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Cocoon silk chemistry of non-cyclostome Braconidae, with remarks on phylogenetic relationships within the Microgastrinae (Hymenoptera: Braconidae)

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Bulk amino acid composition was determined for cocoon silks for 54 species of non-cyclostome braconid wasps collectively representing 14 subfamilies. Little intraspecific variation was encountered either between conspecific individuals of differing origin or between physically different silk layers within a single cocoon. Variation within subfamilies was small except in the Microgastrinae. Most taxa, excluding most microgastrines, had silk of a fairly typical fibroin type with high relative abundances of alanine, serine or glycine (of which either alanine or serine was the most abundant) and usually with moderately low molar concentrations of presumed acidic residues (aspartate/asparagine (As(x)) and glutamate/glutamine (Gl(x))) which ranged from approximately 2% up to nearly 30% (in Helconinae and Blacinae). In the Microgastrinae, members of the genus *Microplitis* (four species) were similar to the other non-cyclostome subfamilies in having 14.3–26.1 molar % As(x), but the other 10 microgastrine genera investigated produced silks with As(x) the most abundant detected residue comprising 32.4–50.5 molar % while glycine represented less than 10% of residues, indicating an α-helical silk. These data are discussed in the light of some recent independent phylogenetic studies on the Microgastrinae that also suggest a basal position for *Microplitis* within the subfamily, despite its apparently highly specialized biology.

KEYWORDS: Cocoons, silk composition, Braconidae, phylogeny, Microgastrinae, *Microplitis*.

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Introduction

Whereas a great deal of research, both pure and applied, has been carried out on the silks made by various moths, spiders, mussels and even aquatic dipterans, virtually nothing is known about those made by the Hymenoptera except for a fairly thorough survey of sawfly larval cocoon silks (Lucas and Rudall, 1968), some scattered data on aculeate silks including those made by adults, and results for a single genus of Braconidae (Rudall and Kenchington, 1971). A small amount of X-ray crystallographic work has also been conducted on the cocoon silks of various sawflies and on that of the braconid *Macrocentrus thoracicus* (Nees von Esenbeck) (Rudall and Kenchington, 1971). The latter revealed the presence of a novel three-dimensional folding structure of the β-pleated sheets, though its amino acid composition indicated that it was a classical fibroin-type silk (see below). Rudall and Kenchington's study, based like the present one on bulk amino acid composition, indicated that even within the sawfly superfamily Tenthredinoidea, three chemically different silks were produced by different species, namely fibroin, collagen and a polyglycine. The occurrence of such different silks among sawflies prompted us to survey a wide range of different cocoon types produced by other hymenopterans and, in particular, by members of the Ichneumonoidea.

Silks may be defined as any structural fibrous proteins, and their structures typically include a considerable proportion of regular protein arrangements including α-helices, parallel and cross-β sheets and collagen-type folding (Rudall and Kenchington, 1971). These structures are in turn permitted by the presence of a large proportion of amino acids with short side chains. Fibroins, as in the case of silk moth (*Bombyx mori* L. and *Antheraea* spp.) silks, are dominated by β-pleated sheet folding (Rudall and Kenchington, 1971; Datta *et al.*, 2001). They have been classified into a number of groups according to their molar proportion of glycine residues, from group 1 with approximately 45% glycine down to group 6 with only 2% glycine (Rudall and Kenchington, 1971). Classical fibroins, i.e. those from silkworm and similar silks, are largely composed of (glycine-X-glycine-X-glycine-X)$_n$ where X is alanine or serine, though many other similar repeating motifs are known in different taxa (Rudall, 1962; Lombardi and Kaplan, 1990; Craig, 1997; Craig *et al.*, 1999; Datta *et al.*, 2001; Nakazawa and Asakura, 2002). The only braconid for which silk chemistry was known is *Macrocentrus*, and it was found to have a fibroin silk with a typically low proportion of acidic residues and serine, alanine and glycine collectively constituting about 80% of the total amino acid residues. X-ray crystallography of this silk showed it to have an unusual tertiary structure with the molecular strands arranged in groups of three, something known elsewhere only in the nematine sawflies *Cladius* and *Trichiocampus*. α-Helical silks had been found only in the cocoon of the sawfly *Arge* (Argidae) as well as in some adult silks secreted during nest construction by vespid wasps and in the oothecae of praying mantids (Rudall, 1962). These are typified by very high molar proportions of acidic residues (glutamate and aspartate) and low levels of glycine. In contrast, polyglycine with up to 66% glycine residues was recorded in some tenthredinid sawfly silks (*Phymatocera*, *Blennocampa* and *Heterarthrus*). Collagen silks have a characteristic structure with three alpha-helical chains wrapped around each other forming a rope-like triple helix, and each chain is made up of repeats of the motif (Gly-X-Y) where Y and often X are the modified amino acid hydroxyproline. Thus collagen silks are typically about 33% glycine.

We have surveyed silk chemistry across a large range of ichneumonoid parasitic

wasps and show that there is marked variation between higher groupings though, in general, members of the same subfamilies and between some closely related subfamilies are relatively consistent. In this paper we present outline data on all non-cyclostome Braconidae surveyed and comment on possible phylogenetic relationships within the Microgastrinae.

Methods

Abbreviations

Standard three-letter abbreviations for the amino acids are used as follows: Ala, alanine; Arg, arginine; As(x), aspartate/asparagine; Gl(x), glutamate/glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine. Hydroxyproline is abbreviated as HyPro.

