1998. P. A. Selden (ed.). *Proceedings of the 17th European Colloquium of Arachnology, Edinburgh 1997.*

Guanine as a colorant in spiders: development, genetics, phylogenetics and ecology

G. S. Oxford

Department of Biology, University of York,
P.O. Box 373, York, YO10 5YW, UK

Summary

The distribution and significance of visible guanine deposits in spiders are re-examined using modern techniques (SEM, X-ray crystallography) and in the light of phylogenetic information. The disposition and extent of guanine deposits can change markedly during development and there is a close and predictable relationship between presence of guanine and overlying hypodermal pigmentation. Various genetic models developed to explore this relationship are described and evaluated. The dichotomy between matt and silver guanine deposits, earlier recognized by Millot, is examined at the EM level; a third type might be present in some families. Preliminary X-ray crystallography results suggest that these two major guanine types have identical crystal structures, implying that their gross morphologies result from different growth conditions. Matt guanine appears to be the ancestral type and, on the basis of recent phylogenies, the silver form appears to have arisen independently from it at least eight times. Analyses of visible guanine presence, habitat types and body size in the British spider fauna confirm Millot's observation that guanine is very unevenly distributed among families. It is generally more common in species occupying open rather than closed environments, and in large spiders rather than small.

Introduction

Coloration in spiders may serve a number of important functions, for example crypsis, mimicry, aposematism and thermoregulation (Oxford & Gillespie, 1998). However, the evolutionary elaboration of bright colours for these purposes is not straightforward. The opisthosomas of spiders have rather thin, elastic cuticles (Barth, 1973; Dalingwater, 1987) to allow for large changes in volume associated with sporadic feeding (Riechert & Harp, 1987) and, in females, the development of eggs. A consequence of this thinness is that the cuticle is virtually transparent. The peripheral volume of the opisthosoma is dominated by the digestive mass which, in most species, is a shade of brown. This, then, dictates the basic background colour upon which pigments have to be deployed. For species in which black or brown coloration is selectively advantageous, sufficient pigment can be deposited in the hypodermis for the optimum overall colour to be achieved. However, lighter pigments, for example yellows and reds, remain almost invisible against the brown background of the digestive mass.

Spiders have evolved two devices to circumvent this problem when bright colours are advantageous. The first involves effectively increasing the thickness, but not the stiffness, of the cuticle by the use of hairs or flattened setae which may contain pigments or appear white or silvery as a result of structural coloration (Hill, 1979; Holl, 1987). This option is exploited in a number of families, for example the Salticidae. A second solution to the problem, adopted by many taxa, is to generate a white background between the brown digestive diverticula and the hypodermis, against which hypodermal pigments are viewed. This is achieved by depositing often massive amounts of guanine, the principal nitrogenous excretory product in spiders (Anderson, 1966), in peripheral cells of the digestive diverticula (Millot, 1926). Although normally confined to the opisthosoma, guanine is sometimes also found in the portion of the digestive mass that extends forwards into

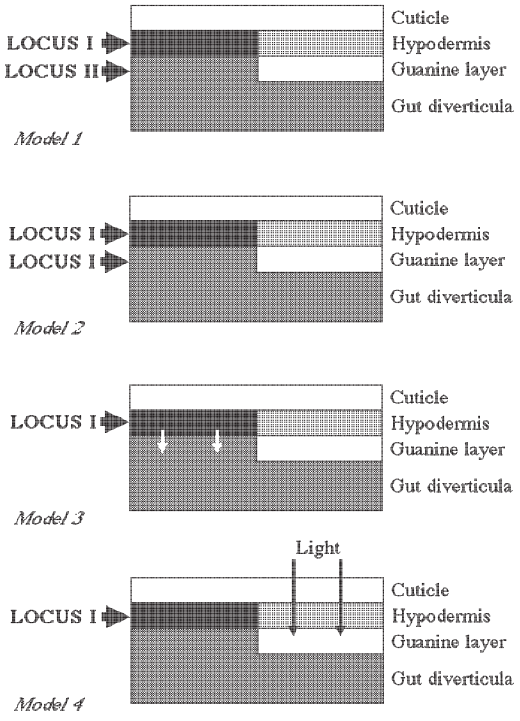


Fig. 1: Possible genetic models to explain the close co-ordination between pigmentation and guanine deposition. Each diagram depicts a vertical section through the opisthosoma, the right half of the section represents a lightly pigmented hypodermis (light grey) underlain by a white guanine layer (white), and the left half a black-pigmented hypodermis (black) without underlying guanine. The brown digestive mass is shown dark grey. The genetic system acting in the hypodermis and peripheral digestive mass is given on the left of each diagram. White arrows in Model 3 signify chemical communication between tissue types. See text for full explanation.

the prosoma (Foelix, 1996), for example in immature *Metellina mengei* (Metidae).

Developmental changes in pigment and guanine distributions—some models

The deposition of guanine in peripheral guanocytes does not indicate that the spider is unable to excrete the product at a fast enough rate. Indeed, spiders possess mechanisms that enable excretory products to be diverted towards storage rather than evacuation (Collatz, 1987; Seitz, 1987). In some species second instar juveniles, newly emerged from the egg sac and prior to feeding, possess no visible guanine (e.g. *Tegenaria* spp. (Agelenidae)) whereas in others massive deposits are found (e.g. *Latrodectus* spp. (Theridiidae) and *Araneus* spp. (Araneidae)). Changes in guanine and in overlying pigments are very tightly orchestrated throughout development. The usual situation is that visible guanine is present under areas of unpigmented hypodermis and where red and yellow pigments occur, but is absent under regions of dark brown or black pigment. The amount and disposition of visible guanine can change markedly during growth, either increasing or decreasing its importance as a colorant. In *Latrodectus mactans*, for example, the heavy deposits of guanine characteristic of the entire opisthosomal surface of second instar juveniles gradually disappear as dark pigments develop in the hypodermis. In mature females, guanine deposits are lost completely except, significantly, from beneath the ventral, red hourglass pattern. In the Hawaiian happy-face spider, *Theridion grallator* (Theridiidae), it has been demonstrated that the very precise one-to-one matching of hypodermal pigmentation and underlying guanine deposits are apparently under the control of a small number of major gene loci (Oxford & Gillespie, 1996a,b).

