogram: *Antrodiaetus apacheus*, *Brachypelma smithi* (Mygalomorphae); *H. pococki* (Hypochilidae), *Kukulcania hibernalis* (Filistatidae); *Waitkera waitkerensis* (Uloboridae); *Latrodectus nactaris maxiaus*, *Latrodectus* sp. (Theridiidae); *Nephila clavipes*, *N. edulis*, *N. madagascariensis*, *N. tetragnathoides*, *N. plumipes* (Tetragnathidae); *Cytophora* sp., *Argiope argentata*, *A. appensa*, *Tegenaria astica*, *T. pocki*, (Agelenidae), *Araneus diadematus*, *A. cucurbitanus*, *A. undatus*.

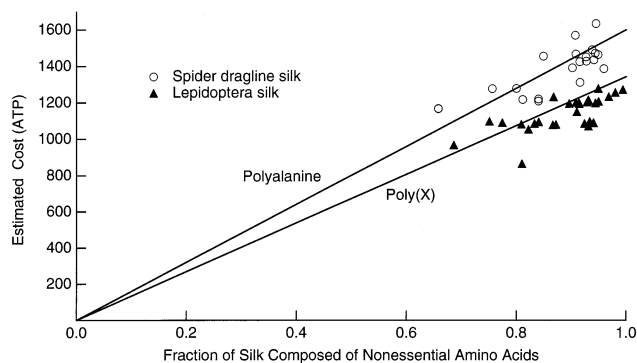


Fig. 3. Estimated synthetic costs of silks are plotted as a function of their proportion of nonessential amino acids. While the silk data shows that cost increases with proportion of amino acids synthesized ($P < 0.001$, $r^2 = 0.32$, $N = 42$), the scatter in the data reveals that variation in amino acid composition can affect total silk cost by as much as 22%. Silks produced by herbivorous Lepidoptera larvae are slightly less costly than silks composed of a random assemblage of amino acids while silks produced by spiders are slightly less than the cost of a fiber composed of entirely of alanine, the putative ancestral polypeptide of spider silk fibroins.

some spiders have equally high demands for silk production, but these demands persist throughout their lives. Even though the diet of predatory spiders is more diverse and protein rich than the diet of an herbivore, it is likely to be energy poor, which may make it difficult to meet silk production needs.

Table 2

Comparisons of synthetic costs of silks between taxa by type of gland. While the limited data may suggest a trend towards reduced silk cost among organisms whose resource base is predictable (herbivores), trends in the cost silks produced by predatory spiders, whose resource base is likely to be variable, are more complex

Ancestral taxa	Derived taxa	Direction of difference between contrasted pairs
<i>Contrasts among silks produced by herbivores (labial gland)</i>		
Psychiidae 1176 ATP, S.D. = 35, $n = 2$ species, two individuals total	Lasiocampidae 1082, S.D. = 95, $n = 4$ species, four individuals total	+
Bombycoidea 1131, S.D. = 116, $n = 5$ species, 12 individuals total	Lycaenidae 1060, S.D. = 20 $n = 2$ species, two individuals total	+
Symphyla (Hymenoptera) 1446, S.D. = 184, $n = 4$	Lepidoptera 1117, S.D. = 110, $n = 20$	+
<i>Contrasts among silks produced by predators and herbivores (colletarial gland)</i>		
Apocrita 1232, S.D. = 160, $n = 4$	Neuroptera + Coleoptera 1251, S.D. = 46, $n = 2$ species, two individuals total	-
<i>Contrasts among ampullate gland silks between predatory spiders</i>		
Hypochilus (Paleocribellatae) 1302 ATP, $n = 1$ species, one individual	<i>Filistata</i> (Haplogynae) 1226 ATP, $n = 1$ species, one individual	+
<i>Tegenaria</i> 1360 ATP, S.D. = 39, $n = 2$ species, three individuals total	<i>Pisaura</i> 1463 ATP, S.D. = 72, $n = 1$ species, three individuals	-
<i>Nephila</i> 1416, S.D. = 124, $n = 5$ species, six individuals total	<i>Araneus</i> 1209 ATP, S.D. = 46, $n = 2$ species, three individuals total	+
<i>Cytophora</i> 1463, S.D. = 15, $n = 2$ species, four individuals total	<i>Argiope</i> 1293, S.D. = 282, $n = 2$ species, 18 individuals total	+
<i>Waitkera</i> 1235, $n = 1$ species, one individual	<i>Latrodectus</i> and <i>Nephila</i> 1421, S.D. = 110, $n = 7$ species, nine individuals total	-

Alanine, serine and glycine are the major components of the silks produced by Lepidoptera larvae and the dragline silks produced by spiders. Nevertheless, the presence of substantial amounts of glutamine and proline (10% or more) differentiate spider silks from silks produced by the Lepidoptera (Fig. 3). As a result, dragline silks produced by the Araneoidea require significantly more ATP than silks produced by larval Lepidoptera. Among insects, large amounts of glutamine or asparagine are present only in the silks produced by the Hymenoptera. In contrast, the ATP cost of silks produced by Lepidoptera larvae is only slightly less than the ATP cost of producing silks from a random assemblage of amino acids. This is, perhaps, not surprising since 97% of the silk produced by the herbivorous, Lepidoptera larvae is composed of alanine, serine and glycine (estimated 16, 14.5 and 8.5 ATP per amino acid respectively), and the average cost of synthesizing any amino acid is equal to 13.3 ATP (S.D. = 9.5).

