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Comparative structural analysis of cocoons and cocoon silk in three spider species through Scanning Electron Microscopy

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Abstract
Spider silk has been the subject of detailed studies for a long time, mostly because of its extraordinary mechanical properties. Until now, most studies have focused on spider web silk and dragline silk. No real research has been conducted yet on the structural properties of cocoon silk. When analysing cocoon threads of different spider species (by means of Scanning Electron Microscopy), we found that different spider species have adapted several strategies to construct their cocoons probably as evolutionary responses to environmental conditions.

Key words: cocoon silk, *Araneus diadematus*, *Pardosa lugubris*, *Zygiella x-notata*, Araneae

INTRODUCTION

Spiders invented silk 400 million years ago for the formation of cocoons (egg protection), possibly through the evolutionary invention of spinnerets (Decae 1984). The building of orb webs to capture prey followed about two hundred million years later (Schultz 1987; Selden 1989). The silk threads (also used in producing the egg-sac) are completely composed of proteins, which have made an irreversible transition from a soluble silk protein solution inside the spider into an insoluble fibre outside the spider. The chemical and physical characteristics of the fibres can be altered as a response to both external, because internal conditions and spiders are capable of inducing a rapid and temporary adaptation of silk properties (Vollrath 1999).

Spider silk has long fascinated men. It is a fibre with a unique combination of enormous tensile strength and elasticity and with an ultra low weight. For centuries, people have been attempting to obtain and use spider silk. But successful mass-production has been elusive mainly because spiders have cannibalistic tendencies and tend to eat each other when thrown together in silk-making colonies. This explains why spider silk does not have such large-scale application possibilities yet as the silk of the silkworm *Bombyx mori* (Linnaeus, 1758).

Until now, most spider silk studies have focused on draglines and web structures (review in Vollrath 2000). Although their strength and elasticity is not questioned (studies have shown almost similar stressstrain properties to that of Kevlar), we focused on possibly the earliest invention of spiders, namely cocoon threads. Almost no literature is known to exist about the detailed structure of cocoon threads. Studies on the structure (morphology) and function of cocoons within spiders include a detailed study within one genus: *Argiope bruennichi* Scopoli, 1772 (Bergthaler 1995) and *A. aurantia* Lucas, 1833 (Hieber 1985, 1992a,b; Foradori et al. 2002), within one family: Uloboridae (Opell 1984) and within this family more specifically *Polenecia producta* (Simon, 1873) (Peters & Kovoor 1989) and between different spider genera and species: *Diguetia canities* (McCook, 1889) (Cazier & Mortenson 1968), *Nephila clavipes* (Linnaeus, 1767) (Christenson & Wenzl 1980) and *Cyrtophora citricola* (Forskal, 1775) (Kullmann 1961). The studies of Barghout et al. (1999, 2001) dealt only with the molecular (crystalline) structure of the cocoon thread (by means of Transmission Electron Microscopy) without describing the structure of the cocoon itself. A more formal structure description (in the framework of egg cocoon parasites) was made for two mimetid spiders (*Mimetus notius* Chamberlin, 1923 and *M. puritanus* Chamberlin, 1923) (Guarisco & Mott 1990; Guarisco 2001) and the linyphiid spider *Pityohyphantes costatus* (Hentz, 1850) (Manuel 1984). Although they are not well studied in detail, we believe that even these fibres have enormous potentials and can even compete with other spider-made fibres (Van Wassenhove 2001; Van Nimmen et al. 2002).

This study investigates the structure of cocoon threads (in relation to draglines in some species), and their particular characteristics and how they can differ between spider families. Other studies of the project include the use of these cocoon threads in medical applications (more specifically the use of cocoon silk in cartilage regeneration). We believe that cocoon threads can be very useful for mankind in several ways (Van Wassenhove 2001; Van Nimmen et al. 2002).

MATERIAL AND METHODS

For this study, we used cocoons from individuals sampled in the field. Threads were pulled out of the cocoon, parts of the cocoons, or were prepared out of the whole cocoon (especially within Lycosidae). All threads were placed upon a small block, coated with gold and further analysed at Ghent University using Scanning Electron Microscopy (JEOL SM 840).