Preparation of silk for analysis

Silk fibres were teased from cocoons, sonicated (using an ultrasonic water bath) in 5% aqueous sodium dodecyl sulphate solution, and washed in distilled water to remove soluble contaminants and dirt particles. The dried samples were sent to Commonwealth Biotechnologies Inc. (Richmond, VA) for bulk amino acid analysis.

Conditions for quantification of standard amino acids

Samples of dry silk fibres (0.001–0.11 mg) were transferred to pyrolysed glass tubes and hydrolysed in 400 μl of 6 N HCl under argon at 100°C for 20 h. Primary amino acids were separated and quantified using the standard 'Amino Quant' high performance liquid chromatography (HPLC) procedure after derivatization with orthopthalaldehyde (OPA). All standards, reagents, columns and software were from HP (now Agilent). The HPLC separation used a reverse-phase C18 column and a 17 min ramp from 100% solvent A (20 mM sodium acetate buffer, 0.018% triethylamine, 0.3% tetrahydrofuran, pH 7.2) to 40% solvent B (20% 100 mM sodium acetate buffer, 40% methanol, 40% acetonitrile, pH 7.2) at a flow rate of 0.45 ml min^{-1}. Residues were detected with a programmable diode array detector.

When necessary, two aliquots at different dilutions were analysed, enabling amino acids present at a wide range of concentrations to be determined accurately; in these cases the lower concentration aliquot was used to quantify serine, threonine, aspartate/asparagine, alanine and glycine, and the remaining amino acids were quantified using a higher concentration run. This method does not allow determination of tryptophan or cysteine, and does not provide totally accurate determination of serine. Of these, only serine was present in the silk samples in appreciable quantities and, given the protocol, standards show that the crude serine quantifications underestimate actual serine by 20–30%. Twin time-point analysis, that in principle would allow more accurate determination of serine by back extrapolation, was not feasible because of the insoluble nature of silk. In our results, serine molar proportions are presented as measured and, in interpreting the nature of the silks, these should be increased by approximately 25%. The technique employed could not distinguish aspartate from asparagine residues nor glutamate from glutamine. Most probably, the great majority of these residues in the silks were aspartate and glutamate, i.e. acid side-chain residues, and this assumption was

used in calculating the ratio of acidic/neutral residues (table 2). These uncertainties are expressed as As(x) and Gl(x) respectively.

Conditions for detection of hydroxyproline

Silks from a subset of nine taxa were additionally analysed for the presence of hydroxyproline, a secondary amino acid characteristic of collagen silk. Each sample was transferred to a pyrolysed glass tube and hydrolysed in 0.5 ml of 6 N HCl for 20 h at 110°C. The samples were vortexed once during hydrolysis to aid breakdown of the material present. Following hydrolysis the samples were taken to dryness, then dissolved in 200 μl of sample loading buffer. Samples were diluted to standardize total amino acid concentration, and 1 μl was then subjected to analysis. Each sample was analysed twice. The first run was the standard 'Amino Quant' protocol that includes all amino acids except hydroxyproline (in the OPA derivitization, a small background peak elutes at the same time as hydroxyproline). Therefore to best detect hydroxyproline with no extraneous background peaks a second run was performed which utilized only 9-fluorenylmethylchloroformate (FMOC) derivatization which only derivatizes secondary amino acids. Chromatograms for the hydroxyproline run were expanded and carefully examined for a small peak with the characteristic retention time of hydroxyproline (14.1 min). Only very small peaks were detected—1.7 pmol of hydroxyproline was the highest amount detected in any of the samples.

Some samples presented baseline irregularities in the proline region of the chromatogram. In these cases, the baseline was redrawn and the peak reintegrated but actual proline content may vary from the detected amount.

Phylogeny

The phylogeny of non-cyclostome braconid subfamilies presented in figure 1 is from the independent molecular studies of Belshaw *et al.* (1998) and Belshaw and Quicke (2002). We have not included the Agathidinae + Sigalphinae in this figure because their position with respect to the other subfamilies was not reliably supported.

Materials

Taxa for which hydroxyproline was measured are indicated by an asterisk.

Agathidinae: *Alabagrus stigma* (Brullé)*, British Guiana; *Bassus javanus* (Bhat and Gupta), Malaysia; *Bassus rufipes* (Nees), UK; *Braunsia fenestrata* Kriechbaumer, Kenya; *Disophrys* sp., India; *Earinus gloriatorius* (Panzer), UK.

Blacinae: *Blacus errans* (Nees), Europe.

Cardiochilinae: *Schoenlandella sahelensis* (Huddleston and Walker), Senegal.

Charmontinae: *Charmon cruentatus* Haliday, UK; *C. extensor* (Linnaeus), UK.

Cheloninae (incl. Adeliinae): *Adelius* sp., UK; *Ascogaster rufidens* Wesmael, UK; *Ascogaster similis* (Nees), no data; *Chelonus* aff. *bifoveolatus* Szepligeti*, India; *C. (Microchelonus) contractus* (Nees), UK; *C. scabrator* (Fabricius), UK; *Phanerotoma* sp., India (ex Teak defoliator).

Euphorinae (incl. Meteorinae): *Aridelus* sp.*, Uganda; *Meteorus gyrator* (Thunberg), UK; *M. unicolor* (Wesmael), UK; *Perilitus sichelus* Giard, UK; *Zele albiditarsus* Curtis, UK.

