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Cocoon silk chemistry in parasitic wasps (Hymenoptera, Ichneumonoidea) and their hosts

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Bulk amino acid compositions of larval cocoon silks of 24 species of ichneumonoid parasitic wasps, representing 13 subfamilies that kill the host in a larval or prepupal stage, are compared with those of their hosts to test the hypothesis that amino acid compositions of major protein products should, in certain cases, be similar on energetic grounds. Although substantial variation in amino acid composition was found among both parasitoids and hosts, suggesting the production of different types of silks, no significant general matching was detected. However, the trend in the degree of similarity observed was in the direction predicted by *a priori* consideration of the nature of the parasitoid – host association. Lack of a general association may be explained by the very simple silk glands of the parasitic wasps and by the fact that, in most cases, their hosts are not completely consumed at a time when they are likely to contain any large reserves of silk proteins. The three species of *Cotesia* (Braconidae: Microgastrinae) investigated stood out in that their silks showed considerable interspecific variation in molar percentage amino acid composition, and this might be associated with their apparent utilization of α-helical silks rather than fibroins.

ADDITIONAL KEYWORDS: amino acids – *Cotesia* – energetics – Lepidoptera.

INTRODUCTION

Natural selection is expected to act in such a way that energetically expensive cell biochemical reactions, such as the metabolic conversion of one type of amino acid to another, are minimized (see Craig & Weber, 1998; McDonald, 2001; or for a Gibbs free energy perspective, Amend & Shock, 1998). Under normal circumstances, many organisms, carnivores and parasitoids in particular, contain approximately the same mix of amino acids as their food sources, and therefore, although some processing of amino acids occurs, this is not necessarily extensive. That is, the wide range of proteins in prey tissues approximates, overall, to that of the predator's own tissues and so the relative proportions of each of the amino acids in both is likely to be broadly similar, consequently the proportion of dietary amino acid molecules that have to be converted into other amino acids may be small (see for example, Barrett & Schmidt, 1991). Nevertheless, parasitism often leads to profound changes in host chemical composition (e.g. Bischof & Ortel, 1996; Falabella, Tremblay & Pennacchio, 2000; see also Quicke, 1997) and it is presumed that these are largely under the control of the parasitoid and are generally aimed at improving the host as a food resource. The opportunity to modify host biochemistry in favour of the parasitoid is particularly evident in the case of those displaying the koinobiont life history strategy in which hosts are allowed to continue their development after parasitism (Askew & Shaw, 1986; see also Shaw & Huddleston, 1991). In contrast, idiobionts, whose hosts do not continue development after parasitism, and particularly ectoparasitic ones, appear to have little opportunity to tailor their hosts' physiology to their advantage (see Baker & Fabrick, 2000).

In some situations, the diets of organisms may be highly biased in terms of their amino acid constitutions and very different from the requirements of the organism, necessitating extensive, and energetically

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expensive, reprocessing. In cases such as aphids feeding on phloem fluids or ticks on blood, much of this amino acid processing relies on symbiotic bacteria (see Prosser & Douglas, 1991; Wilkinson & Douglas, 1996; Rahbe *et al*., 2002). In at least some Lepidoptera, a novel amino acid conversion pathway has evolved that is energetically less expensive than the more typical one (O'Brien, Fogel & Boggs, 2002).

There is evidence that the amino acids of silks produced in spiders are determined in part by their insect diets (Craig *et al*., 2000). That is, spiders can modify their silk production in such a way that the amount of amino acid conversion is reduced (Craig *et al*., 1999). Silk production in spiders, the undoubted masters of the art, is of course complex, involving several different types of silk per species, these being produced by a range of glands and extruded through several pairs of spinnerets (Foelix, 1996).

Parasitic wasps as larvae are essentially a form of predator, and the amino acid composition of their diet (insect soft tissues) seems highly likely to correspond broadly to the amino acid composition of the parasitoid. The only previous comparative study of parasitoid and host gross amino acid composition confirms this for an egg parasitoid (Barrett & Schmidt, 1991). However, there is one biological feature that raises a different requirement, at least for the Ichneumonoidea, and that is cocoon formation. The final instar larva of virtually all ichneumonoids that kill their host in its larval or prepupal stage produce at least some sort of cocoon comprising fibroin or α-helical silk (Quicke *et al*., in press). These silk proteins have a highly biased amino acid composition; in typical fibroins, the short side chain amino acids serine, alanine and glycine can comprise between 60 and 80% of amino acid residues, whilst in α-helical silks, aspartate + glutamate may similarly constitute up to 70% (Rudall & Kenchington, 1971; Craig & Riekel, 2002). These compositions start to assume a major metabolic significance when the mass of the cocoon is compared to the dry mass of the adult parasitoid that will emerge from it. We do not know the maximum but for at least one East African species of euphorine braconid, belonging to the genus *Aridelus*, the cocoon weighs more than twice as much as the adult wasp. This is unusually large, but 1 / 1 dry weight ratios are common (D. L. J. Quicke, M. R. Shaw & E. Baumgart, unpubl. data). Thus the wasp expends a very considerable chemical resource in its cocoon and the amino acid processing that goes into converting a typical soft tissue mix of amino acids into these highly biased silks must be very considerable indeed.