How is the general pattern of guanine presence under pale (or no) pigments, and guanine absence under dark pigments, generated? Figure 1 presents four possible models which explore the genetical control of guanine and pigmentation and their co-ordination. Model 1 involves two separate and independently regulated loci with locus I active in the hypodermis and locus II in the peripheral digestive diverticula. This model recognizes that the major tissues involved in the co-ordination have very different embryological origins: the hypodermis from the ectoderm and the digestive diverticula from the endoderm (Foelix, 1996). It also recognizes that the biochemical pathways involved in guanine and pigment (mostly ommochromes—Seligy, 1972) deposition are quite different. However, the complexity of matching developmental and spatial regulation required by this model, and the necessity for parallel mutations at the loci concerned, weigh heavily against it. Model 2 involves just one locus with independent,

pleiotropic effects in the two tissues. Parallel mutations are therefore not involved, but the problem of developmental co-ordination remains. There is also the additional requirement that a single locus is capable of controlling disparate biochemical pathways. Model 3 also involves one locus, but this acts directly on the hypodermal pigmentation alone. The presence and absence of guanine beneath is a result of chemical communication between the hypodermis and the peripheral digestive diverticula. Yellow and red pigments are predominantly ommatins, whereas black and browns are usually a combination of ommatins and ommins (Seligy, 1972). One possibility, therefore, is that the presence of guanine is constitutive (in those taxa that show visible guanine—see below) except where ommins are present in the overlying hypodermis. If ommins (or components of the pathway leading to ommins) exert an inhibitory effect, then this would explain why unpigmented areas (for example, the white cross of *Araneus diadematus* (Araneidae)) as well as ommatin-only regions are underlain by guanine. The genetic basis for Model 4 is identical to that for Model 3, but here the guanine layer is induced rather than inhibited. Model 4 postulates that guanine is deposited under unpigmented or lightly pigmented areas as a result of the action of light. So, in the example of *Latrodectus mactans* mentioned above, as the depth of pigmentation increases during development under the influence of locus I, light intensities impinging on the peripheral cells of the digestive diverticula decrease and, as a result, so does the amount of stored guanine.

At present, Models 3 and 4 seem the most plausible and need not be mutually exclusive. Tests to differentiate these models might involve finding spiders whose dark coloration is a result of tanning of the cuticle (Cutler & Richards, 1972) rather than pigmentary. If Model 3 is correct, guanine deposition should not be inhibited beneath the tanned areas whereas in Model 4 it should. An easy way to verify Model 4 is to confine spiders in the dark and compare guanine levels with controls maintained in the light. Alternatively, bilaterally symmetrical guanine features can, on one side, be overlain with black paint and compared with the "internal control" features on the other. I am currently conducting these experiments using *Steatoda grossa* (Theridiidae). Quantification of guanine in *Oecobius amboseli* (Oecobiidae) individuals living in light and dark environments lends some support to Model 4 in that those from dark habitats have significantly fewer deposits (D. Penney, unpubl.). However, this is an observation and not an experiment, and the possibility of genetic adaptations to different light levels cannot be discounted.

The models presented refer to the common situation in which guanine is deposited beneath light or no pigmentation, but not beneath dark. However, in many families visible guanine is extremely rare (see below) even though dark pigmentation is patchy or absent. In *Dysdera crocata* (Dysderidae), for example, the opisthosoma is uniformly buff coloured as a result of the digestive mass showing through the integument. In these cases, lack of guanine is presumably caused by one or more of the following mechanisms: (1) biochemical inhibition of guanine accumulation; (2) the inability of digestive cells to store guanine; (3) non-functioning of the control valve in the Malpighian tubules by which spiders are able to divert guanine to these sites (Collatz, 1987; Seitz, 1987). In other taxa, guanine is located under some areas of unpigmented integument but not under others. For example, the lateral surfaces of the opisthosoma of *Zygiella x-notata* (Araneidae) are normally guanine-free, whereas the dorsal surface has extensive deposits. This can only be a result of mechanisms (1) or (2) above. For some species, the situation is more complex still. In *Theridion grallator*, red and black hypodermal pigments are deposited as lines or patches on a translucent yellow opisthosoma (Oxford & Gillespie, 1996a). Guanine is found under these pigment fields (including the black) but nowhere else, despite the lack of ommins or light-shielding pigments. Induction of guanine by xanthommatin pigment (as opposed to inhibition by ommins) is a possibility but would not explain the White morph, in which a solid shield of guanine is laid down on the dorsum without obvious overlying pigmentation. An additional consideration is that, contrary to the rules of interactions between other morphs, lack of pigmentation in White is normally fully dominant to pigmented patterns in heterozygous individuals (Oxford & Gillespie, 1996a,c).

The nature of guanine deposits—matt versus silver

Guanine achieves a physical, as opposed to a pigmentary, whiteness by reflecting incident light. With respect to the optical qualities of the guanine layer, spiders can be divided into two broad categories. If the colours of overlying pigmentation are discounted, in some taxa the guanine layer is matt white while in others it is silvery. There is a third sort of white "pigment" common in some thomisid and philodromid species which appears to be more superficial and may be found in parts of the body e.g. chelicerae and legs, in which digestive diverticula are absent (Oxford, pers. obs.). Whether this too is guanine has yet to be demonstrated. All three types of white colorants retain their characteristics after preservation for long periods in 70% ethanol.