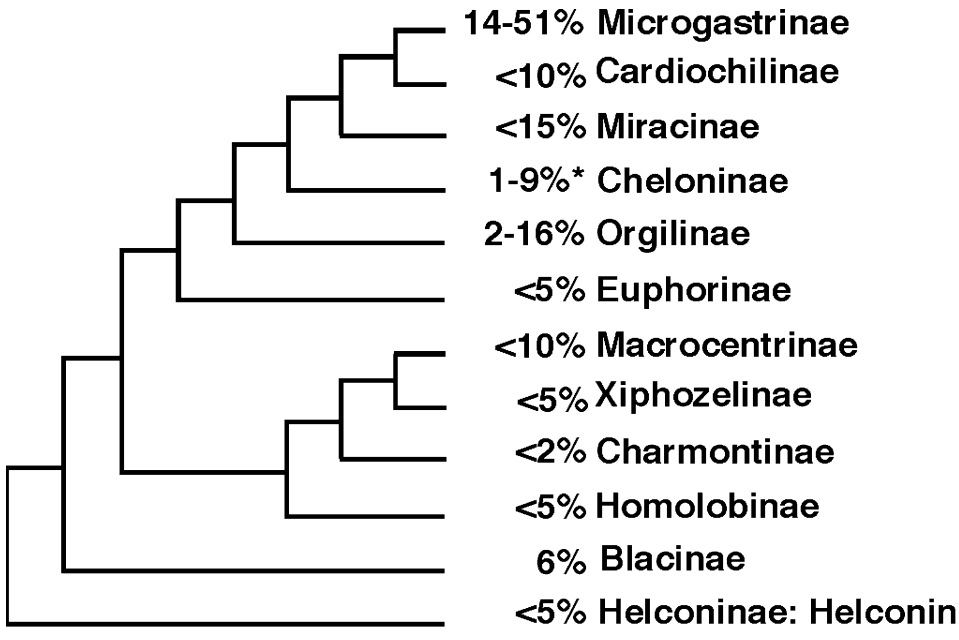

FIG. 1. Phylogenetic relationships of the non-cyclostome subfamilies investigated based on molecular analyses by Belshaw *et al.* (1998) and Belshaw and Quicke (2002) with approximate molar percentages of As(x) in the cocoon silk indicated.

Helconinae: *Helcon angustator* Nees, Netherlands; *Austrohelcon* sp.*, Australia.
Homolobinae: *Homolobus discolor* (Wesmael), UK.
Macrocentrinae: *Austrozele filicornis* (Cameron), Fiji; *Macrocentrus cingulum* Brischke, UK; *M. infirmus* (Nees)*, UK; *M. linearis* (Nees), UK; *M. pallipes* (Nees), UK.
Microgastrinae: *Choeras dorsalis* (Spinola), UK; *Cotesia cajae* (Bouché)*, UK; *Cotesia glomerata* (Linnaeus), UK; *Cotesia rubecula* (Marshall), UK; *Cotesia tibialis* (Curtis), UK; *Diolcogaster alvearia* (Fabricius), France; *Dolichogenidea* sp., no data; *Fornicia* ?*chalcoscelidis* Wilkinson*, Malaysia; *Fornicia* sp., no data; *Glyptapanteles fulvipes* (Haliday), UK; *Microgaster alebion* Nixon, UK; *Microgaster grandis* Thomson, UK; *Microgaster subcompletus* Nees, UK; *Microplitis fordi* Nixon, UK; *Microplitis ocellatae* (Bouché), UK; *Microplitis tuberculifer* (Wesmael), France; *Microplitis tristis* (Nees), UK; *Nyereria mlange* (Wilkinson), Uganda; *Pholetesor circumscriptus* (Nees), UK; *Sathon falcatus* (Nees), UK.
Miracinae: *Mirax* sp., UK.
Orgilinae: *Orgilus leptocephalus* (Hartig), Poland and Netherlands; *O. pimpinellae* Niezabitowski (aggregate), UK; *Stantonia agroterae* Nixon, India.
Sigalphinae: *Acampsis alternipes* (Nees)*, France.
Xiphozelinae: *Xiphozele compressiventris* Cameron*, India.

Results

Amino acid compositions of the analysed silks are given in table 1 as molar percentages.

Intraspecific variation

Three possible sources of intraspecific variation were investigated: (1) between unrelated individuals collected at the same time of year in widely separated localities; (2) between different cocoon morphotypes, i.e. summer versus over-wintering forms; and (3) between different layers of silk within the same cocoon.

For examination of intraspecific variation, silk was analysed for two individuals each for five species: *Chelonus* aff. *bifoveolatus*, *Microgaster subcompletus*, *Microplitis ocellatae*, *Orgilus leptocephalus* and *Xiphozele compressiventris*. In each case, the two samples were very similar in their bulk amino acid composition (table 1).

Inner and outer layers were separated from cocoons and analysed separately for amino acid composition for five of the surveyed species (*Macrocentrus cingulum*, *Zele albiditarsus*, *Xiphozele compressiventris*, *Disophrys* sp. and *Acampsis alternipes*). The compositions of inner and outer layers of each were very similar within species despite the considerable differences in colour and texture, and between-species differences were clearly dominant (table 1). We also analysed separately the cocoon silk proper and the looser and darker brown silk between the cocoons of the gregarious macrocentrine, *Macrocentrus infirmus*, and again found that despite the conspicuous differences in texture and colour, the bulk silk chemistry was very similar (table 1).

Subfamily-level variation

Visual inspection of table 1 shows that members of most subfamilies were generally similar in their overall amino acid compositions. Within the Microgastrinae, however, we observed two distinct patterns of amino acid composition. Cocoon silk was analysed for 19 species within this subfamily, representing 11 genera which are collectively widely distributed through the subfamily in terms of its current classification (Mason, 1981). Members of the Microgastrinae, with the exception of the four species of *Microplitis*, differ from the other species examined in that they have far higher molar percentages of As(x) and approximately half as much glycine (table 1).