RESULTS AND DISCUSSION

Pardosa lugubris (Walckenaer, 1802) (Lycosidae, Araneae)

Pardosa lugubris is a widespread species in Europe and occurs in a variety of forest types ranging from oak (*Quercus* spp.) and beech (*Fagus sylvatica*) to pine (*Pinus sylvestris*) woods. The species can also be found in woodland clearings, on forest borders and along hedges (ecotones) (Töpfer-Hofmann et al. 2000), which are also the preferential habitat types of the spider species in Belgium (Maelfait et al. 1998). In contrast to the sister species *P. saltans* Töpfer-Hofmann, 2000, *P. lugubris* lives in more drier situations mainly in the north of Belgium (Hendrickx et al. 2001). The species makes a cocoon at the beginning of the summer (June-August) (Alderweireldt & Maelfait 1990), and as a consequence the cocoons are subjected to high temperatures. The spiderlings emerge in summer and hibernate as subadults during winter, so the cocoons are not subjected to low temperatures. Since confusion is possible with the sister species (*P. saltans*), we made sure that the investigated cocoon was derived from a *Pardosa lugubris* s.s. female (Hendrickx et al. 2001).

The cocoon of this species is a tightly woven structure consisting of two structures separated by a white septum. The tightly woven structure is composed of two parts: an inner and an outer layer. Both structures were analysed with SEM. The outer layer is tightly woven and consisted of thin threads intermingled with some thicker threads and embedded in some kind of cement (Figs. 1,2), which possibly explains the brown colour (also seen in other Lycosidae like *Pardosa amentata,* but absent in *Pirata*-species). The tightness of the structure is a possible adaptation to a warm climate. The layer might be present to prevent eggs from desiccation. This could also explain why the *Pirata* species (which usually live in fairly wet environments) do not construct these cement-like substances but produce a more uniform (mostly white) cocoon without a septum. An overview of the inner layer (Fig. 3) shows that it consists of several thicker and thinner threads, but when looked upon in detail (Fig. 4) we observe that these thicker threads are in fact several equally built threads that are sometimes glued together. The threads are without cement-like substances. The space between the threads is ideal for holding air, so this layer is possibly used to maintain internal temperatures in the cocoon. The septum in between is composed of threads woven in parallel. A possible function of the ' thin' septum is to hold the two parts

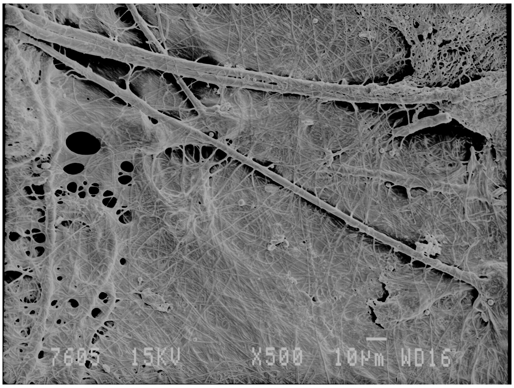

Fig. 1. Structure of the outer layer from the cocoon of *Pardosa lugubris* where several thread types are embedded in some kind of cement. Scale bar and magnification (X500) are indicated on the picture.

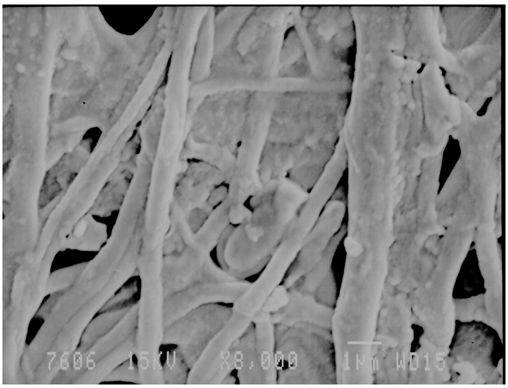

Fig. 2. Details of Fig. 1 (outer layer of cocoon from *Pardosa lugubris*) where it is clear that several types of threads are embedded together in cement. Scale bar and magnification (X8000) are indicated on the picture.

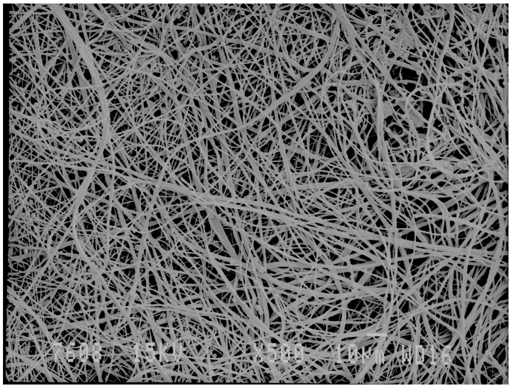

Fig. 3. Structures of the inner layer (cocoon of *Pardosa lugubris*) where on the first view several thread types seem to appear (this time no embedding in cement). Scale bar and magnification (X500) are indicated on the picture.

