

Structure and function of the silk-gland system in Oxyopidae (Araneae)

J. Kovoov and A. Muñoz-Cuevas

Laboratoire de Zoologie–Arthropodes,
CNRS–MNHN, 61 rue de Buffon,
75231 Paris Cedex 05, France

Summary

The silk-gland system of *Peucetia* and *Oxyopes* (Oxyopidae) comprises four gland types: ampullate (2 pairs), aciniform (30–50 pairs), piriform (50–80 pairs), and cylindrical (15–35 pairs). From the behaviour of these spiders, which catch prey by sight and do not build a web, such a large set of silk glands is unexpected. Most of the silks produced are used in construction of complex egg sacs and for their attachment to vegetation. In males, cylindrical glands are absent, but the number of their aciniform glands is equal to that of the cylindrical and aciniform glands combined in females. In male *Peucetia*, but not in *Oxyopes*, small epiandrous glands open on the anterior lip of the epigastric furrow. The very thin silk they produce is a component of the sperm web. The silk-gland system in *Peucetia* and *Oxyopes* genera shows anatomical as well as histochemical characteristics which, in combination, seem specific to the family Oxyopidae. Close relationships with other hunting spiders of the Lycosoidea group (Lycosidae, Ctenidae) are not obvious.

Introduction

It seems well established that the use of silk by the Oxyopidae (lynx spiders) is less important for predation or mating behaviour than are visual cues (Gerhardt, 1927, 1933; Gertsch, 1949; Whitcomb *et al.*, 1963; Whitcomb & Eason, 1965; Young & Lockley, 1985). Adult oxyopids do not generally spin a web. However, early instars of *Peucetia viridans* (Hentz) have been observed “making an irregular web of loose threads” (Kaston, 1972), and female oxyopids lay several bunches of eggs in complex silken bags, fixed beneath leaves by some threads, which are vigilantly guarded (Willey & Adler, 1989). On the other hand, the earlier suggestion by Rovner (1980) that oxyopids may have “a linyphiid- or theridiid-like web weaver” as an ancestor has been supported by Griswold (1983) in his study of *Tapinillus longipes* (Taczanowski), a tropical oxyopid spider which builds a web.

The silk-gland system of Oxyopidae has not been studied so far, except in its ontogenetic aspect (Kovoov & Muñoz-Cuevas, 1995). The results of the present study will be combined

with those on the venom-gland and visual systems of several oxyopid species in order to compare their respective roles in behaviour related to certain characteristics of habitat illumination (Kovoov & Muñoz-Cuevas, 1997).

Material and methods

The silk-gland system of *Oxyopes lineatus* Latreille, 1806 was studied in 18 adult or subadult females and 10 adult or subadult males collected in Pyrenean mid-mountain prairies (France). Four species of *Peucetia* Thorell, 1869 were studied: *Peucetia gerhardi* Van Niekerk & Dippenaar, 1994 (4 adult males, 3 adult females) from Nigeria; *P. cauca* Lourenço, 1990 (6 juveniles, 3 adult females) from Colombia; *P. graminea* Pocock, 1900 (1 adult male, 1 adult female) from Thailand; and *P. viridis* (Blackwall, 1858) (2 adult females) from southern Spain.

External structures of spinnerets were examined on specimens fixed in alcohol, dehydrated and dried at room temperature, then coated with gold and palladium, prior to observation by scanning electron microscopy (Jeol JSM 840).

Species	<i>Peucetia gerhardi</i>	<i>Peucetia viridis</i>	<i>Oxyopes lineatus</i>
Epigastric furrow			
male	10–11	–	–
Anterior-lateral spinnerets			
MA male	1	1	1
female	1	1	1
Pi male	70–71	–	–
female	70–71	75–76	46
Posterior-median spinnerets			
mA male	1	1	1
female	1	1	1
Ac male	20–22	–	–
female	9–10	15–16	13–11
Cy female	10	17–19	6
Posterior-lateral spinnerets			
Ac male	40–41		
female	25–26	32	23–24
Cy female	12–14	15	9

Table 1: Number of spigots on spinnerets of three species of Oxyopidae (adults). Numbers refer to spigots on one side, or two sides if they are different. Ac = aciniform, Cy = cylindrical, MA = major ampullate, mA = minor ampullate, Pi = piriform glands.

For histological study, opisthosomas were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Sections, 5–6 μm thick, were stained using Heidenhain's azan, phosphotungstic haematoxylin, and Gabe's one-step trichrome, alone or in combination with aldehyde fuchsin. Histochemical reactions were used to visualize (1) *anionic groups and polysaccharidic substances*: P.A.S., alcian blue at pH 2.5, toluidine blue at pH 4.2; (2) *proteins*: Danielli's coupled tetrazonium reaction, Morel and Sisley's reaction for tyrosine, lead haematoxylin staining for carboxyl groups; (3) *reducing groups*: ferric ferricyanide reaction. Technical procedures are detailed in the handbooks by Gabe (1968) and Lillie & Fullmer (1976).