While very few parasitic wasps kill their hosts at a time when they are likely to have large amounts of silk in their salivary/silk glands, it is not unreasonable to suspect that, for host taxa that make cocoons, the precursors of the host silks will have been stored in a form requiring rather minimal processing so that silk can be produced quickly when needed. That is, it is to be expected that much of the amino acid processing will have been completed before the various storage proteins are made.

Craig & Weber (1998) have made a strong case that selection can function to reduce metabolic costs associated with amino acid conversions, and this can be achieved either through modification of metabolic pathways or selective use of less costly amino acids, especially in proteins that are produced in bulk. Although selection pressures at the level of the individual amino acid based on its individual energetic cost is extremely small, the collective selection pressure posed by production of a vast quantity of silk with a radically different amino acid composition is high. Thus we would expect that if a parasitoid could evolve a silk that, all other things being equal, was energetically relatively inexpensive to produce, then it would be selected to do so. However, silk genes appear to show a form of evolution with apparently some constraints operating on the repeat motifs (Hayashi & Lewis, 2000); that is, the motifs of short side-chain amino acid coding regions in any given silk gene are repeated many times with considerable fidelity. This implies that if a mutation in one amino acid motif occurs, it is either quickly eliminated or else quickly repeated through the whole gene thus maintaining the similarity of all the repeat units and magnifying the effect in terms of energetics.

We have carried out a survey of amino acid compositions of silks of a number of parasitoid wasps belonging to the superfamily Ichneumonoidea (which comprises the Ichneumonidae and Braconidae) together with those of their hosts (in our case, primarily Lepidoptera). Our choice of parasitoid-host pairs was restricted to cases in which the host produces significant amounts of silk. On the one hand we have concentrated on parasitoid genera (*Agrothereutes*, *Phobocampe* and *Cotesia*) in which different species attack taxonomically widely separated hosts whose silks are likely to differ markedly in their amino acid compositions. Thereby, we hoped to detect any host-related shifts in parasitoid silk chemistry. On the other hand, we have examined parasitoids that collectively exhibit substantial variation in exploitation of their hosts, affording a range of potentially differing accesses to their host's silk chemistry. Three situations that could bear on silk relationships between the host and parasitoid can be discerned: (i) the host is not attacked until its use of silk is over (i.e. the parasitoids are idiobionts ovipositing into cocoons); (ii) the parasitoid is present in or on the host before the host uses its silk for cocoon construction, but it

doesn't extensively consume and kill the host until the host's use of silk is over (i.e. the parasitoids are koinobionts killing the host in a cocooned stage); (iii) the parasitoid completes its growth and kills the host before the latter has started its cocoon construction (in our cases these were koinobionts that kill the host as an incompletely grown larva). Our classification of examined taxa in these categories are given in the Materials Examined section. We predict *a priori* that if there has been selection on parasitoids to produce silks that match availability of host amino acids, then it will be least detectable in group (i), and most apparent in group (iii). Below we show that the observed trend is in the predicted direction but that matching *per se* was not significant even in the case of group (iii) associations.

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; HyPro, hydroxyproline.

Preparation of silk for analysis

Silk fibres were teased from cocoons, sonicated (using an ultrasonic water bath) in aqueous, dilute (5%) sodium dodecyl sulphate solution, and washed in distilled water to remove soluble contaminants such as dirt particles and sericins (see Garel, Deleage & Prudhomme, 1997). Dried samples were sent to Commonwealth Biotechnologies Inc. (Richmond, Virginia) for bulk amino acid analysis. In nearly all cases, it was easy to obtain pure samples of host and parasitoid silks, and especially great care was applied in cases (notably *Rhysipolis* spp.) where cocoons were closely apposed to those of their hosts – differences in colour and texture were helpful in this matter.