The dichotomy between matt and silver guanine was commented on by Millot (1926) who suggested that, in the former, the guanine crystals are small and cuboid, whereas silver guanine is composed of thinner plates. These observations at the light microscope level have now been confirmed by scanning electron microscopy. By carefully peeling back the integument from ethanol-preserved specimens, the guanine layer is exposed and can be directly coated and viewed *in situ*. An example of guanine crystals producing a matt white structural colour is shown in Figure 2A. These crystals, from *Nephila* sp. (Tetragnathidae), are up to 1.3 μm long (but more typically *c.* 1 μm) and *c.* 0.7 μm wide, and vary in depth from *c.* 0.1 μm upwards, possibly in quantum units. In some places stacks of crystals are obvious, similar in size and shape to those illustrated by Seitz (1972) in his transmission electron microscope study of guanine deposits in *Araneus diadematus* (Araneidae). Generally the guanine blocks are arranged haphazardly so that light is reflected from them at all angles, thus producing the matt appearance.

The structuring of deposits in *Nephila* is in marked contrast to the disposition of silver guanine in *Tetragnatha polychromata* (Tetragnathidae) (Fig. 2B). Here, the crystals form plates up to 4.5 μm long and approximately half as wide. Each plate has a uniform thickness of *c.* 0.1 μm and they are stacked parallel to each other in layers up to 5 μm deep (Fig. 2C). In this configuration they form a highly effective reflective system, possibly as a result of the multiple thin-film interference phenomenon (Denton, 1970; Land, 1972; Fujii, 1993). The stacks of guanine crystals in spiders are very similar to those observed in the scales of fish and in the reflectors of crustaceans and molluscs (reviewed by Herring, 1994), but may be less well organized. Some fish also produce a matt white structural colour with non-orientated guanine crystals (e.g. in the belly of sharks—Herring, 1994). The guanine plates of *T. polychromata* faithfully follow the contours of the digestive lobes such that the major plane of each plate is always parallel to the surface (Fig. 2D).

A third species, *Misumena vatia* (Thomisidae), also has guanine deposits that generate a matt white appearance. This species apparently lacks the more superficial white "pigmentation" of many thomisids mentioned above. The crystal structure of guanine in *Misumena* may vary according to position on the spider, although this has not yet been investigated systematically. Thus, in some places small cuboid crystals are found, similar to those of *Nephila* in depth (*c.* 0.1 μm upwards) but somewhat smaller in other dimensions (*c.* 0.8 μm by *c.* 0.4 μm). In other areas of the same individual larger structures are found interspersed with the small crystals. These may be roughly cuboid (*c.* 2.0 μm long) (Fig. 2E) or in the form of rods more than 10 μm in length, and have a distinctive, layered appearance. Whether these represent another form of crystalline guanine, or are chemically unrelated, requires micro-dissection and spectrophotometric analyses.

How are the matt white and silver forms of guanine related? There are three possibilities: (1) they are chemically identical and represent two different stable crystalline states; (2) they are chemically *and* crystallographically identical but differ in their habit (growth environment); or (3) they are chemically non-identical (differing, perhaps, in side groups) and as a consequence have different crystal structures. Standard spectrophotometric analyses suggest that both matt and silver forms are pure guanine, but the technique might not be sensitive enough to reveal small differences in side-groups that may influence crystal structure. Guanine