Results

Spinnerets

Silk glands of Oxyopidae open on three pairs of spinnerets through four types of spigots. The

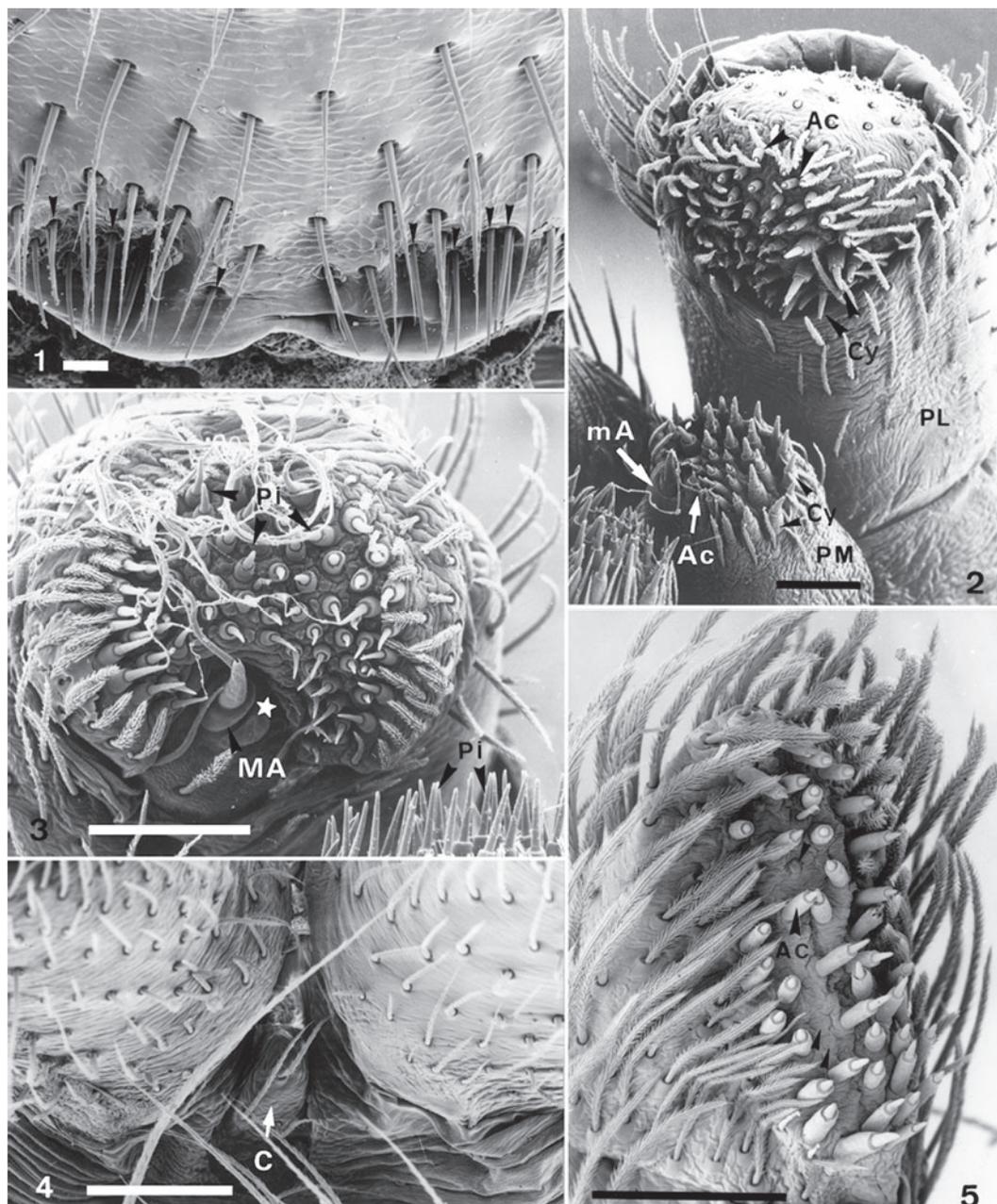
anterior-median pair of spinnerets is reduced to a conical colulus (Fig. 4) which is flanked by two setae near its base and bears a single median seta at the tip. Spinnerets of males at any developmental stage, and females until the second instar before reaching sexual maturity, exhibit three types of spigots corresponding to three gland types. A fourth type of spigot appears on the posterior-median and lateral spinnerets of subadult and adult females. On the other hand, adult males of *Peucetia* show two sets (10–11) of fine spigots in two symmetrical depressions on the anterior lip of the epigastric furrow (Fig. 1).

Anterior-lateral spinnerets (AL) are the largest. The oblique internal surface of their apical article shows two types of spigot: one large cone (MA), corresponding to a major ampullate gland, in a depressed area, and a remnant of a second one which is functional in all juvenile stages; regularly spaced piriform gland spigots of different sizes surround this area (Fig. 3).