Collagen

The reported presence of collagen in the cocoon silks of numerous sawflies prompted us to test for the presence of hydroxyproline in a small but phylogenetically diverse subset of the taxa examined (see table 1). The largest amount detected was 0.3 molar %. Further, collagens typically have approximately 30% glycine and have Gly + Ala + Ser = 50%. Most of the silks investigated here had less than 16% glycine (all non-*Microplitis* Microgastrinae, Miracinae, Orgilinae, most Euphorinae, Agathidinae, Sigalphinae, Blacinae and Helconinae), and those that had rather larger proportions of glycine (e.g. Macrocentrinae, Xiphozelinae, Charmontinae), also had high concentrations of serine and/or alanine such that Gly + Ala + Ser $>>$ 50% and, therefore, at least the bulk of their silk could not be collagen (see table 2).

Table 1. Relative abundances of 16 amino acids in cocoon silks (the most abundant amino acid is indicated in bold; analyses duplicated as part of hydroxyproline detection are indicated by an asterisk).

	Amino acid (molar percentage)															
	As(x)	Gl(x)	Ser	Ala	Gly	His	Thr	Arg	Tyr	Val	Met	Phe	Ile	Leu	Lys	Pro
Microgastrinae																
Choeras dorsalis	**37.03**	1.09	29.15	13.96	9.97	0.94	1.37	0.68	0.86	1.00	0.65	0.70	0.80	0.89	0.55	0.35
Cotesia cajae	**35.7**	2.36	28.9	15.3	4.34	0	1.86	1.76	3.54	1.36	0	0.67	1.2	1.3	1.1	0.71
*Cotesia cajae**	**43.65**	3.37	9.72	15.61	4.69	0.46	1.42	1.36	4.57	2.06	4.70	1.06	1.60	1.81	1.17	2.74
Cotesia glomerata	**37.9**	1.85	29.67	10.8	6.15	1.11	1.39	0.73	3.25	0.79	0	1.69	1.19	0.92	0.42	2.16
Cotesia rubecula	**37.94**	2.27	28.25	13.35	5.85	0.65	2.29	1.03	3.4	1.17	0	0.70	0.99	0.98	0.72	0.37
Cotesia tibialis	**40.0**	1.53	30.05	12.77	8.1	0.39	0.96	0.89	0.64	1.9	0.26	0.31	0.65	0.85	0.68	0.95
Diolcogaster alvearia	**44.79**	1.94	11.76	17.27	7.45	0.38	1.06	0.97	4.82	1.20	0.32	1.12	1.41	1.12	1.04	3.44
Dolichogenidea sp.	**41.0**	1.19	27.76	16.38	5.41	0	0.68	0	3.34	0.92	0	1.0	0.69	0.94	0.22	0.29
Fornicia ?*chalcoscelidis*	**34.73**	1.42	39.35	7.49	6.81	0	2.49	2.36	0	1.00	0	0.56	0.82	1.34	0.83	0.78
Fornicia sp.*	**47.57**	1.97	19.24	9.31	8.85	0.26	2.32	2.97	0.16	1.37	0.16	0.58	1.15	1.94	1.09	0.95
Glyptapanteles fulvipes	**35.97**	1.36	26.39	15.92	7.00	0.52	1.51	0.96	3.00	1.34	0.34	0.46	0.66	0.93	0.72	2.58
Microgaster alebion	**39.95**	1.41	21.90	18.96	6.22	0.39	1.14	1.04	0.57	1.67	0.17	0.19	0.85	1.07	0.90	3.56
Microgaster grandis	**42.67**	1.30	25.11	20.2	3.40	0.33	0.61	0.51	1.16	0.52	0	0.36	0.70	0.65	0.57	1.89
Microgaster subcompletus (specimen 1)	**47.07**	0	24.04	22.95	5.01	0	0	0	0	0	0	0	0	0	0.98	0
Microgaster subcompletus (specimen 2)	**45.19**	1.47	25.13	21.60	5.58	0	0	0	0	0	0	0	0	0	1.18	0
Microplitis fordi	17.42	3.11	**36.00**	2.51	15.37	0.67	1.79	4.48	3.79	2.70	0.51	1.21	1.84	4.46	1.23	2.71
Microplitis ocellatae (specimen 1)	14.36	2.52	**24.32**	15.32	15.34	0.99	4.14	2.82	3.92	3.31	0.67	2.32	1.85	4.43	1.79	1.88
Microplitis ocellatae (specimen 2)	15.11	2.43	17.10	**17.35**	16.20	1.80	3.51	3.14	3.68	3.91	0.89	2.80	2.20	4.85	1.48	3.56
Microplitis tristis	**26.11**	6.31	7.62	13.13	21.88	1.14	0.77	5.92	2.72	4.35	0.58	0.70	2.12	2.65	1.96	2.04
Microplitis tuberculifer	14.95	5.43	**34.73**	6.39	16.90	0	2.86	1.56	3.47	2.22	0.44	0.68	2.87	1.42	0.74	5.36
Nyereria mlange	**32.38**	2.74	24.98	13.3	7.62	0.96	2.14	1.37	3.91	2.09	0.57	1.78	1.39	1.62	0.99	2.12
Pholetesor circumscriptus	**50.50**	0.63	13.13	24.06	6.94	0.78	0.68	0.61	0	0.79	0	0	0.34	1.09	0.44	0
Sathon falcatus	**45.52**	1.72	12.21	21.5	4.70	0.76	0.76	1.00	3.02	1.58	0	0.48	1.01	1.14	1.00	3.69
Miracinae																
Mirax sp.	13.28	13.36	18.40	**31.19**	6.57	0	2.94	4.96	0	2.59	0	0	0	4.26	0.41	0
Cardiochilinae																
Schoenlandella sahelensis	9.77	1.82	**32.98**	24.6	13.52	0	0.98	3.01	0	1.26	0	0	0.99	1.16	1.02	1.14

Table 1. (*Continued*).