Conditions for quantification of standard amino acids

Samples of dry silk fibres (0.023–1.56 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' HPLC (high performance liquid chromatography) procedure after derivatization with OPA (orthopthalaldehyde). All standards, reagents, columns and software were from Agilent. The HPLC separation used a reverse phase C18 column and a 17 minute 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 per minute. 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 would allow more accurate determination of serine by back extrapolation, was not feasible because of the insoluble nature of silk. In our results, only measured serine molar proportions are presented 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, because the other amino acids are relatively rare and especially so in silks studied to date.

Data analysis

To test whether host and parasitoid silks in host-parasitoid relationships had more similar chemistries than expected by chance alone we employed a randomization procedure in Microsoft Excel. Only five amino acids were considered (As(x), Gl(x), Gly, Ala and Ser) because, firstly, these were the only ones that were ever the most abundant amino acid in any sample and, secondly, these were the only amino acids present that ever exceeded 10 molar per cent (Table 1). The relative abundance of these five were ranked for both parasitoid and host and absolute values of the rank differences for these five were summed for each host-parasitoid pair. These values were then summed for all pairs and for selected subsets of taxa. For example, for *Agrothereutes leucorhaeus* and its host *Lasiocampa quercus* (Table 1; first species pair) the rank orders of the molar proportions for the As(x), Gl(x), Ser, Ala and Gly were 5, 4, 1, 2, 3 for the parasitoid and 4, 5, 3, 2, 1 for the host, respectively. The

Table 1. Molar percentages of individual amino acids in silk proteins of various parasitic ichneumonoid wasps and their hosts. The most abundant amino acid in each silk is indicated in bold type face

	Amino acids														
Parasitoid/host associations	As(x)	Gl(x)	Ser	Ala	Gly	His	Thr	Arg	Tyr	Val	Phe	Ile	Leu	Lys	Pro
ICHNEUMONIDAE															
Cryptinae															
Agrothereutes leucorhaeus	2.43	14.13	**33.67**	19.2	15.6	1.34	1.15	5.48	0	1.07	0	0.84	0.99	1.92	2.21
Host – *Lasiocampa quercus*	4.79	2.45	14.84	28.55	**36.3**	0.97	2.08	2.67	2.29	1.19	0	1.03	0.84	0.45	1.53
Agrothereutes mandator	2.85	12.16	**33.05**	17.0	16.6	1.34	1.47	5.15	0.69	1.42	0.38	1.41	1.85	1.49	3.31
Host – *Trichiosoma lucorum*	4.43	2.17	10.72	**44.37**	26.34	0	2.13	2.09	3.33	0.95	2.28	0	1.16	0	0
Agrothereutes saturniae	2.01	14.03	**35.24**	19.9	16.75	0	1.04	3.96	0	0.82	0	0.70	0.88	2.26	2.45
Host – *Saturnia pavonia*	5.00	2.07	14.33	**31.95**	27.1	0.89	1.00	2.47	4.47	2.57	0.65	0.53	5.63	0.28	1.06
Hemiteles similis	7.83	5.06	**36.09**	17.23	19.51	3.4	0.84	5.87	0	0.89	0	0.61	0.91	1.18	0.57
Host – *Zygiella x-notata*	3.38	8.76	22.32	**25.06**	16.94	0	2.83	1.45	2.27	3.51	2.8	1.69	5.0	0.56	3.49
Adelognathinae															
Adelognathus sp.	**44.66**	2.34	13.85	28.12	4.68	0	1.31	0.55	1.01	0.91	0	0.79	1.11	0.69	0
Host – *Nematus leucotrochus*	11.6	9.25	3.05	12.25	**33.37**	0.80	2.57	4.70	2.87	3.51	0.52	2.31	2.03	2.32	8.86
Tryphoninae															
Exenterus abruptorius	7.48	10.40	23.03	**42.55**	6.18	0	4.97	0	0	1.68	0	1.04	1.20	0.71	0.75
Host – *Neodiprion sertifer*(outer)	1.50	1.13	5.52	**50.07**	35.76	0	1.14	0	3.11	0.60	0	0	0.70	0.45	0
Host– *Neodiprion sertifer* (inner)	3.48	3.19	8.38	**45.02**	31.36	0	2.21	0	3.0	1.09	0	0	1.06	1.18	0
Netelia vinulae (inner)	9.16	9.87	27.73	**34.72**	7.52	1.94	0.86	4.37	0.13	0.57	0.11	0.38	0.58	1.22	0.84
Netelia vinulae (outer)	11.2	10.87	21.28	**34.67**	8.67	2.47	0	4.34	0	0.9	0	0.6	0.9	1.69	0
Host – *Cerura vinula*	9.44	10.32	30.43	**35.53**	7.57	1.72	0	4.11	0	0	0	0	0	0.69	0.19
Banchinae															
Lissonota stigmator (pale form)	16.43	4.82	12.42	**52.91**	1.40	2.37	1.60	3.34	0	0	0	0	0	3.11	1.61
Host – *Anthophila fabriciana*	5.77	4.27	12.83	29.37	**29.4**	0.77	1.57	3.03	2.73	2.25	0	2.08	0.68	1.07	4.20
Syzeuctus fuscator	6.0	2.24	**40.85**	21.03	14.86	1.23	0	1.7	0	0	0	0	0	3.3	0
Host – *Pempelia genistella*	2.17	1.62	5.99	**43.68**	32.08	0	1.33	0	2.99	7.54	0	0.50	0.84	0.68	0.56
Campopleginae															
Phobocampe crassiuscula	4.57	1.52	**42.83**	11.59	22.79	1.6	0.69	4.6	3.09	2.81	0.52	1.19	0.6	0.44	0.94
Host – *Opisthograptis luteolata*	10.7	3.64	16.29	19.12	**23.74**	0.39	2.72	2.98	5.59	3.48	0.73	2.54	5.14	1.02	1.87
Phobocampe sp.	3.87	1.59	**42.57**	10.36	22.03	1.8	0.94	4.6	3.67	3.62	0.47	1.37	0.88	0.44	1.15
Host – *Nycteola revayana*	6.3	2.33	22.86	13.59	**35.35**	0.36	3.21	1.37	3.33	2.38	0.6	1.7	3.19	0.54	2.64
Phobocampe unicincta	4.09	1.88	**42.68**	10.47	21.16	1.63	0.85	4.62	3.68	3.56	0.48	1.42	0.89	0.53	1.07