The *posterior-median spinnerets* (MS) are short, conical and flattened on their inner side; they bear at their tip one cone of a minor ampullate gland (mA), about 30% smaller than the MA cone, two remnants of the same type (reduced at the last moult), small spigots of aciniform glands and, in females, an outer array of larger spigots corresponding to cylindrical glands, absent in males (Fig. 2).

Posterior-lateral spinnerets (PS) comprise a long and cylindrical basal article and a much shorter apical one which bears on its inner side only one kind of spigot (aciniform glands) in males (Fig. 5), and two kinds (aciniform and cylindrical glands) in females (Fig. 2). Between spigots of piriform and aciniform glands, numerous small remnants of these spigots are present, which are functional during earlier stages, but regress and are replaced by new ones in adults (Figs. 3, 5).

The shape of the spinnerets and spigots was the same in both species of *Peucetia* as in *Oxyopes lineatus*. The number of spigots of piriform, aciniform and cylindrical glands differed from one species to another, as shown in Table 1. The combined number of aciniform and cylindrical spigots in females was found to be equal to that of aciniform spigots alone in males.



Figs. 1–5: Spinnerets. **1** Frontal view of the anterior lip of the epigastric furrow of a male *Peucetia gerhardi*; arrow heads = spigots of epiantrous glands. **2** posterior lateral (PL) and median (PM) spinnerets of a female *P. viridis*; Ac = aciniform, Cy = cylindrical, mA = minor ampullate. **3** anterior lateral spinneret of a female *P. gerhardi*; MA = major ampullate, Pi = piriform gland spigots, * = remnant of a second MA. **4** colulus (C) of *P. gerhardi*. **5** posterior lateral spinneret of a male *P. gerhardi*; Ac = aciniform gland spigots, small arrow heads = remnants of Ac. All spinnerets were artificially spread out. Scale lines = 20 μm (1), = 100 μm (2–5).

Silk glands

Silk glands of Oxyopidae belong to three (male) or four types (female). Each silk gland ends in one spigot. All glands secrete two distinct proteins.

Ampullate glands are the most extended silk glands in immature as well as young mature females and in males at all stages except at the very end of their life cycle. In juveniles of both sexes, four pairs of ampullate glands are developed: two pairs of major and two pairs of minor ampullate glands. At the adult moult, one pair of each type disappears, though a small remnant of the corresponding cone is still present on each anterior-lateral and posterior-medial spinneret (Figs. 2–3). The second remnant on the posterior-medial spinnerets (Fig. 2) might correspond to a third pair of minor ampullate glands which regressed before the fourth instar.

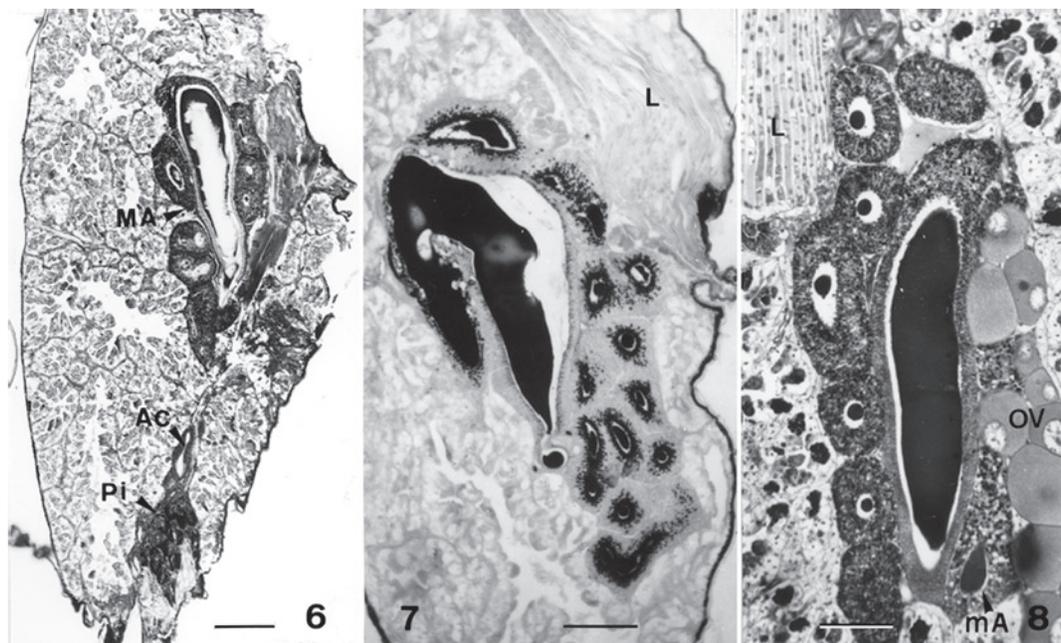
The shape of ampullate glands is that of a long, rather large, convoluted tube undulating