	Amino acid (molar percentage)															
	As(x)	Gl(x)	Ser	Ala	Gly	His	Thr	Arg	Tyr	Val	Met	Phe	Ile	Leu	Lys	Pro
Cheloninae (incl. Adeliinae)																
Adelius sp.	7.14	25.88	13.54	**30.64**	5.6	0	5.6	0	1.09	2.37	0	1.27	1.63	3.67	1.58	0
Ascogaster rufidens	1.40	13.90	**32.40**	22.30	17.52	0	0	5.76	0	1.67	0	0	0	4.42	0	0
Ascogaster similis	8.49	3.73	19.92	15.89	**28.67**	1.80	0.58	7.72	0.34	2.16	0.32	2.45	1.68	1.37	2.25	2.65
Chelonus aff. *bifoveolatus*	7.71	5.99	7.97	**36.93**	19.72	1.53	0	8.71	0	3.93	0	0	1.58	1.98	1.51	3.00
Chelonus aff. *bifoveolatus**	8.13	5.49	14.63	**33.38**	18.62	1.41	0.73	8.19	0.31	3.50	0.35	0.29	1.01	1.80	1.33	0.80
Chelonus contractus	3.12	6.1	**33.77**	24.02	16.79	1.18	0.50	3.40	0	2.78	0	0	0.76	4.44	0	0
Chelonus scabrator	**38.3**	1.65	29.0	17.98	5.0	0	1.86	0.81	0.87	2.0	0	0.42	0.72	0.79	0.52	0
Phanerotoma sp.	2.11	14.44	**29.75**	28.16	13.21	0.39	0.63	4.77	0	1.27	0	0.43	0.68	1.75	0.51	1.89
Orgilinae																
Orgilus leptocephalus (specimen 1)	5.39	25.5	24.9	**26.97**	13.3	0	0	0	0	0	0	0	0	0	1.62	2.26
Orgilus leptocephalus (specimen 2)*	15.42	6.29	2.01	**35.39**	11.6	0	2.67	3.82	0	5.25	1.07	0	4.67	7.04	2.91	1.85
Orgilus pimpinellae aggregate	5.34	27.43	23.4	**28.45**	8.63	0	0.54	1.11	0	0.46	0	0	0.57	0.56	1.00	2.47
Stantonia agroterae	2.67	24.31	24.17	**40.57**	3.65	0	0	0	0	0	0	0	0	2.37	0	2.36
Homolobinae																
Homolobus discolor	3.18	22.48	20.26	**43.49**	5.16	0.82	1.1	0.78	0	0.65	0	0	0.45	0.58	0.73	0.36
Euphorinae + Meteorinae																
Meteorus gyrator	1.26	9.64	20.99	**43.53**	13.26	0.77	0.94	0.56	3.6	1.04	2.78	0	0.37	0.53	0.22	0.52
Meteorus unicolor	1.81	1.91	13.71	**52.0**	22.04	1.10	0	0	4.13	0.76	0	0	0	0.49	0	2.05
Zele albiditarsus (outer)	4.09	26.81	22.53	**40.52**	6.04	0	0	0	0	0	0	0	0	0	0	0
Zele albiditarsus (inner)	3.07	25.6	21.68	**34.73**	5.14	0.76	0.73	0	0.51	0.69	0	0	0.69	0.90	0.46	1.98
Aridelus sp.	2.26	9.45	18.08	**56.4**	8.31	0	1.92	0	0	1.72	0	0	1.44	0	0	0.42
Aridelus sp.*	2.54	10.10	16.04	**56.18**	8.37	0.2	1.95	0.12	0.21	1.79	0	0.12	1.50	0.47	0.15	0.21
Perilitus sichelus	4.11	17.72	21.39	**36.67**	7.70	1.50	1.99	0.33	0	1.40	0	0.90	0.51	0.51	0.74	0
Charmontiinae																
Charmon cruentatus	1.58	5.4	**35.3**	24.4	24.9	2.96	0	0.96	0	0.48	0	0	0	0	3.98	0
Charmon extensor (inner layer only)	1.48	5.42	**39.65**	23.03	23.31	1.70	0	0.62	0	0.35	0	0	0	0.34	3.28	0.78
Xiphozelinae																
Xiphozele compressiventris (outer)	1.10	0.83	**28.39**	25.77	28.13	0	0.47	0.2	0	4.75	0	0	2.88	4.64	2.48	0.37
*Xiphozele compressiventris** (outer)	1.06	0.98	16.72	29.94	**30.81**	1.50	0.41	0.22	0.22	5.41	1.18	0.14	3.06	5.36	2.98	0
Xiphozele compressiventris (inner)	4.61	3.46	**29.76**	24.01	25.85	0	0	0	0	4.54	0	0	0	4.74	3.03	0

Table 1. (*Continued*).