Host – *Calliteara pudibunda*	10.5	4.26	**20.94**	16.28	20.31	0.55	5.14	4.37	3.85	2.37	1.43	1.6	1.78	1.87	4.62
Hyposoter carbonarius	8.51	2.40	**42.33**	10.7	17.94	1.68	1.15	3.9	3.04	2.01	0.52	0.92	1.14	1.88	1.19
Host – *Dicallomera fascelina*	9.68	6.27	19.18	16.76	**20.83**	1.22	5.16	3.05	4.55	0.21	0.73	1.51	1.95	2.0	5.03
Ctenopelmatinae															
Lamachus eques	12.85	9.73	**23.43**	16.67	15.53	1.97	2.21	5.92	1.20	1.54	0	1.90	4.10	3.07	0
Host – *Neodiprion sertifer*	2.17	1.62	5.99	**43.69**	32.08	0	1.33	0	2.99	7.54	0	0.50	0.84	0.68	0.56
Cremastinae															
Trathala sp.	2.44	7.76	**32.16**	30.56	16.38	2.31	0	4.44	0	0.77	0	0	0.73	1.25	1.21
Host – indet. (Lepidoptera)	11.91	5.86	**31.20**	14.9	16.31	0	2.65	0	0	2.49	2.17	1.37	1.30	1.07	8.75
BRACONIDAE															
Rhysipolinae															
Rhysipolis hariolator	3.56	1.95	22.23	6.90	**57.62**	0.93	0.89	0.58	0.37	1.00	0	0.54	1.26	0.50	1.74
Host – *Parornix devoniella*	20.50	9.51	9.15	10.26	**24.70**	0	3.20	1.52	1.50	1.74	0.85	2.29	2.20	2.67	9.93
Rhysipolis sp.	1.86	3.34	39.27	0	**53.64**	0	0	0	0	0	0	0	0	0	1.94
Host – *Mompha raschkiella*	2.01	6.21	9.10	**41.15**	25.86	0.32	1.5	0.74	1.04	2.64	0.24	2.22	2.57	1.3	2.91
Rogadinae															
Clinocentrus exsertor	3.18	1.82	12.6	12.3	**55.82**	0.52	0.27	3.75	0	1.30	0	0.75	1.76	2.25	3.62
Host – Mompha conturbatella	2.63	6.99	6.84	**38.63**	26.88	0	1.24	0.77	0	3.09	0	4.30	5.23	1.25	2.18
Microgastrinae															
Cotesia cajae	**43.65**	3.37	9.72	15.61	4.69	0.46	1.42	1.36	4.57	2.06	1.06	1.60	1.81	1.17	2.74
Host – *Arctia caja*	10.54	9.64	8.07	**19.22**	15.07	1.83	4.54	4.68	5.27	3.87	1.76	2.46	2.87	3.09	6.75
Cotesia zygaenarum	**31.03**	3.58	28.85	7.66	8.0	0.6	3.38	1.75	3.92	2.02	1.35	1.54	1.71	1.06	3.07
Host – *Zygaena lonicerae*	7.27	4.65	14.57	20.23	**31.12**	0.1	3.48	1.68	5.21	3.0	1.65	1.43	2.67	0.34	2.46
Cotesia orestes	**36.3**	2.09	29.07	11.59	6.45	0.51	1.63	1.52	2.42	1.53	1.14	1.28	1.29	0.72	2.06
Host – *Euthrix potatoria*	10.28	3.33	19.06	22.71	**22.79**	0.36	4.15	5.71	4.62	1.12	0.41	0.87	0.93	1.0	2.49
Euphorinae															
Meteorus unicolor	1.81	1.91	13.71	**52.0**	22.0	1.10	0	0	4.13	0.76	0	0	0.49	0	2.05
Host – *Zygaena lonicerae*	7.27	4.65	14.57	20.23	**31.12**	0.1	3.48	1.68	5.21	3.0	1.65	1.43	2.67	0.34	2.46
Orgilinae															
Orgilus pimpinellae	5.34	27.43	23.4	**28.45**	8.63	0	0.54	1.11	0	0.46	0	0.57	0.56	1.00	2.47
Host – *Mompha miscella*	5.56	7.53	3.72	**34.59**	27.07	0	1.64	2.39	0.55	3.61	0	3.62	1.92	4.07	3.81
Agathidinae															
Disophrys sp. (inner)	4.89	8.13	**44.3**	16.7	16.7	0	0	7.51	0	0	0	0	1.21	0	0.83
Disophrys sp. (outer)	4.03	7.53	**45.84**	17.03	15.23	0	0.67	6.63	0	0.55	0	0.48	0.95	0	0.45
Host – *Euproctis* sp.	9.40	7.42	19.0	17.76	**22.83**	0	5.51	5.03	3.65	2.36	0.568	2.07	1.67	1.0	1.74