from beneath the lung plates, along the ovary, up to the posterior third of the opisthosoma where aciniform, cylindrical (in females) and piriform glands form a compact mass. *Major ampullate glands* are the most anterior and lateral. The distal tube (tail) forms a hairpin bend anteriorly, and then widens slightly forming the ampulla which ends at half the length of the opisthosoma. The excretory duct runs at first towards the spinnerets and reverses its direction once at the level of the aciniform glands and a second time near the end of the ampulla, to finally reach the anterior-lateral spinnerets. *Minor ampullate glands* extend medially on either side of the sagittal plane of the opisthosoma. In *Peucetia* species, the sizes of major and minor ampullate glands do not differ much, in *Oxyopes lineatus*, minor ampullate glands of males or females are clearly smaller than major ampullate ones.

In all species studied of both sexes, ampullate glands secrete two distinct proteinic substances: one is produced in the distal tube and the initial

Glands	Acidophily	Basic aminoacids	Tyrosine	Reducing	Carboxyl	Polysaccharides
		Proline, Tyrosine, Tryptophan	(2)	groups	groups	Mucosubstances Anionic groups
		(1)	(2)	(3)	(4)	(5, 6)
MA	dist.: cyanoph.	++	+++	++++	0	0
	prox.: cyanoph.	++	++, +, 0	+++	+	+ (acidic)
mA	dist.: complex	++	+++	+, ++, +++	0	0
	prox.: cyanoph.	+++	0	++++	0	+ (acidic)
Pi	dist.: complex	+	+	+	0	0
	prox.: basoph.	++++	0	0	++++	+
Ac	dist.: complex	++	+	+	0	0
	prox.: acidoph.	+	+	0	+	0
Cy	A: cyanoph.	+	+	++	0	0
	B: erythroph.	+++	0	0	+++	0
Epi	dist.: complex	+	+	++	0	0
	prox.: basoph.	++++	0	0	++++	+

Table 2: Main staining affinities and histochemical characteristics of silk glands in *Peucetia gerhardi*. MA = major ampullate, mA = minor ampullate, Pi = piriform, Ac = aciniform, Cy = cylindrical, Epi = epiandrous glands. Technical references: (1) Danielli's coupled tetrazonium reaction (Pearse, 1968); (2) Morel and Sisley's reaction (Lillie, 1965); (3) ferric ferricyanide reaction (Adams, 1956); (4) plumbic haematoxylin (Solcia *et al.*, 1969); (5) P.A.S.-reaction (McManus, 1946); (6) alcian blue (Mowry, 1956).



Figs. 6–8: Ampullate glands in *Oxyopes lineatus*. **6** parasagittal general view of the opisthosoma of a male; Ac = aciniform glands, L = lung, MA = major ampullate gland, Pi = piriform glands, phosphotungstic haematoxylin, orange-yellow filter. **7** reducing compounds (black) in a major ampullate gland of a male; L = lung, ferric ferricyanide reaction, orange filter. **8** major ampullate gland and a small part of the tail of a minor ampullate (mA) of a female; L = lung, Ov = ovary, Danielli's coupled tetrazonium reaction for proteins, green filter. Scale lines = 200 μm (6), = 100 μm (7–8).

part of the ampulla, the other in the rest of the ampulla (Figs. 6–8). Major ampullate glands may be distinguished from minor ones by the staining affinities and the histochemical characteristics of their secretory products (Table 2). But the largest secretory granules are secreted in the distal tube of minor ampullate glands: this characteristic alone makes major and minor ampullate glands easily distinguishable (Fig. 9). In all cases, the second secretory product appears in the cells of the ampulla as finer granules, the size of which gradually decreases towards the excretory duct. The two proteins form respectively the core and the coat of the silk fibre that issues from each type of ampullate glands.

Piriform glands. Fifty to seventy-five pairs of piriform glands are compactly piled in two packs along the ventral surface of the posterior part of the opisthosoma, above the anterior-lateral spinnerets inside which their excretory

ducts are collected. In *Peuceitia* species, they appear as short, sinuous tubes (Figs. 10, 14) of different sizes. They are more rounded and of about the same size in *Oxyopes lineatus* (Figs. 16–17). All piriform glands are bipartite, but a variation of the respective extent of each part (from one-quarter to three-quarters) was found in all species studied. The histochemical characteristics of the two proteins secreted contrast strongly (Table 2; Figs. 16–17). The volume of piriform glands decreases in males at the end of the life cycle only; in adult females, during the first few days after mating, synthesis of proteins takes place in small piriform glands, the size of which is at a maximum just at the time of egg laying, when cylindrical glands are also full of secretion (Figs. 16–17).