	Amino acid (molar percentage)															
	As(x)	Gl(x)	Ser	Ala	Gly	His	Thr	Arg	Tyr	Val	Met	Phe	Ile	Leu	Lys	Pro
Macrocentrinae																
Austrozele filicornis	9.19	8.5	30.79	**31.1**	8.25	1.20	1.06	5.12	0.69	0.87	0	0.36	0.58	0.91	1.09	0.29
Macrocentrus cingulum (outer)	5.9	6.87	25.2	**30.1**	16.0	2.6	0.84	1.98	1.21	1.15	0	0	0.6	1.56	2.55	3.4
Macrocentrus cingulum (inner)	6.55	6.27	27.8	**34.9**	15.6	3.05	0	1.81	0	0.82	0	0	0	1.0	2.28	0
Macrocentrus infirmus (cocoon proper)	5.65	7.10	21.94	**39.0**	20.76	0	0.95	0	0	0	0.746	0	0.67	0.82	1.83	0.51
*Macrocentrus infirmus** (cocoon proper)	6.83	8.81	11.96	**40.30**	21.38	1.06	0.71	0.74	0.49	1.47	0.42	0.48	0.89	1.57	1.90	0.90
Macrocentrus infirmus (fluff between cocoons)	5.48	7.82	23.39	**41.35**	18.38	0	0.63	0	0	0.647	0	0	0	0.977	1.38	0
Macrocentrus linearis	1.49	1.41	23.05	**41.38**	29.90	0	0	0	0	1.11	0	0	0	0	3.64	0
Macrocentrus pallipes	9.45	7.89	19.49	**44.81**	26.73	1.49	0	0	0	0	0	0	0	0.665	5.73	0
Blacinae																
Blacus errans	6.28	20.24	21.00	**41.31**	8.37	0	0	1.39	0	0	0	0	0	0	1.39	0
Helconinae																
Helcon angustator	3.97	19.78	23.98	**36.35**	2.98	1.81	1.07	0.77	0.30	1.06	0	1.07	1.29	1.41	1.71	2.44
Austrohelcon. sp.	3.65	20.73	25.77	**39.03**	2.16	1.43	1.09	0.41	0.11	0.68	0	0.17	0.7	0.79	1.73	1.56
Austrohelcon. sp.*	4.57	25.31	14.12	**44.65**	2.57	1.57	0.83	0.50	0.24	0.87	0	0.24	0.89	0.96	1.67	0.94
Agathidinae																
Alabagrus stigma[†]	3.71	7.73	26.15	**28.04**	12.94	0.6	0.77	7.86	0.52	1.70	0.07	0.86	1.13	5.56	0.68	1.66
Bassus javanus	4.26	11.9	**31.6**	26.30	14.78	0	0	6.32	0	0.91	0	0	0	2.07	0.97	0.93
Bassus rufipes	8.69	4.25	**31.71**	26.48	13.04	0	0.79	9.52	0	1.40	0	0	1.06	2.51	0.72	0
Braunsia fenestrata	8.76	3.07	**36.27**	21.76	10.23	2.0	1.09	8.98	0	0.93	0	1.37	0.90	2.83	1.05	0.74
Disophrys sp. (outer)	4.03	7.53	**45.84**	17.03	15.23	0	0.67	6.63	0	0.55	0.625	0	0.48	0.95	0	0.45
Disophrys sp. (inner)	4.89	8.13	**44.3**	16.7	16.7	0	0	7.51	0	0	0	0	0	1.21	0	0.83
Earinus gloriatorius	2.48	3.53	22.14	**25.57**	11.71	1.6	0	4.37	2.02	2.96	0	0	0.67	1.07	21.71	0
Sigalphinae																
Acampsis alternipes (outer)	2.20	1.97	27.83	**31.99**	15.24	2.14	0.66	7.95	0.31	2.75	0	2.52	0.92	1.49	1.01	0.92
*Acampsis alternipes** (inner)	2.51	2.10	24.77	**33.66**	15.56	1.94	0.72	8.11	0.32	2.96	0	0	2.92	0.96	1.41	1.29

[†]Specimen was relatively resistant to hydrolysis and required longer treatment which would result in a loss of serine.

Discussion

This study focuses on silk chemistry within the non-cyclostome clade of the parasitic wasp family Braconidae and provides the first comparative data on bulk amino acid composition within the family. Previous studies within the Hymenoptera have been restricted to larval sawfly and adult aculeate wasp silks, in both of which considerable variation has been encountered.

Three factors, taken together, suggest that the majority of braconids produce silk that comprises predominantly a single basic type of protein. These are: (1) the consistency in bulk amino acid composition both between conspecific individuals and, more especially, between the physically markedly different layers of the cocoons in all the species investigated in that respect; (2) the very simple tubular silk glands of ichneumonoid wasp larvae; and (3) the amino acid compositions of the silks themselves, which in many taxa were strongly dominated by a single amino acid. This is in contrast to the data on sawflies that suggest that their larval silks often comprise two or more different classes of fibrous protein (Rudall, 1962).

Table 2 provides some summary features of the silk amino acid composition that can be used to help to interpret the nature of the silks involved. In conjunction with other compositional features, the molar % short side-chain amino acids can indicate particular fibrous proteins. For example, in collagen, short side-chain amino acids typically account for 50% of residues, glycine alone comprising 30%, and, at least in vertebrates, with hydroxyproline abundant. The Ala/Gly ratio is approximately 1 in classic (group 1) fibroins, and Ala + Gly account for approximately 80% of residues, though the proportion of Gly is lower in groups 2–6. A high ratio of acidic residues to glycine (Asp + Glu/Gly), or in our case As(x)/Gly as we detected very little Gl(x) in most of our samples, can indicate α-helical silk. Further, hydrophilicity (as indicated by As(x) + Gl(x) + Ser/Ala + Gly) might be expected to be lower with a greater requirement to exclude water, or even to retain water (see Tagawa, 1996).