absolute values of the differences were thus 1, 1, 2, 0 and 2, giving a total sum of rank differences of 6. Relevant null distributions were then generated by recalculating the value for between 100 and 500 data sets in which parasitoid-host pairs were randomly assigned, and the probability of an event at least as extreme as the observed value presented.

MATERIALS EXAMINED

Parasitic wasps in this study were divided into three groups on the basis of life history features that are thought likely to influence the selective pressures to harmonize their silk chemistry with those of their hosts (see Introduction). These groups are as follows and the classification is also given below:

(i) all *Agrothereutes* spp. and *Hemiteles*;
(ii) *Exenterus*, *Netelia*, *Lissonota*, *Syzeuctus*, *Lamachus*, *Trathala*, all *Rhysipolis* spp., *Clinocentrus*, *Orgilus*, and *Disophrys*;
(iii) *Adelognathus*, all *Phobocampe* spp., *Hyposoter*, *Meteorus*, and all *Cotesia* spp.

ICHNEUMONIDAE

CRYPTINAE

Agrothereutes leucorhaeus (Donovan) (M) ex *Lasiocampa quercus* (Linnaeus) (Lepidoptera: Lasiocampidae). Category (i).

Agrothereutes mandator (Linnaeus) (O) ex *Trichiosoma leucorum* (Linnaeus) (Hymenoptera: Cimbicidae). Category (i).

Agrothereutes saturniae (Boie) (M) ex *Saturnia pavonia* (Linnaeus) (Lepidoptera: Saturnidae). Category (i).

Hemiteles similis (Gmelin) (O) ex egg cocoon of *Zygiella x-notata* (Clerck) (Aranae: Araneidae). Category (i).

ADELOGNATHINAE

Adelognathus sp. (M) ex *Nematus leucotrochus* Hartig (Hymenoptera: Tenthredinidae). Category (iii).

TRYPHONINAE

Exenterus abruptorius (Thunberg) (M) ex *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae) (outer and inner cocoon of host analysed separately). Category (ii).

Netelia vinulae (Scopoli) (M) ex *Cerura vinula* (Linnaeus) (Lepidoptera: Notodontidae) (outer and inner cocoon of host analysed separately). Category (ii).