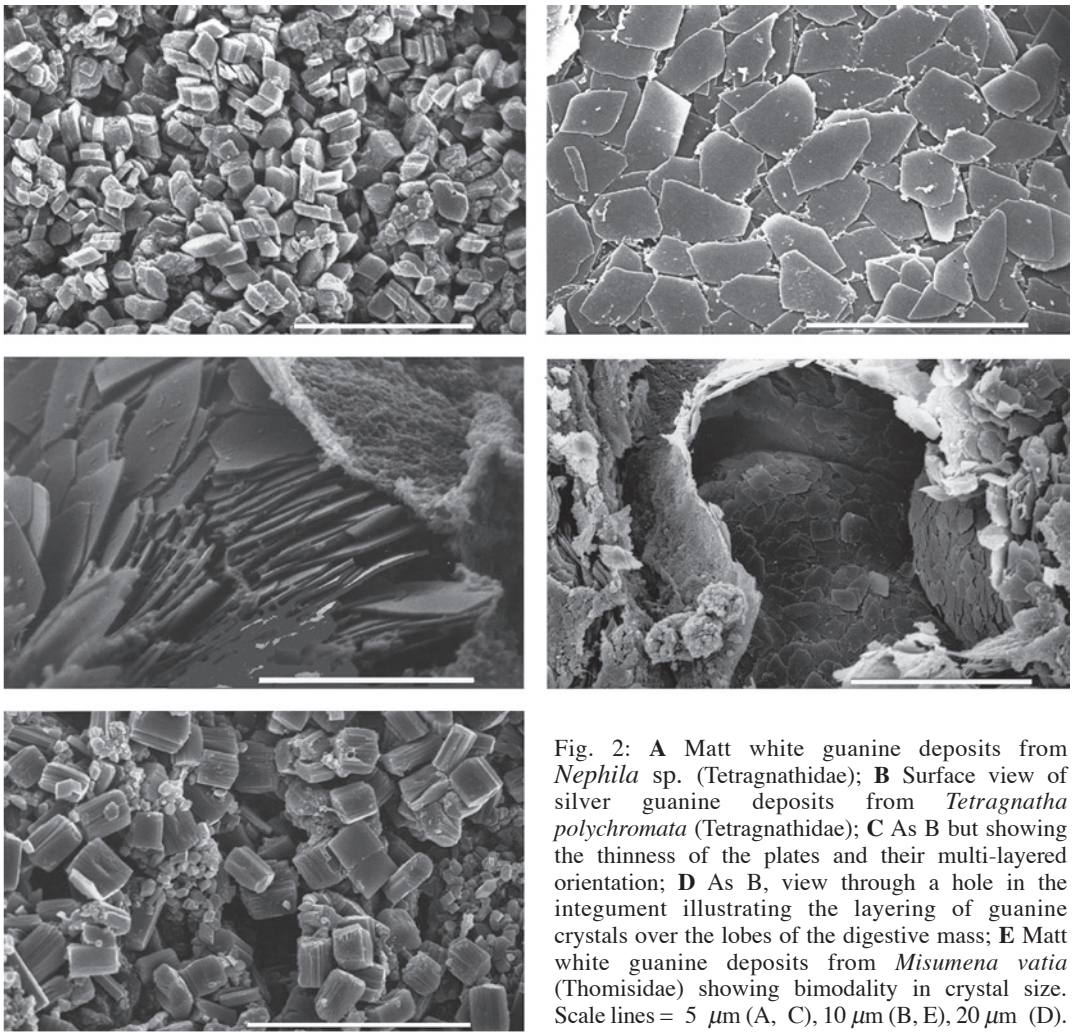
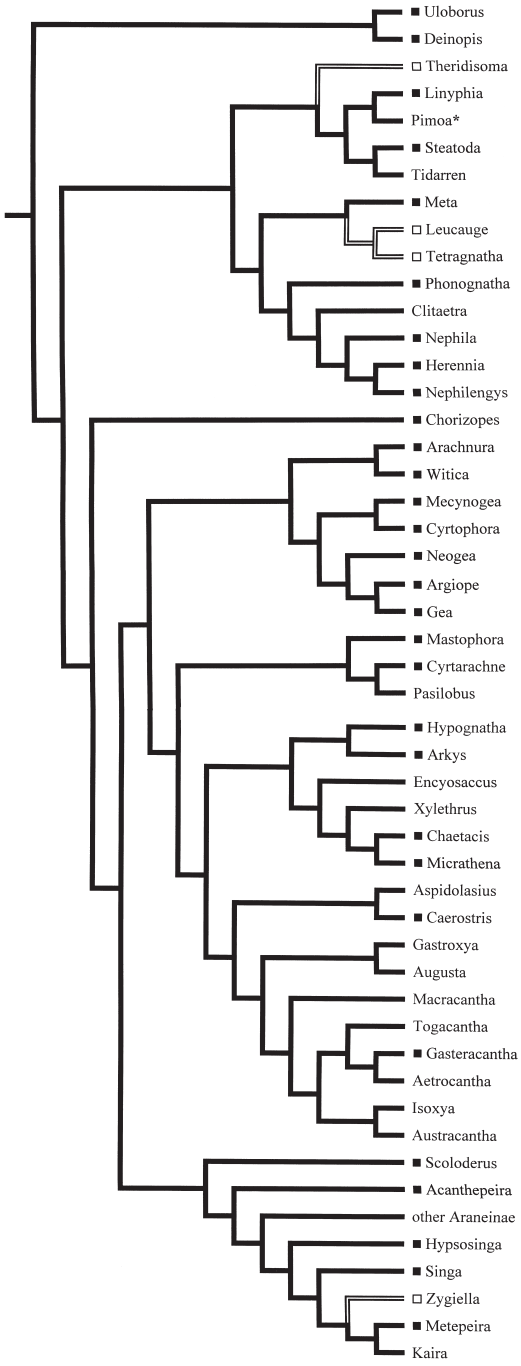


Fig. 2: **A** Matt white guanine deposits from *Nephila* sp. (Tetragnathidae); **B** Surface view of silver guanine deposits from *Tetragnatha polychromata* (Tetragnathidae); **C** As B but showing the thinness of the plates and their multi-layered orientation; **D** As B, view through a hole in the integument illustrating the layering of guanine crystals over the lobes of the digestive mass; **E** Matt white guanine deposits from *Misumena vatia* (Thomisidae) showing bimodality in crystal size. Scale lines = 5 μm (A, C), 10 μm (B, E), 20 μm (D).

crystals are not large enough for normal X-ray crystallographic investigations but some information can be gained by examining a mass of crystals packed into capillary tubing. Preliminary results using this technique suggest that the basic crystallographic unit of matt guanine from *Nephila* sp. (Tetragnathidae—the same specimen illustrated in Fig. 2A) and silver guanine from *Tetragnatha montana* (Tetragnathidae) is probably identical. Silver and matt guanine may, therefore, result from differences in their growth habit within guanocytes. An exactly parallel situation exists for aragonite in mollusc shells where different growth forms result in different optical properties (Bandel, 1990)

Phylogenetic distribution of guanine types

I have examined alcohol-preserved specimens from many of the genera used in the construction of three recent spider phylogenies for the presence of matt or silver guanine. Drawings and photographs are unreliable sources of information for determining guanine type. Many families of spiders show little or no visible guanine (Millot, 1926), and this is discussed more fully below. In species that do deposit visible guanine, the taxonomic distribution of silver and matt types shows that the latter is by far the more widespread within the Entelegynae (*sensu* Coddington & Levi, 1991). This suggests that matt guanine is ancestral, a view reinforced by



examining specific phylogenies. In material examined so far there is only one example of guanine stored in the peripheral digestive mass of a species in the Haplogynae division of the Araneoclada (Coddington & Levi, 1991). Matt guanine was present in a single specimen of a *Modisimus* sp. (Pholcidae) from Panama although other individuals of apparently the same species lacked visible deposits.