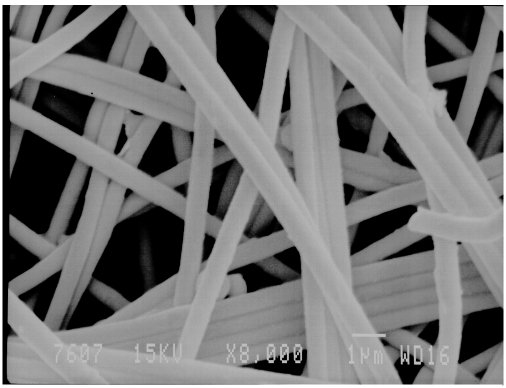

Fig. 4. Detail of the inner layer (cocoon of *Pardosa lugubris*) where thicker threads seem to be composed of several smaller threads glued together. Inner layer is actually constructed from one type of thread. Scale bar and magnification (X8000) are indicated on the picture.

together and it probably also plays a major role as an easy escape place for juveniles when they come out of the cocoon.

Araneus diadematus Clerck, 1757 (Araneidae, Araneae)

The common garden spider *Araneus diadematus* is one of the most common and best-known orb weavers. It is a very common spider in Europe and can be found in a wide variety of habitats. It is common in woodlands, heathlands and gardens although human activity in gardens discourages them (Van Wassenhove 2001). The spiders are adult from summer to autumn (September-October) and the cocoons are deposited in hidden places (in vegetation, under the gutter of buildings). The cocoons overwinter and spiderlings emerge around May, the following year. Hence the cocoon is exposed to lower temperatures but because it is hidden away, it is somewhat protected against the harshest conditions during winter.

The cocoon has two layers. An outer layer consists of some loose threads while the threads of the egg sac itself has a more tightly woven structure. Nevertheless, the fibres were of similar thickness and were perfectly round. So it is clear that the spider uses only one type of silk gland (more specifically the cylindrical or tubuliform gland) to make the cocoon silk. Thus, the cocoon fibres of *A. diadematus* seemed to be quite uniform and did not seem to be composed of several threads like the dragline which seems to be composed of thicker and thinner threads that are stuck together (Van Wassenhove 2001) (Fig. 5). Quite surprisingly the cocoon silk did have a structure on the surface (Fig. 6). Figure 5 (magnification x500) shows a smooth fibre, whereas a larger magnification of x8000 (Fig. 6) makes the surface structure visible. On the surface there are some longitudinal superficial cavities with a length of 1μm or more. But also on places without cavities, some kind of relief could be seen. The precise function of these holes is unclear. This is different from the structure of a normal dragline, which is smoother without holes. It has to be further investigated why this difference is present.

Zygiella x-notata (Clerck, 1757)(Araneidae, Araneae)

Zygiella x-notata is also a very common species in Europe (with the exception of north-east Europe) and can also be found in a wide variety of habitats, mainly in the neighbourhood of houses. In more southern parts of Europe, the species can also be found on rocks and trees (Roberts 1995). The spider normally makes a cocoon in late autumn (November-December) and hides its cocoon (similarly to *A. diadematus*) from direct environmental exposure. The cocoons overwinter and spiderlings emerge around May, the next year. It is one of the few spiders that continue to make webs during winter (Van Wassenhove 2001).

The cocoons of *Z. x-notata* are very complex and consist of several layers. We could mainly distinguish two types of threads: white and yellowish ones. The latter form the actual cocoon while the white threads cover the others and are also responsible for attachment to the substrate (Gheysens 2003). We investigated only the threads that directly surround the egg sac (yellowish threads).