Almost all the silks analysed showed the typical high relative abundance of short side-chain amino acids of fibrous proteins with molar proportions of Gly + Ala + Ser ranging from 38–74% in the 'microgastroid' subfamilies (Cardiochilinae, Cheloninae, Microgastrinae, Miracinae) to 51–94% in the 'helconoid' group (Blacinae, Charmontinae, Helconinae, Macrocentrinae, Xiphozelinae) (table 2). Previously, *Macrocentrus* cocoon silk has been described as a typical fibroin, with short side-chain amino acids making up approximately 80% of residues and with very low proportions of acidic residues (Rudall and Kenchington, 1971). Our results are largely consistent with this except that in two species, *M. cingulum* and *M. infirmus*, Ala + Gly constituted only 46–61% and the highest detected levels (up to 71%) were found in *M. linearis* and *M. pallipes*.

The great majority of the non-cyclostome braconids investigated produce silks that can be classified as fibroins (i.e. short side-chain amino acids constituting 45–95 molar %; see table 2), and with low molar % of acidic amino acids. At least some of these presumably had long poly-alanine repeats as the molar % of Ala was sometimes very high (56% in *Aridelus*) (table 1). A few taxa have relatively high concentrations of glycine (22% in *Meteorus unicolor*, $>15\%$ in four species of Cheloninae and in all species of *Microplitis*).

Perhaps one of the most interesting and surprising findings is that the microgastrines investigated all had silks with very low molar % Gly and, with the exception of *Microplitis* which appears to have a plesiomorphic silk composition (see figure 1) with respect to the other surveyed microgastrines, very high levels of

Table 2. Summary chemistries and physicochemical properties of bulk silk amino acid composition.

	Molar % short side-chain residues: Gly + Ala + Ser	Major hydrophilic/hydrophic residues: (Asp + Glu + Ser)/(Ala + Gly)	Ala + Gly	As(x)/Gly	As(x)/Gl(x)	Ala/Gly
Microgastrinae						
Choeras dorsalis	53.08	2.81	23.9	3.71	33.97	1.40
Cotesia cajae	48.54	3.41	19.6	8.23	15.13	3.53
Cotesia glomerata	46.62	4.10	20.3	6.16	20.49	1.76
Cotesia rubecula	47.45	3.57	16.9	6.49	16.71	2.28
Cotesia tibialis	50.92	3.43	19.2	4.94	26.14	1.58
Diolcogaster alvearia	36.48	2.37	20.9	6.01	23.09	2.32
Dolichogenidea sp.	59.55	3.21	21.8	7.58	34.45	3.03
Fornicia ?chalcoscelidis	53.65	5.28	14.3–18.2	5.10	24.46	1.10
Glyptapanteles fulvipes	49.31	2.78	22.9	5.13	26.45	2.27
Microgaster alebion	47.08	2.51	25.2	6.42	28.33	3.05
Microgaster grandis	48.71	2.93	23.6	12.55	32.82	5.94
Microgaster subcompletus (specimen 1)	52.0	2.54	27.9	9.40	∞	4.58
Microgaster subcompletus (specimen 2)	52.29	2.64	27.2	8.10	30.74	3.87
Microplitis fordi	53.88	3.16	17.9	1.13	5.60	0.16
Microplitis ocellatae (specimen 1)	54.98	1.34	30.7	0.92	5.70	1.00
Microplitis ocellatae (specimen 2)	50.65	1.03	33.6	0.93	6.22	1.07
Microplitis tristis	42.63	1.14	35.0	1.19	4.14	0.60
Microplitis tuberculifer	58.02	2.37	23.3	0.885	2.75	0.38
Nyereria mlange	45.9	2.87	20.9	4.25	11.82	1.75
Pholetesor circumscriptus	44.23	2.07	31.0	7.27	80.16	3.47
Sathon falcatus	38.41	2.27	26.2	9.69	26.47	4.57
Miracinae						
Mirax	56.26	1.19	36.8	2.02	0.99	4.75
Cardiochilinae						
Schoenlandella sahelensis	71.1	1.17	38.1	0.72	5.37	1.82

Table 2. (*Continued*).

	Molar % short side-chain residues: Gly + Ala + Ser	Major hydrophilic/hydrophic residues: (Asp + Glu + Ser)/(Ala + Gly)	Ala + Gly	As(x)/Gly	As(x)/Gl(x)	Ala/Gly
Cheloninae						
Adelius sp.	49.78	1.29	36.2	1.27	0.28	5.47
Ascogaster rufidens	72.12	1.20	39.8	0.08	0.10	1.27
Ascogaster similis	64.48	0.72	44.6	0.30	2.28	0.55
Chelonus aff. *bifoveolatus*	64.62	0.38	56.6	0.39	1.29	1.87
Chelonus contractus	74.58	1.05	40.8	0.19	0.51	1.43
Chelonus scabrator	51.98	3.0	23.0	7.66	23.21	3.60
Phanerotoma sp.	71.12	1.11	41.3	0.16	0.15	2.13
Orgilinae						
Orgilus leptocephalus (specimen 1)	65.17	1.39	40.2	0.41	0.21	2.03
Orgilus leptocephalus (specimen 2)	49.00	0.50	47.0	1.33	2.45	3.05
Orgilus pimpinellae aggregate	60.48	1.51	37.1	0.62	0.19	3.30
Stantonia agroterae	68.39	1.16	44.2	0.73	0.11	11.1
Homolobinae						
Homolobus discolor	68.91	0.94	48.7	0.62	0.14	8.43
Euphorinae + Meteorinae						
Meteorus gyrator	77.78	0.56	56.8	0.09	0.13	3.28
Meteorus unicolor	87.75	0.24	74.0	0.08	0.95	2.36
Zele albiditarsus (outer)	69.09	1.15	46.6	0.68	0.15	6.71
Zele albiditarsus (inner)	61.65	1.26	39.9	0.60	0.12	6.76
Aridelus sp.	82.79	0.46	64.7	0.27	0.24	6.79
Perilitus sichelus	65.76	0.97	44.4	0.53	0.23	4.76
Charmontiinae						
Charmon cruentatus	84.6	0.86	49.3	0.06	0.29	0.980
Charmon extensor (inner)	85.99	1.00	46.3	0.06	0.27	0.988
Xiphozelinae						
Xiphozele compressiventris (outer)	82.29	0.56	53.9	0.04	1.33	0.916
Xiphozele compressiventris (inner)	79.62	0.76	49.9	0.18	1.33	0.929

Table 2. (*Continued*).