If the phylogenies used here are correct, silver guanine has apparently appeared independently at least eight times within the Entelegynae: three times in the Theridiidae, twice in the Tetragnathidae and Araneidae and once in the Theridiosomatidae (Figs. 3–5). Figure 3 (after Coddington *et al*., 1997) shows the broad phylogenetic relationships between guanine types in the Theridiosomatidae, Tetragnathidae and Araneidae. Within the Araneidae, *Mangora pia* from Panama (not shown in Fig. 3) has silver guanine. All individuals exhibit pale patches of tissue within the dorsal opisthosoma which contain a variable number of small silver plates. In some cases detection requires very close scrutiny; the pale patches could, at a cursory glance, be mistaken for matt guanine. Figure 4 (after Hormiga *et al*., 1995) provides a phylogeny of the Tetragnathidae and out-groups in more detail. All genera within the Tetragnathinae examined so far possess silver guanine (including *Dyschiriognatha* and *Agriognatha*, not shown in Fig. 4). Within the "Metinae" (demonstrated by Hormiga *et al*. to be a paraphyletic assemblage) *Chrysometa* has silver guanine but *Meta* and *Metellina* do not. The most parsimonious explanation is that silver guanine has evolved independently in *Chrysometa*. Finally, Figure 5 illustrates a phylogeny of selected species within the Theridiidae (after Gillespie & Tan, unpubl.). The silver-guanine genera *Euryopis*, *Argyrodes* and *Chrysso* are apparently rather distantly related and again represent independent convergences from matt-guanine ancestors. Within *Chrysso*

Fig. 3: Guanine types superimposed on a phylogeny of a taxonomic sample of species from the Tetragnathidae and Araneidae plus their outgroups (redrawn from Coddington *et al*., 1997). Matt guanine is indicated by black branches and squares; silver guanine by open branches and squares. Species scored in a genus followed by an asterisk did not exhibit visible guanine deposits. Genera without squares or an asterisk have not been examined for guanine type. Species scored for guanine are not necessarily those used to construct the phylogeny. Matt guanine appears to be the ancestral type and is assumed unless there is evidence to the contrary.

species there is great variation in the amount and disposition of guanine, from large, highly reflective plates to scattered deposits similar to those described in *Mangora* above.

Current information indicates that the type of guanine, silver or matt, deposited within guanocytes is characteristic of a genus and in some cases higher taxonomic groupings (e.g. Tetragnathinae—Fig. 4). It therefore has significance as a taxonomic character state that has hitherto not (or rarely) been recognized or used. The absolute reliability of guanine type in this respect will emerge as more taxa are examined. At present, it is futile to speculate on what has driven the conversion from matt to silver guanine on at least eight separate occasions. Certainly there is nothing obvious in the general habitat preferences of the genera concerned to suggest an adaptive advantage.

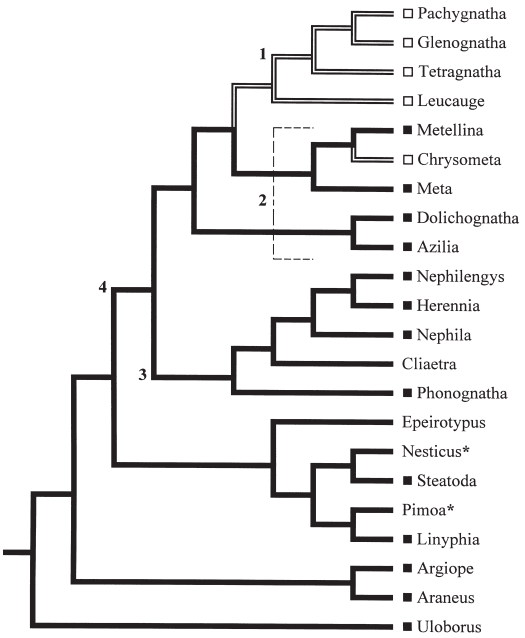


Fig. 4: Cladogram of the Tetragnathidae based on 60 morphological (not including guanine), behavioural and web characters (after Hormiga *et al*., 1995), with guanine types superimposed. Tree length, 130 steps. Conventions as for Figure 3. The species used in the construction of the phylogeny are not necessarily those scored for guanine type. Numbered nodes: 1 = Tetragnathinae, 2 = "Metinae", 3 = Nephilinae, 4 = Tetragnathidae.

Guanine and ecology

If stored guanine is important for its influence on the appearance of a species, one might expect relationships between the presence of visible guanine and aspects of ecology. I have sought such associations using the well-characterized British spider fauna in the first instance. Information for this study was gleaned principally from the identification guides of Jones (1983) and Roberts (1985, 1987, 1995). The first guide is photographic and the other three based on coloured drawings. Guanine was classified as "present" (score 3; sample size, n = 127), "absent" (score 1; n = 300) or "unknown" (score 2; n = 17). The last category includes examples where the glare of the photographic flash could be confused with guanine or where white coloration did not show the characteristic structure of guanine deposits (for example in some thomisids). Information on mean body lengths (average for males and females) and habitats was obtained from the same guides and from Locket & Millidge (1951, 1953). Habitats were coded as: within houses, under stones, in leaf litter, under bark, in holes, on open ground, on low vegetation, on bushes, on trees, on wall surfaces, and in caves. Many species were scored under more than one category. To simplify the analysis, and to increase sample sizes within categories, habitats were amalgamated into two broad classes, "open" (open ground, low vegetation, bushes, trees, wall surfaces) and "closed" (houses, under stones, leaf litter, under bark, holes, caves). Species occupying only "open" habitats were scored 1 and those found only in "closed" habitats were scored 3. Where species occurred in both habitats they were scored 2.