BANCHINAE

Lissonota stigmator Aubert (M) ex *Anthophila fabriciana* (Linnaeus) (Lepidoptera: Choreutidae). Category (ii).

Syzeuctus fuscator (Panzer) (U) ex *Pempelia genistella* (Duponchel) (Lepidoptera: Pyralidae). Category (ii).

CAMPOPLEGINAE

Phobocampe crassiuscula (Gravenhorst) (U) ex *Opisthograptis luteolata* (Linneaus) (Lepidoptera: Geometridae). Category (iii).

Phobocampe sp. (U) ex *Nycteola revayana* (Scopoli) (Lepidoptera: Noctuidae). Category (iii).

Phobocampe unicincta (Gravenhorst) (O) ex *Calliteara pudibunda* (Linnaeus) (Lepidoptera: Lymantriidae). Category (iii).

Hyposoter carbonarius (Ratzeburg) (M) ex *Dicallomera fascelina* (Linnaeus) (Lepidoptera: Lymantriidae). Category (iii).

CTENOPELMATINAE

Lamachus eques (Hartig) (M) ex *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae). Category (ii).

CREMASTINAE

Trathala sp. (U) ex undetermined host (Lepidoptera). Category (ii).

BRACONIDAE/

RHYSIPOLINAE

Rhysipolis hariolator (Haliday) (M) ex *Parornix devoniella* (Stainton) (Lepidoptera: Gracillariidae). Category (ii).

Rhysipolis sp. (O) ex *Mompha raschkiella* (Zeller) (Lepidoptera: Momphidae). Category (ii).

ROGADINAE

Clinocentrus exsertor (Nees) (M) ex *Mompha conturbatella* (Hübner) (Lepidoptera: Momphidae). Category (ii).

EUPHORINAE

Meteorus unicolor (Wesmael) (O) ex *Zygaena lonicerae* (Scheven) (Lepidoptera: Zygaenidae). Category (iii).

ORGILINAE

Orgilus pimpinellae Niezabitowski (U) ex *Mompha miscella* (Denis & Schiffermüller) (Lepidoptera: Momphidae). Category (ii).

AGATHIDINAE

Disophrys sp. nr. *kandyensis* (Cameron) (U) ex *Euproctis* sp. (Lepidoptera: Lymantriidae) on *Careya arborea* Roxb. (Lecythidaceae). Category (ii).

MICROGASTRINAE

Cotesia cajae (Bouchè) (O) ex *Arctia caja* (Linnaeus) (Lepidoptera: Arctiidae). Category (iii).

Cotesia zygaenarum (Marshall) (O) ex *Zygaena lonicerae* (Scheven) (Lepidoptera: Zygaenidae). Category (iii).

Cotesia orestes (Nixon) (M) ex *Euthrix potatoria* (Linnaeus) (Lepidoptera: Lasiocampidae). Category (iii).

The indications (M) (O) and (U) following the parasitoid names above refer to their host-specificity: respectively, monophagous, oligophagous (on closely related hosts) or unknown. Host range assessments are made in relation to the faunal environment concerned (e.g. British Isles).

RESULTS AND DISCUSSION

Considerable variation in amino acid composition was found between the various parasitoid taxa and between the hosts (Table 1) though, with the exceptions of *Adelognathus* and *Cotesia* species, all were dominated by the short side chain amino acids As(x), Gl(x), Ser, Ala and/or Gly. Which particular amino acid was dominant varied between taxa but most silks could be best interpreted as fibroins (i.e. similar to silks of the silk moths *Bombyx* and *Antheraea*). This was particularly extreme in the case of both *Rhysipolis* species in which Ser + Gly constituted 90% or more of all amino acid residues present. The silk of *Adelognathus* was extremely rich in As(x) (presumed to be aspartate) and very low in Gly. This is characteristic of α-helical silks which have previously been found only in mantid oothecae, cocoon silk of the sawfly genus *Arge* (Lucas & Rudall, 1968; Rudall & Kenchington, 1971) and in non-*Microplitis* microgastrine braconid wasp larval cocoons (Quicke *et al*., in press).