Aciniform glands. About forty pairs of simple elongated sacs, with a short narrow tail in *Peuceitia*, are grouped in the posterior quarter of the opisthosoma, above the median posterior

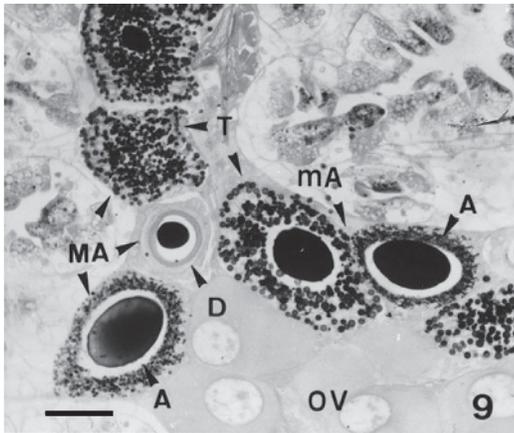


Fig. 9: Transverse sections of major (MA) and minor (mA) ampullate glands of *O. lineatus* (female). A = ampulla, D = excretory duct, Ov = ovary, T = tail, ferric ferricyanide reaction, orange filter. Scale line = 50 μ m.

spinnerets. These glands are variable in size; the largest ones are the most anterior and median ones (Fig. 11). A noticeable variation of their volume occurs according to their physiological state. In immature spiders, aciniform glands are relatively larger than in adult females or males (Figs. 10, 12). Aciniform glands are reduced in old males and in females after several egg layings (Figs. 14–15). These glands secrete one protein in their distal part, and another one in their proximal collar (Table 2; Fig. 10).

Cylindrical glands. The development of cylindrical glands, which are fairly numerous in Oxyopidae (Table 1), especially in *Peucetia cauca* (Fig. 13), correlates with the maturation of eggs in the ovary. In subadult females, they appear to be in an embryonic state, and do not secrete any product (Fig. 12). From the beginning of the secretion, two proteinic substances (A and B) are produced in their single category of secretory cells. Protein A, which appears homogeneously cyanophilic in *Peucetia* species, and with double affinities in *Oxyopes lineatus*, is surrounded by a thin layer of protein B in the lumen of the glands (Table 2; Figs. 12, 14).

Epiandrous glands. Each long setiform spigot on the anterior lip of the epigastric furrow of male *Peucetia* contains the excretory duct of a small gland, the anatomical and histochemical characteristics of which strongly resemble those

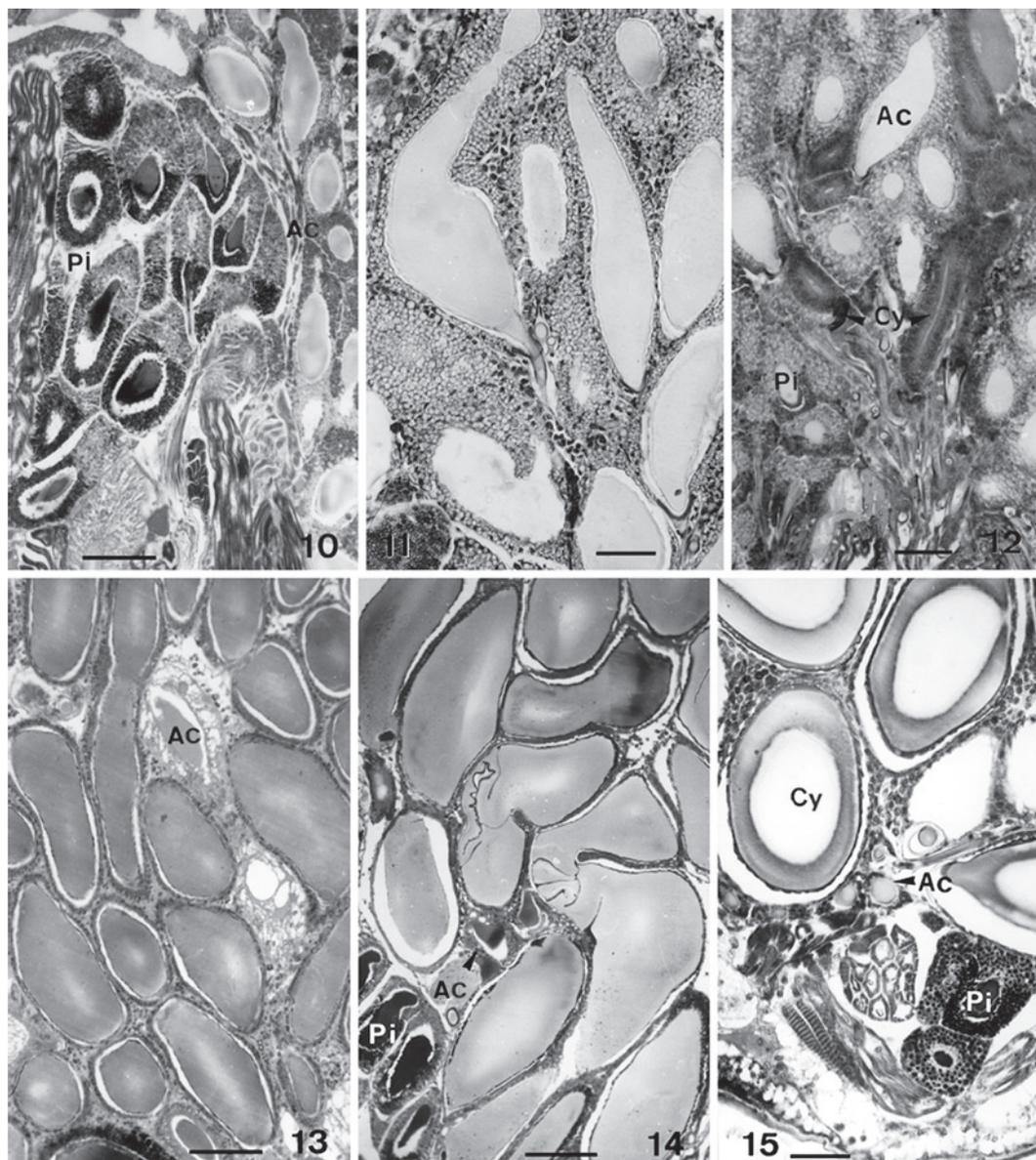
of piriform glands (Table 2; Fig. 18). Such glands are missing in male *Oxyopes lineatus*.