The reliability with which the presence of visible guanine can be determined from pictorial sources was assessed by subsequently examining preserved specimens of 196 of the species illustrated. For the photographic guide (Jones, 1983) 84.0% were correctly classified (sample size, n = 131, 44/59 correctly scored positive, 66/72 correctly scored negative) whereas the figure for the drawings (Roberts, 1985, 1987, 1995) was 93.4% (n = 167, 54/61 correctly scored positive, 102/106 correctly scored negative). Differences between photographs and

drawings, classified as either "right" or "wrong" in comparison with the specimens, are highly significant ($\chi^2_{(1)} = 6.83$, $0.01 > P > 0.001$). With the two sources combined (with each species counted once only and, where the sources differed, erring on the side of detection), the overall proportion of spiders correctly classified was 92.3% ($n = 196$). The discrepancy between illustrated and actual specimens arises for several reasons. For example, in some species there is genuine variation between individuals in whether or not they displayed visible guanine. In other cases, guanine may be present but not visible dorsally, the aspect normally illustrated.

Table 1 lists the mean guanine and habitat scores for families represented in the database by five or more species. There is clearly great variation in the extent to which different families, on average, use guanine as a colorant. For the species scored, none in the families Gnaphosidae, Liocranidae, Salticidae, Agelenidae and Hahnidae exhibit visible guanine. The mean of 1.03 for the Lycosidae is a result of a single species being scored as "unknown". Other families yielded very high guanine scores, notably the Thomisidae, Philodromidae, Theridiidae, Tetragnathidae, Metidae and Araneidae. Differences in the presence of visible guanine between families have long been recognized (e.g. Millot, 1926), but not previously quantified. Mean guanine scores and mean habitat scores are significantly negatively correlated ($r = -0.585$, d.f. = 13, $P = 0.02$), such that families that live in "open" habitats tend to exhibit a higher guanine score than those living in "closed" environments. Similar negative correlations are found when species scores are averaged at the level of genus ($r = -0.257$, d.f. = 208, $P < 0.001$). At the species level, where scores are 1, 2 or 3 for each variable, a contingency chi-squared test was highly significant ($\chi^2_{(4)} = 22.3$, $P < 0.001$). The percentage of species with visible guanine (score 3) in "open", "closed" and both habitats are 35.6% (sample size, $n = 264$), 13.6% ($n = 103$) and 24.7% ($n = 77$), respectively. The correlation between mean guanine score and mean body size for families with five or more species was not significant. At the genus level a significant relationship between these variables was found ($r = 0.170$, d.f. = 208, $P = 0.014$) but at the level of species comparisons were again not significant (ANOVA). All three levels of analysis agree in suggesting a general trend such that larger spiders have a greater tendency to possess visible guanine than smaller ones. Only at the species level was a significant association found between habitat score and body size (ANOVA, $F_{(2,441)} = 6.43$, $P = 0.002$). Subsequent pairwise comparisons (making allowance for multiple testing) indicated that mean size was significantly larger in "closed" habitats (score 3) than in the other two categories.

Family	**No. species**	**Mean guanine score**	**Mean habitat score**
Dictynidae	10	1.70	1.70
Gnaphosidae	19	1.00	2.53
Clubionidae	15	1.13	1.67
Liocranidae	8	1.00	2.00
Thomisidae	16	2.69	1.37
Philodromidae	13	2.77	1.00
Salticidae	27	1.00	1.33
Lycosidae	30	1.03	1.27
Agelenidae	14	1.00	2.57
Hahnidae	5	1.00	2.60
Theridiidae	40	2.55	1.75
Tetragnathidae	9	3.00	1.00
Metidae	8	2.62	2.00
Araneidae	26	2.88	1.08
Linyphiidae	173	1.29	1.61

Table 1: Mean guanine and habitat scores for the fifteen families from the British spider fauna represented in the database by five or more species. Guanine scores range from 1 = absent to 3 = present; habitat scores range from 1 = "open" to 3 = "closed" (see text for more details).

Simple analyses of this sort could be misleading because they do not take the confounding effects of phylogenetic non-independence of sampling units into account. There are several techniques proposed to circumvent this problem (e.g. Harvey & Pagel, 1991) but many require good phylogenies at lower taxonomic levels, and for spiders these are generally not available. In the present case differences among families may represent lineage-specific variation which could affect associations at genus and species levels. However there are many examples of

closely related species differing in both guanine and habitat scores, suggesting that there is the potential for adaptive divergence at or below the level of genus. Under these circumstances Stearns's method of phylogenetic subtraction is appropriate (Stearns, 1983; Harvey & Pagel, 1991; Gage, 1994; for an alternative viewpoint see Ridley, 1989). Individual species or genus scores for the characters of interest have subtracted from them the appropriate mean character score for their family (calculated from species scores or genus means, respectively). In this way, the scores of lower taxa are scaled such that they are free of differences associated with families. Using this method, relationships between scaled guanine scores and scaled habitat scores are still significant at the level of the genus ($r = –0.145$, d.f. = 208, $P = 0.036$) and of the species ($r = –0.095$, d.f. = 442, $P = 0.045$), although the significance is much reduced. Scaled guanine scores are significantly correlated with scaled body sizes at both genus ($r = 0.261$, d.f. = 208, $P < 0.001$) and species ($r = 0.150$, d.f. = 442, $P = 0.001$) levels. Scaled body size was not significantly correlated with scaled habitat score at either level.

These analyses, although rough and ready, show that the presence of visible guanine is not randomly distributed amongst spider families in Britain but tends to be found in those that, on average, live in more open habitats. This conclusion is reinforced by analyses at genus and species levels, even after allowing for family effects. The fact that the strength of association between presence of visible guanine and habitat is reduced when inter-family variation is removed may indicate that there are constraints at higher taxonomic levels (Stearns, 1983; see also discussion in Harvey & Pagel, 1991). For example, coloration in British members of the Salticidae is produced predominantly by scales and not by hypodermal pigments: consequently, guanine as a colorant is redundant and absent (within the present dataset). Thus the presence of scales is a family-level feature that could be regarded as a "constraint", with implications for guanine usage. This view is reinforced by the observation that salticids that do not use scales for coloration, e.g. *Chapoda* (*panama*?), do possess visible (matt) guanine deposits. Associations between guanine score and mean body size were stronger at both genus and species levels after phylogenetic subtraction. It is worth noting that different tests were used in species level analyses before and after phylogenetic subtraction, and for different variables, and this may have had an influence on the degree of statistical significance detected.