	Molar % short side-chain residues: Gly + Ala + Ser	Major hydrophilic/hydrophic residues: (Asp + Glu + Ser)/(Ala + Gly)	Ala + Gly	As(x)/Gly	As(x)/Gl(x)	Ala/Gly
Macrocentrinae						
Austrozele filicornis	70.14	1.23	39.4	1.11	1.08	3.77
Macrocentrus cingulum (outer)	71.3	0.82	46.1	0.37	0.86	1.88
Macrocentrus cingulum (inner)	78.3	0.80	50.5	0.42	1.04	2.24
Macrocentrus infirmus (cocoon proper)	81.7	0.58	59.8	0.27		1.88
Macrocentrus infirmus (fluff between cocoon)	83.02	0.61	61.8	0.30	0.70	2.24
Macrocentrus linearis	94.33	0.36	71.3	0.05	1.06	1.38
Macrocentrus pallipes	91.03	0.51	71.5	0.35	1.20	1.68
Blacinae						
Blacus errans	70.68	0.96	49.7	0.750	0.31	4.93
Helconinae						
Helcon angustator	63.31	1.21	39.3	1.33	0.20	12.20
Austrohelcon	66.96	1.22	41.2–47.3	1.69	0.18	18.07
Agathidinae						
Alabagrus stigma[†]	67.13	0.92	40.1	0.287	0.48	2.17
Bassus javanus	72.68	1.16	41.1	0.288	0.36	1.78
Bassus rufipes	71.23	1.13	39.5	0.666	2.04	2.03
Braunsia fenestrata	68.26	1.50	32.0	0.856	2.85	2.13
Disophrys sp. (outer)	78.0	1.78	32.2	0.265	0.54	1.12
Disophrys sp. (inner)	77.7	1.72	33.4	0.293	0.60	1.00
Earinus gloriatorius	59.42	0.76	37.3	0.212	0.70	2.18
Sigalphinae						
Acampsis alternipes (outer)	75.06	0.677	47.2	0.144	1.12	2.10
Acampsis alternipes (inner)	74.0	0.678	49.3	0.161	1.14	2.16

[†]Specimen was relatively resistant to hydrolysis and required longer treatment which would result in a loss of serine.

As(x). The amino acid compositions of the silks of these non-*Microplitis* microgastrines can best be interpreted as indicating α-helical silks which, in the Hymenoptera, have only previously been detected in the larval silk of the sawfly *Arge* and in the nest-constructing silks made by the adults of some vespoid wasps (Rudall, 1962).

The phylogeny of the Microgastrinae has been investigated semi-formally (Mason, 1981; Walker *et al*., 1990; Maetô, 1996) and more formally analysed by Dowton and Austin (1998), but there is still no firm consensus. Although a molecular study (Dowton and Austin, 1998), based on the combined analysis of two gene fragments and also a combined molecular and morphological analysis, also suggested that *Microplitis* may be a basal microgastrine, the comparative biology of this genus suggests otherwise (cf. Shaw and Huddleston, 1991; Austin and Dangerfield, 1993, and references therein). All Microgastrinae attack Lepidoptera larvae, and almost all known hosts belong to the Ditrysia. Most ditrysian families are extensively attacked by microgastrines overall, but *Microplitis* species are particularly associated with the most apomorphic families, particularly Noctuidae and to a much lesser extent Notodontidae, Sphingidae and Geometridae. In these hosts, which characteristically feed exposed, the *Microplitis* larvae consume largely haemolymph and fat bodies, leaving much of the host unconsumed and alive after the parasitoid larva(e) have egressed for cocoon formation. Most solitary *Microplitis* egress from premature hosts and in some way prevent loss of host fluid as they do so (possibly by leaving the exuvium of the last feeding instar's skin in their exit hole). The final instar appears not to feed but rather to be specialized for leaving the host and cocoon formation (cf. Mason, 1981). Cocoons are often tough, fluted structures specialized for overwintering (in cool areas) and are often made beneath the still living host (especially in the case of aposematic hosts of solitary species) or are carried more or less dorsally on the hapless host which may then wander off to die in a secluded site that is presumably advantageous to the parasitoid. Gregarious *Microplitis* species more often leave hosts later, for example in subterranean pupation sites in which case the defunct host sometimes subsequently moves away from the parasitoids' cocoon mass in a way that again appears to be adaptive for the parasitoid, as it prevents their cocoons from lying next to the corpse that will tend to rot or decompose septically at high humidity. Similar levels of sophistication in the way hosts are manipulated by *Microplitis* are seen in some genera of Mason's (1981) Cotesiini (cf. Shaw and Huddleston, 1991), but are in sharp contrast to the presumably plesiomorphic habits seen in some genera of Mason's (1981) Apantelini and Microgastrini, which not only tend to parasitize much more basal Ditrysia but also have final instar larvae that engage in external feeding (cf. Shaw and Huddleston, 1991) and construct the simplest cocoons.

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