Discussion

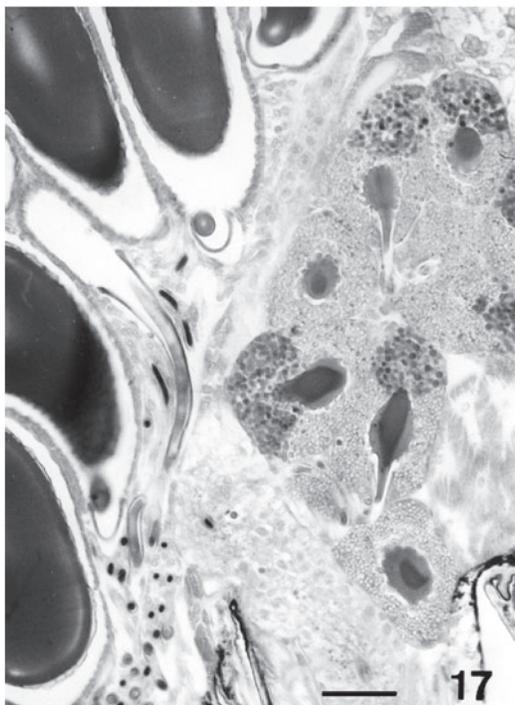
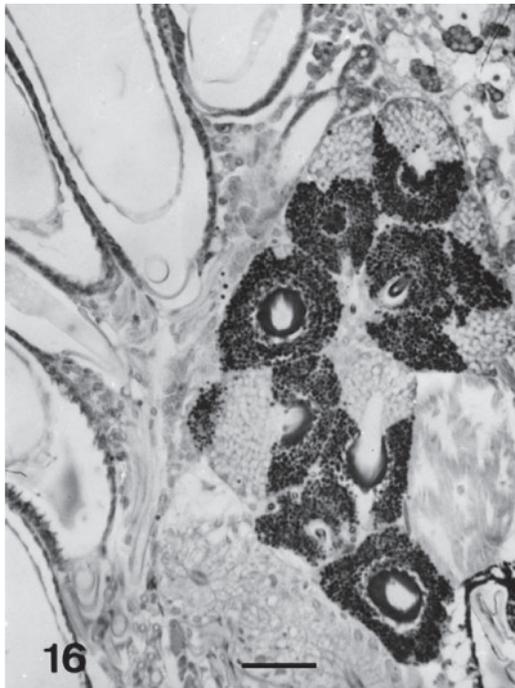
The silk-gland system in the two main genera of the family Oxyopidae comprises the four types of glands, all well developed, that are usually present in a number of other families, i.e. Agelenidae, Lycosidae and Thomisidae (Kovoor, 1977). The only two pairs of ampullate glands in adults are distinguishable from each other, both anatomically and histochemically, this distinction being more obvious in *Oxyopes lineatus* than in *Peucetia* species. Their characteristics correspond to a greater specialization of *Oxyopes* absent from Lycosidae so far studied (Richter, 1970; Kovoor, 1976) and from Salticidae, the ampullate glands of which comprise four very similar pairs (unpubl. data).