No data are currently available regarding the use of the intermediate metabolite, alanine, as the amino donor group for the synthesis of silks in any organism other than Lepidoptera. The specialized pathway for glycine synthesis available to Lepidoptera results in an energy saving of 6 ATP, and a comparison of means test shows that mean costs of silks spun by Lepidoptera and spiders (only taxa where sample size is adequate) are significantly different (ANOVA, $P < 0.001$, $n = 42$).

To determine if the difference in cost between spider and Lepidoptera silks is due to glycine alone, we set the synthetic cost of glycine equal to 8.5 ATP for spiders and re-analyzed the data. The mean costs still differed, but the significance of the ANOVA dropped to $P = 0.08$. While this result may indicate the importance of the specialized glycine pathway for the synthesis of silks spun by Lepidoptera, it also suggests that the larger amounts of the amino acids proline, glutamine and asparagine present in silks spun by spiders are important to overall silk cost. Moreover, the costs of silks produced by spiders and Lepidoptera may additionally reflect the composition of the different dietary pools of amino acids that are available to predators and herbivores but about which we have no information.

Arthropods can synthesize ten of the 20 amino acids that they need [12]. Nonessential amino acids that are available externally may be collected using less energy than would be required to synthesize them. Greenstone [12] detailed the nutritional ecology of the hunting spider, *Pardosa ramulosa* (a member of the amauroboid clade), and found that it foraged selectively to optimize the proportion of essential amino acids provided by the three different species of insects that made up most of their prey [12]. Spiders also obtain nonessential amino acids, stored sugars and partially digested metabolites directly from the body tissues and hemolymph of their prey. As Greenstone found in *P. ramulosa*, the specific mixtures of nutrients are likely to differ among insect groups. In particular, the hemolymph of holometabolous insects (such as Hymenoptera, Lepidoptera, Diptera and Coleoptera) retains a greater diversity and volume of free-circulating amino acids than does the hemolymph of hemimetabolous insects (Orthoptera Hemiptera, Homoptera) [31]. This could suggest that spiders foraging in a flowering field and catching predominately pollinating insects (i.e. bees, some beetles and flies, holometabolous insects) will obtain a different and more diverse assemblage of amino acids than spiders foraging in non-flowering sites that attract predominately herbivorous insects (i.e. grasshoppers, bugs and leaf hoppers, hemimetabolous insects). Because the complex of amino acids and the amount of energy available to synthesize silks are likely to vary both locally and seasonally, a silk-producing predator may encounter limiting amounts of different amino acids at different times during its life. In contrast, herbivorous Lepidoptera larvae that feed on plant material such as leaves and stems are assured an unlimited supply of carbohydrates, but relatively low diversity of amino acids.

We propose that the diverse silk-producing systems of the orb-spinning spiders may have evolved in response to the unpredictable composition of their diet coupled with selection to produce a large volume of silk. In particular, we suggest that competition for

limited or fluctuating supplies of amino acids has resulted in the evolution of two different kinds of glands, those that secrete protein glues (the piriform, aggregate and aciniform glands) and those that secrete silk fibroins (the flagelliform, ampullate and tubuliform glands). By retaining six glands that synthesize these two different classes of silks, orb-spinning spiders may be able to produce as large a volume of silk daily as herbivorous Lepidoptera larvae produce just once, prior to pupation. When considered from the perspective of metabolic efficiency, these data could suggest that the diverse functional properties of silks produced by spiders evolved partly as a consequence of selection to make efficient use of fluctuating amino acid availability.

5. Conclusion

In conclusion, we have found that the amino acid composition of silks produced by arthropods is highly variable and, at least across orders, shows no overarching trends suggesting phylogenetic constraint. Nevertheless, comparison of the cocoon silks spun by herbivorous Lepidoptera and dragline silks spun by predatory spiders suggests that differences in dietary energy versus dietary diversity may be important to silk expression. The high alanine, serine and glycine composition of silks produced by Lepidoptera larvae may reflect the predictable and energy rich diet of an herbivore. In contrast, the multi-gland silk producing system of spiders and variable composition of silks suggest that the diet of predatory spiders is both unpredictable and more diverse, reflecting the different types of prey on which they feed.

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