For the whole data set of 24 host-parasitoid pairs, the sum of absolute rank differences of the five dominant silk amino acids (As(x), Gl(x), Gly, Ala and Ser) was 128. Randomization of the host – parasitoid associations gave the distribution shown in Figure 1A; the probability of obtaining a value at least as low as 128 by chance is 0.126, indicating that there is a non-significant trend towards a similarity between parasitoids and host silks. Consideration of the data, however, especially in the light of the discovery that most Microgastrinae, apart from the genus *Microplitis*, produce silk rich in acidic amino acids and low in glycine and therefore indicative of α-helical silk, suggests that the presence of three *Cotesia* species in the data set might obscure any relationship (see Quicke *et al*. in press). Carrying out the same randomization procedure on the braconid and ichneumonid parasitoid-host data separately (Fig. 1B, C, respectively), however, showed that although there was a stronger association between host and parasitoid silks within the Ichneumonidae, it was still not significant ($P = 0.153$), though an ANOVA showed that ichneumonids and braconids differed significantly in the sum of ranks ($F = 7.09$; d.f. = 1,22; $P = 0.014$).

We further analysed the data partitioned according to the three biological features (see Introduction and Methods) and the results are presented in Figure 1D–F. No significant relationship was found within any of the partitions: group (i) taxa if anything showed a trend in the opposite direction (observed sum of absolute ranks = 24; $P = 1.0$), group (ii) taxa showed a weak trend in the predicted direction (observed sum of absolute ranks = 60; $P = 0.167$) and group (iii) taxa showed the strongest trend (observed sum of absolute ranks = 44; $P = 0.079$).

Consideration of the three parasitoid genera for which we had data from three representative species shows that interspecific variation in amino acid composition is far less in two of the species than between their hosts (Fig. 2), viz *Agrothereutes* in group (i), and *Phobocampe* in group (iii), whereas in *Cotesia (*in group iii) there is a considerable amount of variation even though all three species have silk dominated by As(x) residues. It is not, however, clear if this means that it is possible for some parasitoid genera to evolve different silks reasonably easily, and the variation within *Cotesia* might be peculiar because of its atypical putative α-helical silk. Barrett & Schmidt (1991) found that the total adult amino acid composition of members of the chalcidoid egg-parasitoid genus *Trichogramma* was somewhat less variable than that of their host eggs, which, as in the present results for *Agrothereutes* and *Phobocampe* silks, is not surprising given the greater taxonomic spread of the host taxa.

The lack of significant matching, in contrast to the apparent ability of spiders to modify the chemistries of their silks in response to diet, may be due to the far simpler structures of the silk glands in parasitic wasp larvae (Sehnal & Akai, 1990; see also Quicke, 1997) or because hosts do not vary greatly in their bulk amino