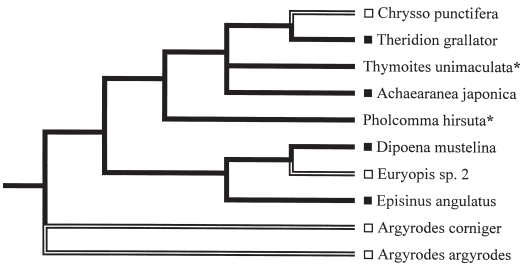


Fig. 5: Guanine type superimposed on a partial phylogeny of the Theridiidae based on Cytochrome Oxidase I amino acid sequences. The single most parsimonious tree length was 54, CI 0.833 (Gillespie & Tan, unpubl.). Conventions as for Figure 3. Species used to construct the tree are named. Guanine information is from other, congeneric, species except for *Theridion grallator*, *Thymoites unimaculata*, *Pholcomma hirsuta* and *Episinus angulatus*.

Visible guanine is used by only a minority (*c*. 30%) of British spiders. The results above suggest that guanine is deployed to a greater extent in species living in more exposed "open" environments and in those with relatively large body sizes, although the correlation coefficients are low. Both of these conclusions make biological sense. For example, Vollrath (1987) reported on two kleptoparasitic *Argyrodes* species (Theridiidae), *A. elevatus* and *A. caudatus*, which share the webs of the same *Nephila* (Tetragnathidae) hosts. The former *Argyrodes* has an opisthosoma covered with silver guanine and is day-active, whereas the latter is dull-brown and active mainly at night. The silver of *A. elevatus* may, in this situation, act in a thermoregulatory capacity and/or make the spider resemble a drop of water on the web. With regard to spider size, if guanine is deployed to produce a disruptive and/or cryptic pattern it may only be advantageous if the size of the spider is greater than the average visible patch size of the background on which it rests or forages (Endler, 1978). Very small species (e.g. many of the tiny, uniformly brown linyphiids) may be close to, or below, the patch size of their

environment and visible patterns would, in these cases, be to no avail. An additional consideration is that small spiders have larger surface area to volume ratios than large ones. As guanine deposits are essentially surface features, small spiders have to store *relatively* more to achieve the same visible effect and this might incur a greater metabolic cost.

Conclusions and future directions

Modern techniques (SEM, X-ray crystallography) and knowledge (phylogenetic relationships) have enabled a re-evaluation and extension of Millot's pioneering work (Millot, 1926) on the nature and distribution of visible guanine in spiders. The general absence of stored guanine in situations in which it has no effect on the appearance of a spider, for example where coloration is a result of surface hairs or scales, or under dark hypodermal pigmentation, leads to two conclusions. First, guanine is stored primarily (or exclusively) as a colorant and not for other, metabolic reasons. Second, the maintenance of stored guanine is energetically costly. During development, guanine is removed immediately it no longer acts as an effective colorant, suggesting that metabolically the most energy efficient destination for nitrogenous excretory products is evacuation rather than storage. There is obviously great complexity in the precise developmental co-ordination of guanine storage and hypodermal pigmentation in spiders, and understanding the genetical and biochemical mechanisms involved is an important and challenging goal for future work. The phylogenetic distributions of the two major types of guanine, matt and silver, show that silver guanine has apparently evolved from matt at least eight times during the history of the Araneoclada. Examination of more species within genera, and of more genera, will further inform this conclusion and demonstrate how reliable guanine type is as a taxonomic character. Additional studies of the crystal structure of matt and silver guanine types are required, with special attention paid to the homogeneity of crystal type within these two main groups. Of particular interest is whether independently evolved silver guanine is identical in its crystalline state and gross morphology. The link between guanine deposition and ecology is clearly multifaceted and to explore it more fully requires a much larger database, quantification of guanine usage, and a more subtle division of environmental and behavioural characteristics. However, there is no substitute for examining a series of phylogenetically independent ecological/behavioural contrasts between closely related species with different levels of visible guanine utilization (e.g. Vollrath, 1987) in order to understand the selective factors involved (Burt, 1989).

Acknowledgements

I would like to thank: Andrea Gillmeister for German translations; Harriet Mitchell for the SEM photographs; Rosemary Gillespie and An-Ming Tan for the theridiid phylogeny; Chris Felton, Charles Griswold, Stephen Hartley, John Murphy and David Penney for access to specimens; Zbyszek Dauter and Madelaine Moore for advice and for performing the X-ray crystallographic analyses; Terry Crawford, Peter Croucher, John Currey, David Penney and John Sparrow for discussion.

References

ANDERSON, J. F. 1966: The excreta of spiders. *Comp. Biochem. Physiol.* **17**: 973–982.

BANDEL, K. 1990: Shell structure of the Gastropoda excluding Archaeogastropoda. *In* J. G. Carter (ed.). *Skeletal biomineralization: patterns, processes and evolutionary trends,* **I**. New York: Van Nostrand Reinhold: 117–134.

BARTH, F. G. 1973: Microfiber reinforcement of an arthropod cuticle. Laminated composite material in biology. *Z. Zellforsch. microsk. Anat.* **144**: 409–433.

BURT, A. 1989: Comparative methods using phylogenetically independent contrasts. *In* P. H. Harvey & L. Partridge (eds.). *Oxford surveys in evolutionary biology, 6*. Oxford: Oxford University Press: 33–53.