Juveniles of *Peucetia*, as well as of *Oxyopes*, show a double set of ampullate glands, and large aciniform and piriform glands, which all together can produce more than enough silk to build a rudimentary web, as was observed by Kaston (1972) with *P. viridans*. However, it is noteworthy that none of the proteins secreted by these glands, except the piriform, seem to be adhesive, from their histochemical characteristics (see Table 2). The web of young *Peucetia* is not made to catch prey. Adult *Peucetia* spin only a kind of retreat made up of some scarce threads fixed on leaves, and *Oxyopes* does not even occupy any silken waiting place. Predation is visually guided; success in predation depends on good eyesight, active venom and the velocity of these spiders. However, *Tapinillus longipes*, an oxyopid spider from moist tropical forest edges in America, is known to be a sheet-web builder (Griswold, 1983; Mora, 1986): prey is ensnared in the web prior to being attacked by this spider. This behaviour, supposed to be primitive, is reminiscent of that of linyphiids; it has been completely lost by *Oxyopes*, while it can be traced as a remnant in the use of a retreat by *Peucetia*. Parallel to this gradual reduction of the role of silk in predation, the optical characteristics of the visual system appear better in *Oxyopes lineatus* than in *Peucetia* species (Kovoor & Muñoz-Cuevas, 1997).

In adult males and females of both genera, the silk-gland system is devoted to reproduction,



Figs. 10–15: **10** Aciniform (Ac) and piriform (Pi) glands in *P. gerhardi* (male); one-step trichrome, green filter. **11** aciniform glands in *P. gerhardi* (male); negative reaction of the two secretory products to P.A.S.-reaction, green filter. **12** aciniform (Ac), cylindrical (Cy) and piriform (Pi) glands in *O. lineatus* (subadult female); P.A.S.-reaction, green filter. **13** cylindrical glands in *P. cauca* adult female; moderate positive reaction of product A tyrosin (grey), Ac = aciniform glands, Morel and Sisley's reaction, green filter. **14** Cylindrical glands in *P. gerhardi*; Ac = aciniform, Pi = piriform glands, Azan, green filter. **15** part of cylindrical glands on *O. lineatus*; Ac = aciniform, Cy = cylindrical, Pi = piriform glands, phosphotungstic haematoxylin, green filter. Scale lines = 100 μm (10, 13), = 50 μm (11–12, 15), = 150 μm (14).



Figs. 16–17: Cylindrical (Cy) and piriform (Pi) glands in *O. lineatus*, just before the last egg laying. **16** Lead haematoxylin staining, green filter; **17** ferric ferricyanide reaction, orange filter. Scale lines = 50 μm .

from the mating of the couple suspended on a silk thread up to the egg laying of females which develop a complementary type of silk gland (cylindrical), the silk of which is the main component of the egg-cases. Males build triangular (*Peucetia*) or square (*Oxyopes*, Gerhardt, 1927) sperm webs; but only male *Peucetia* possess epiandrous glands secreting very thin, probably adhesive fibres. These glands are absent in *Oxyopes lineatus* and *O. heterophthalmus* (Lopez & Emerit, 1988), and their function in *Peucetia* is not clear.

The histophysiological study of the silk-gland system in some *Peucetia* and *Oxyopes* species does not provide an unequivocal answer to the phylogenetic relationships of the Oxyopidae. According to Griswold (1993), the family Oxyopidae, included in the Lycosoidea, is the sister group of the Senoculidae. These spiders need to be studied because close relationships of Oxyopidae with other hunting spiders, such as Lycosidae or Ctenidae, are not obvious.

Acknowledgements

Our thanks go to Magdalena Perez (Altea, Spain) who gave a warm reception to and kindly guided A.M.C. in the field to collect *P. viridis*. We are indebted to R. Cleva (MNHN, Paris) for his invaluable assistance in scanning electron microscopy.

References

- ADAMS, C. W. M. 1956: A stricter interpretation of the ferric ferricyanide reaction, with particular reference to the demonstration of protein-bound sulphhydryl and disulphide groups. *J. Histochem. Cytochem.* **4**: 23–35.
- GABE, M. 1968: *Techniques histologiques*. Paris: Masson.
- GERHARDT, U. 1927: Neue biologische Untersuchungen an einheimischen und auslaendischen Spinnen. *Z. Morphol. Ökol. Tiere* **8**: 96–186.
- GERHARDT, U. 1933: Neue Untersuchungen zur Sexualbiologie der Spinnen insbesondere an Arten der Mittelmeerlaender und der Tropen. *Z. Morphol. Ökol. Tiere* **27**: 1–33.
- GERTSCH, W. J. 1949: *American spiders*. 2nd ed. New York: Van Nostrand Reinhold.
- GRISWOLD, C. E. 1983: *Tapinillus longipes* (Taczanowski), a web-building lynx spider from American tropics (Araneae: Oxyopidae). *J. nat. Hist.* **17**: 979–985.