Comparing the cocoon fibre to that of *A. diadematus*, we saw (with low magnification of x500, Fig. 7) no differences between thread structures in the two species. The only difference found was that *Zygiella* threads tended to be about half as thin as those of *A. diadematus*. It was very interesting, however, that with a magnification of up to 8000 times (Fig. 8), a similar longitudinal structure like in *Araneus* appeared. Here the structure could easily be interpreted as being a very round bundle of fibres wrapped in a thin layer, in such a way that the shape of the outer fibre structure consisting of fine fibres could still be distinguished. The structures visible on the thread itself (Fig. 8) still have an unknown origin and function (possible preparation artefacts?). SEM photographs of cross sections of these fibres

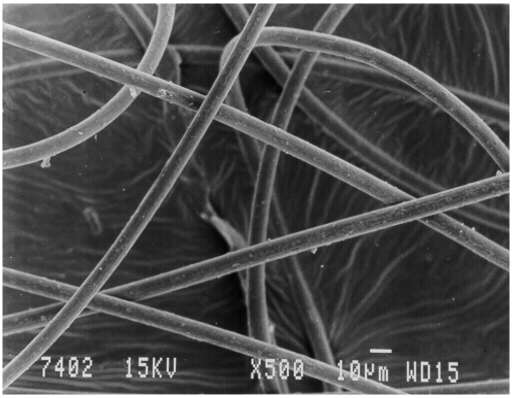

Fig. 5. Overview of cocoon threads of *Araneus diadematus* with equally built threads. Scale bar and magnification (X500) are indicated on the picture.

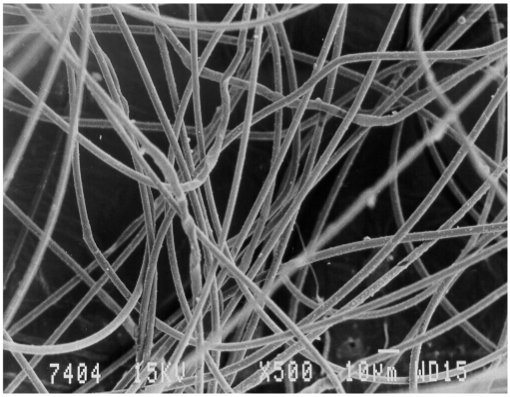

Fig. 7. Overview of cocoon threads from the inner layer of *Zygiella x-notata* with morphologically equally built threads. Scale bar and magnification (X500) are indicated on the picture.

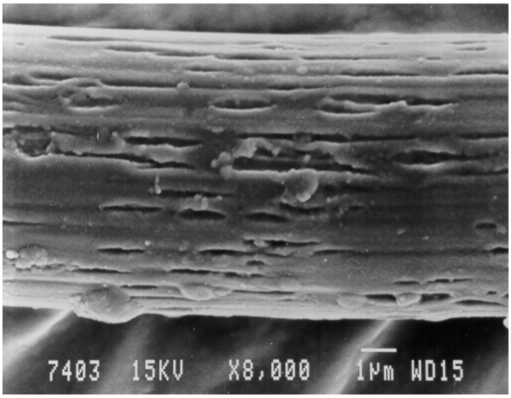

Fig. 6. Detail of a cocoon thread of *Araneus diadematus* with indication of the longitudinal cavities. Scale bar and magnification (X8000) are indicated on the picture.

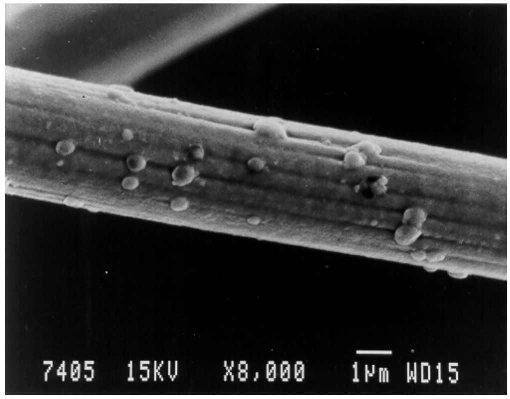

Fig. 8. Detail of a cocoon thread from the inner layer of *Zygiella x-notata*. Particles whose function is still unclear are present on the thread. Scale bar and magnification (X8000) are indicated on the picture.

were not taken yet which could confirm the 'composite' nature of these threads. However, this will certainly be investigated in the near future.

PERSPECTIVES

Spider cocoons seem to differ enormously between families. A detailed analysis of the structures can certainly be helpful in spider phylogeny. This study is only a preliminary analysis. Cocoons of more families and species will be discussed in the future in order to better understand which is the ancestral construction. This study clearly points out that cocoon threads can be equally or maybe even more interesting than other threads produced by spiders.

Further research will involve the comparison of the cocoon silk structures of other spider species, the chemical composition of these threads between spider species, and the use of cocoon fibres in biomedical applications with a detailed study of their chemical and physical properties.

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