CODDINGTON, J. A. & LEVI, H. W. 1991: Systematics and evolution of spiders (Araneae). *A. Rev. Ecol. Syst.* **22**: 565–592.

CODDINGTON, J. A., HORMIGA, G. & SCHARFF, N. 1997: Giant female or dwarf male spiders? *Nature, Lond.* **385**: 687–688.

COLLATZ, K.-G. 1987: Structure and function of the digestive tract. *In* W. Nentwig (ed.). *Ecophysiology of spiders.* Berlin: Springer-Verlag: 229–238.

CUTLER, B. & RICHARDS, A. G. 1972: Sclerotization and localisation of brown and black colours in chelicerates (Arthropoda). *Zool. Jahrb. Anat.* **89**: 404–421.

DALINGWATER, J. E. 1987: Chelicerate cuticle structure. *In* W. Nentwig (ed.). *Ecophysiology of spiders*. Berlin: Springer-Verlag: 3–15.

DENTON, E. J. 1970: On the organization of reflecting surfaces in some marine animals. *Phil. Trans. R. Soc. Ser. B* **258**: 285–313.

ENDLER, J. A. 1978: A predator's view of animal color patterns. *Evol. Biol.* **11**: 319–364.

FOELIX, R. F. 1996: *Biology of spiders*. 2nd ed. Oxford: Oxford University Press.

FUJII, R. 1993: Cytophysiology of fish chromatophores. *Int. Rev. Cytol.* **143**: 191–255.

GAGE, M. J. G. 1994: Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc. R. Soc. Ser. B* **258**: 247–254.

HARVEY, P. H. & PAGEL, M. D. 1991: *The comparative method in evolutionary biology*. Oxford: Oxford University Press.

HERRING, P. J. 1994: Reflective systems in aquatic animals. *Comp. Biochem. Physiol.* **109A**: 513–546.

HILL, D. E. 1979: The scales of salticid spiders. *Zool. J. Linn. Soc.* **65**: 193–218.

HOLL, A. 1987: Coloration and chromes. *In* W. Nentwig (ed.). *Ecophysiology of spiders*. Berlin: Springer-Verlag: 16–25.

HORMIGA, G., EBERHARD, W. G. & CODDINGTON, J. A. 1995: Web-construction behaviour in Australian *Phonognatha* and the phylogeny of Nephiline and Tetragnathid spiders (Araneae: Tetragnathidae). *Aust. J. Zool.* **43**: 313–364.

JONES, D. 1983: *The Country Life guide to spiders of Britain and Northern Europe*. London: Hamlyn.

LAND, M. F. 1972: The physics and biology of animal reflectors. *Prog. Biophys. molec. Biol.* **24**: 75–106.

LOCKET, G. H. & MILLIDGE, A. F. 1951: *British Spiders*, **I**. London: Ray Society.

LOCKET, G. H. & MILLIDGE, A. F. 1953: *British Spiders*, **II**. London: Ray Society.

MILLOT, J. 1926: Contribution à l'histophysiologie des aranéides. *Bull. biol. Fr. Belg., Suppl.* **8**: 1–238.

OXFORD, G. S. & GILLESPIE, R. G. 1996a: Genetics of a colour polymorphism in *Theridion grallator* (Araneae: Theridiidae), the Hawaiian happy-face spider, from Greater Maui. *Heredity, Lond.* **76**: 238–248.

OXFORD, G. S. & GILLESPIE, R. G. 1996b: Quantum shifts in the genetic control of a colour polymorphism in *Theridion grallator* (Araneae: Theridiidae), the Hawaiian happy-face spider. *Heredity, Lond.* **76**: 249–256.

OXFORD, G. S. & GILLESPIE, R. G. 1996c: The effects of genetic background on the island-specific control of a colour polymorphism in *Theridion grallator* (Araneae: Theridiidae), the Hawaiian happy-face spider. *Heredity, Lond.* **76**: 257–266.

OXFORD, G. S. & GILLESPIE, R. G. 1998: Evolution and ecology of spider coloration. *A. Rev. Ent.* **43**: 619–643.

RIDLEY, M. 1989: Why not to use species in comparative tests? *J. theor. Biol.* **136**: 361–364.

RIECHERT, S. E. & HARP, J. M. 1987: Nutritional ecology of spiders. *In* F. Slansky & J. G. Rodriguez (eds.). *Nutritional ecology of insects, mites, spiders, and related invertebrates*. New York: Wiley & Sons: 645–672.

ROBERTS, M. J. 1985: *The spiders of Great Britain and Ireland*, **3**. Colchester, Essex: Harley Books.

ROBERTS, M. J. 1987: *The spiders of Great Britain and Ireland*, **2**. Colchester, Essex: Harley Books.

ROBERTS, M. J. 1995: *Collins field guide. Spiders of Britain and Northern Europe*. London: HarperCollins.

SEITZ, K.-A. 1972: Elektronenmikroskopische Untersuchungen an den Guanin-Speicherzellen von *Araneus diadematus* Clerck (Araneae, Araneidae). *Z. Morph. Tiere*. **72**: 245–262.

SEITZ, K.-A. 1987: Excretory organs. *In* W. Nentwig (ed.). *Ecophysiology of spiders*. Berlin: Springer-Verlag: 239–248.

SELIGY, V. L. 1972: Ommochrome pigments of spiders. *Comp. Biochem. Physiol.* **42A**: 699–709.

STEARNS, S. C. 1983: The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *Oikos* **41**: 173–187.

VOLLRATH, F. 1987: Kleptobiosis in spiders. *In* W. Nentwig (ed.). *Ecophysiology of spiders*. Berlin: Springer-Verlag: 275–286.