- GRISWOLD, C. E. 1993: Investigations into the phylogeny of the lycosoid spiders and their kin (Arachnida: Araneae: Lycosoidea). *Smithson. Contr. Zool.* **539**: 1–39.
- KASTON, B. J. 1972: Web making by young *Peucea viridans* (Hentz) (Araneae: Oxyopidae). *Notes Arachnologists Southwest* **3**: 6–7.
- KOVOOR, J. 1976: Caractères adaptatifs et caractères familiaux des glandes séricigènes dans le genre *Hippasa* Simon (Araneae, Lycosidae). *C. R. 3ème Réunion Arachnol. expr. fr.*: 83–96.
- KOVOOR, J. 1977: La soie et les glandes séricigènes des Arachnides. *Année biol.* **16**: 97–171.
- KOVOOR, J. & MUÑOZ-CUEVAS, A. 1995: Embryonic and postembryonic morphogenesis of the visual, venom- and silk-gland systems in two species of *Peucea* (Araneae, Oxyopidae). *Eur. J. Ent.* **92**: 565–571.
- KOVOOR, J. & MUÑOZ-CUEVAS, A. 1997: Comparative structure of the visual system of lynx spiders (Oxyopidae) and its relation to habitat and behaviour. *Zool. Anz.* **235**: 133–145.
- LILLIE, R. D. 1965: *Histopathologic technic and practical histochemistry*. New York: McGraw-Hill.
- LILLIE, R. D. & FULLMER, H. M. 1976: *Histopathologic technic and practical histochemistry*. New York: McGraw-Hill.
- LOPEZ, A. & EMERIT, M. 1988: New data on the epigastric apparatus of male spiders. *Bull. Br. arachnol. Soc.* **7**: 220–224.
- McMANUS, J. F. A. 1946: The histological demonstration of mucus after periodic acid. *Nature, Lond.* **158**: 202.
- MORA, G. 1986: Use of web by *Tapinillus longipes* (Araneae: Oxyopidae). In W. G. Eberhard, Y. D. Lubin & B. C. Robinson (eds.). *Proceedings of the Ninth International Congress of Arachnology, Panama 1983*. Washington, DC: Smithsonian Institution Press: 173–175.
- MOWRY, R. W. 1956: Alcian blue technics for the histochemical study of acidic carbohydrates. *J. Histochem. Cytochem.* **4**: 407–408.
- PEARSE, A. G. E. 1968: *Histochemistry. Theoretical and applied*. 3rd ed. Boston: Little, Brown & Co.
- RICHTER, C. J. J. 1970: Morphology and function of the spinning apparatus of the wolf spider *Pardosa amentata* (Cl.) (Araneae, Lycosidae). *Z. Morphol. Tiere* **68**: 37–68.
- ROVNER, J. S. 1980: Adaptations for prey capture in oxyopid spiders: phylogenetic implications. In J. Gruber (ed.). *8. Internationaler Arachnologenkongress Wien 1980 Verhandlungen*. Vienna: H. Egermann: 233–237.

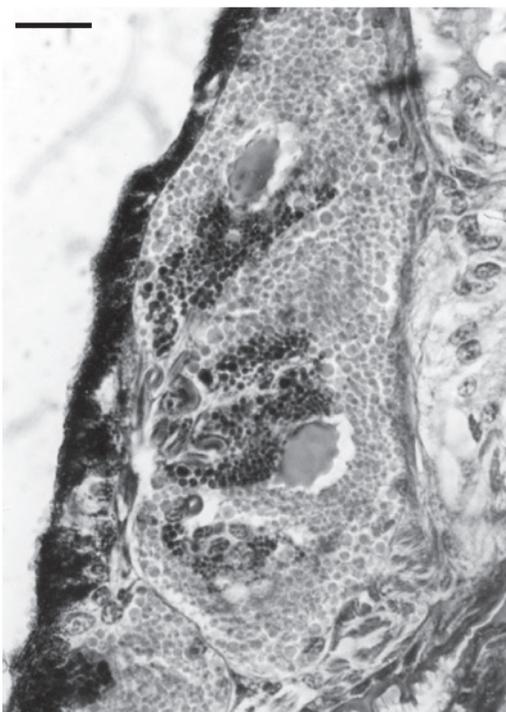


Fig. 18: Epiandrous glands of a male *P. gerhardi*. Danielli's coupled tetrazonium reaction, green filter. Scale line = 20 μ m.

- SOLCIA, E., CAPELLA, C. & VASSALLO, G. 1969: Lead haematoxylin as a staining for endocrine cells. Significance of staining and comparison with other selective methods. *Histochemie* **21**: 116–126.
- WHITCOMB, W. H. & EASON, R. R. 1965: The mating behavior of *Peucea viridans* (Araneae: Oxyopidae). *Fla Ent.* **48**: 164–167.
- WHITCOMB, W. H., EXLINE, H. & HUNTER, R. C. 1963: Spiders of the Arkansas cotton field. *Ann. ent. Soc. Am.* **56**: 653–660.
- WILLEY, M. B. & ADLER, P. H. 1989: Biology of *Peucea viridans* (Araneae, Oxyopidae) in South Carolina, with special reference to predation and maternal care. *J. Arachnol.* **17**: 275–284.
- YOUNG, O. P. & LOCKLEY, T. C. 1985: The striped lynx spider, *Oxyopes salticus* (Araneae: Oxyopidae), in agroecosystems. *Entomophaga* **30**: 329–346.