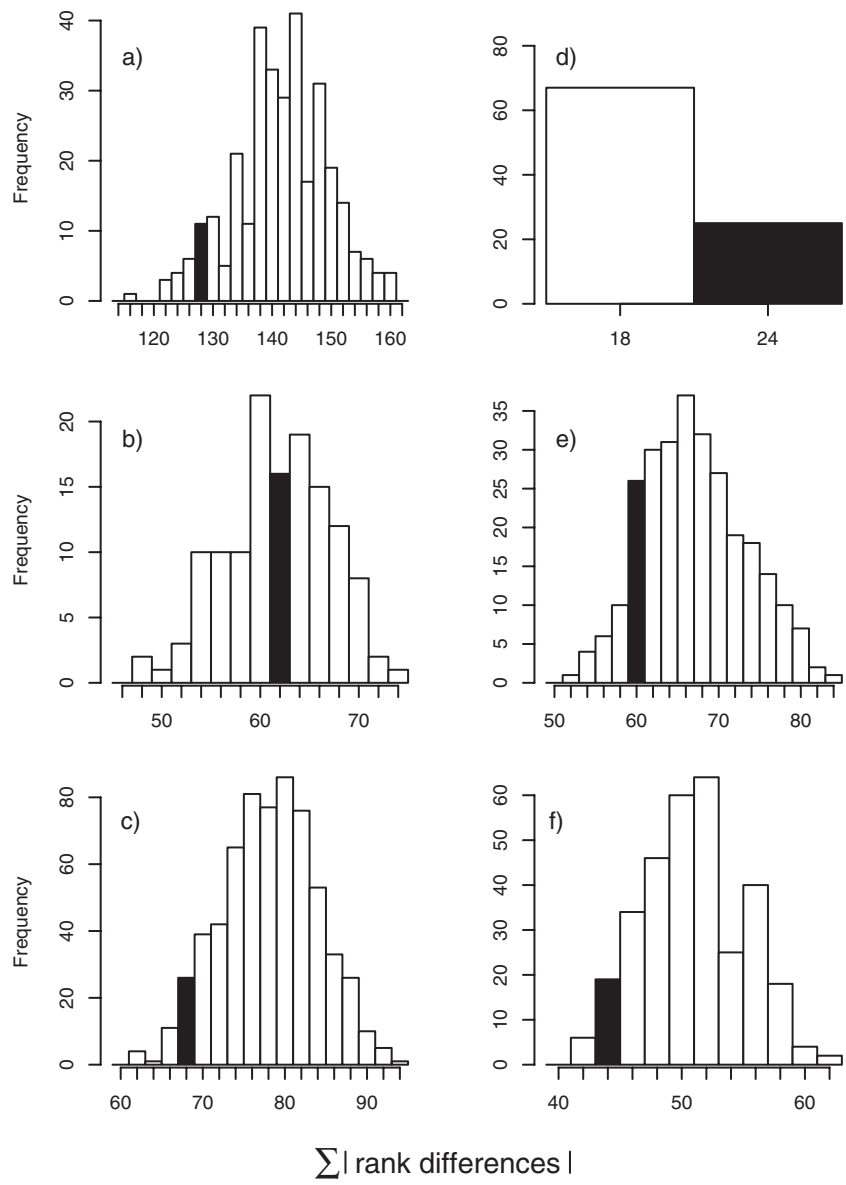

Figure 1. Frequency plots of sums of differences in rank molar abundance of the five most prevalent silk amino acids (As(x), Gl(x), Gly, Ser and Ala) in randomised host parasitoid associations; the value for the natural associations is indicated by the black bar. A, all taxa; B, Braconidae and their hosts only; C, Ichneumonidae and their hosts only; D, group (i) taxa and their hosts; E, group (ii) taxa and their hosts; F, group (iii) taxa and their hosts.

acid composition at the time they are killed, even by parasitoids in our category (iii).

One possibility is that the weak trends observed are not the result of selection on the wasp to match its silk with that of its host *per se*, but rather that silk chemistry might be under independent selection because of environmental conditions where the cocoons are made, and that when wasps and hosts produce cocoons in similar places they will tend towards having to produce silks with similar properties. This could account for the observed interspecific similarities within both *Agrothereutes* and *Phobocampe* silks, in contrast to the differences noted between those of the three *Cotesia* species.

Finally, we would like to point out that silk chemistry appears to vary markedly between different Lepidoptera taxa in agreement with previous findings (see Craig & Riekel, 2002) indicating that it could be of potential phylogenetic significance.

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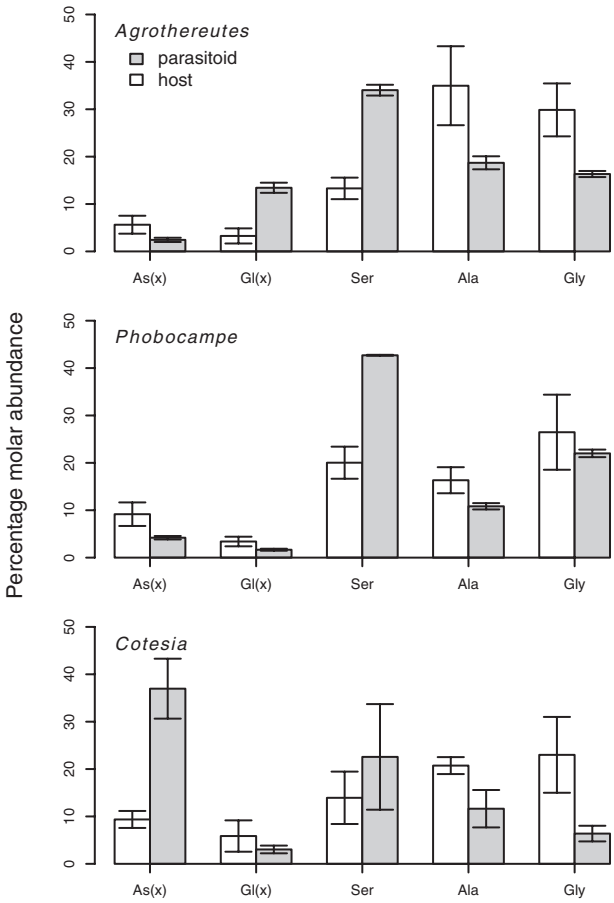

Figure 2. Comparison of variation in molar percentage representations of the five most prevalent silk amino acids in members of three parasitoid genera (each represented by three species) (shaded bars) and their respective hosts (open bars); standard errors are indicated.

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