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Spermatodesmata of the Sawflies (Hymenoptera: Symphyta): Evidence for Multiple Increases in Sperm Bundle Size

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Abstract.-We present the first survey of spermatodesmata (bundles of spermatozoa connected at the head by an extracellular 'gelatinous' matrix) across the sawfly superfamilies. Spermatodesmata occur in all examined taxa within the sawfly grade (Xyelidae-Orussidae inclusive), but are not found in the Apocrita. Using DAPI staining, the numbers of individual sperm per spermatodesm were calculated and the values obtained are mapped on to the current phylogenetic hypothesis. The plesiomorphic spermatodesm in the Hymenoptera, based on that observed in the putatively basal family Xyelidae, contains relatively few sperm, approximately 16. However, in the Tenthredinoidea and in the Siricidae, far larger numbers are found, reaching up to 256 in the Cimbicidae.

In many insects, mature sperm released from testicular follicles are neither free individuals nor packaged into variously complex spermatophores, but are arranged in organised bundles with their anterior ends embedded in an extracellular cap. These structures, called spermatodesmata (spermatodesm singular), occur, amongst others, in at least some members of the Collembola, Orthoptera, Diptera, Coleoptera, Lepidoptera and Hymenoptera (Jamieson 1987). Within the Hymenoptera, spermatodesmata appear to be limited to the basal sawflies (Quicke et al. 1992, Quicke 1997), and they have not been observed in members of the 'Evaniomorpha' (Stephanoidea, Megalyroidea, Evanioidea and Ceraphronoidea examined), proctotrupoid *sensu lato* (Diapriidae, Proctotrupidae, Heloridae and Scelionidae examined), chalcidoid, cynipoid, ichneumonoid or aculeate groups (Quicke et al. 1992, Newman and Quicke 1998, 1999a,b, 2000, Lino-Neto et al. 1999, 2000a,b).

Until now, spermatodesmata have only been characterised in a few sawflies, almost entirely as part of ultrastructural investigations using transmission electron microscopy (Quicke et al. 1992, Newman and Quicke 1999a), but the data obtained are not normally easily interpreted in terms of the actual size and structure of the spermatodesm. However, it was apparent that spermatodesmata vary in both size (i.e. number of individual sperm involved) and shape. For example, in most taxa examined the spermatodesmata resemble a tuft of grass with their acrosomes embedded in an extracellular cap and the nuclei and tails splaying out posteriorly. However in the cephid, *Cephus pygmaeus,* the whole spermatodesm is very elongate, several times longer than an individual sperm, and sperm are inserted along a thin central extracellular matrix core (Quicke et al. 1992).

Sperm produced in a given follicle are all derived from cell divisions from a sin-

gle spermatogonial cell (see Quicke 1997). Because these cell divisions occur synchronously within a follicle and each sperm mother cell in each follicle undergoes a fixed number of cell division rounds, the numbers of sperm per spermatodesm are expected to be 2^n where n is the number of rounds of spermatocyte division.

Counting the numbers of sperm in each spermatodesm was not straight-forward for most taxa because when stained using traditional dyes, the mass was so opaque with overlapping nuclei and tails that individual cells could not be distinguished and counted. We have therefore employed a fluorescence staining technique in order to measure the total DNA content of the spermatodesm and divided that by the DNA content of an individual sperm nucleus. For a few taxa that were no longer available for the current study, we have included some crude estimates of sperm number obtained from transmission electron microscopy of transverse sections (Quicke et al. 1992, Newman and Quicke 1999a). However, these are likely to be underestimated, because more posteriorly inserted sperm may not have been sectioned.

MATERIALS AND METHODS

Materials.—The following taxa were examined. Xyelidae: *Xyela* sp., Colorado; Tenthredinidae: *Tenthredo xantha* Norton, N California; *Strongylogaster distans* Norton, S California; *Dolerus tejoniensis* (Norton), S California; Pergidae: *Acordulecera* sp., Illinois; Cimbicidae: *Trichiosoma triangulum* (Kirby), NW California; *Cimbex americanum* Leach, NW California; Anaxyelidae: *Syntexis libocedrii* Rohwer, California; Xiphydriidae: *Xiphydria abdominalis* Say, Illinois; *Xiphydria maculata* Say, Illinois; Orussidae: *Orussus thoracicus* (Ashmead), N California; *Orussus occidentalis* (Cresson), N California. It should be noted that males of many sawflies are taxonomically difficult to segregate and at present species-level identifications are not always possible. Voucher specimens have therefore been deposited in the United States National Museum (Washington D.C.).

Light microscopy.-Vas deferentia and testes were dissected from living sawflies in insect saline (Clark et al. 1979) and teased apart on a clean microscope slide. After a few minutes to allow the sperm/spermatodesmata to swim free of the disrupted tissue, the slides were heat fixed on an hot plate at approximately 80°C. These slides were stained with a 0.007mg/ml solution of 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and viewed with a Leitz epifluorescence microscope at × 1000 (as in Flemming *et al.* 2000). This stain specifically binds to double stranded DNA and fluoresces under UV light (Figs. 1, 2). Images of stained spermatodesmata were captured using a CV-M300 video camera, and a Scion LG3 frame-grabber mounted in a Power Macintosh running Scion Image 1.62a. Care was taken to ensure that, in each frame, both a complete spermatodesm and an isolated sperm cell was present (This was not possible for *Acordulecera* where only one isolated sperm nucleus could be found). Before capturing, the image was adjusted to minimise pixel saturation and these adjustments affected the spermatodesm and isolated sperm cell in each image equivalently. A densitometric value for both the spermatodesm and the sperm nucleus was determined (the product of area measured in pixels and staining intensity) and the number of sperm in the spermatodesm derived by dividing the two values. On average four images per species were analysed. For *one* species, *Xiphydvia maculata,* it was possible to make a direct count of sperm nuclei present in the spermatodesm. Comparison of this value with that obtained densitometrically (35 ± 7 (95% confidence interval) and 33 ± 3 (95% confidence interval) respectively) confirms the accuracy of the technique. Basic statistics were calculated using Excel 98 (Microsoft).

Making the assumption that DNA

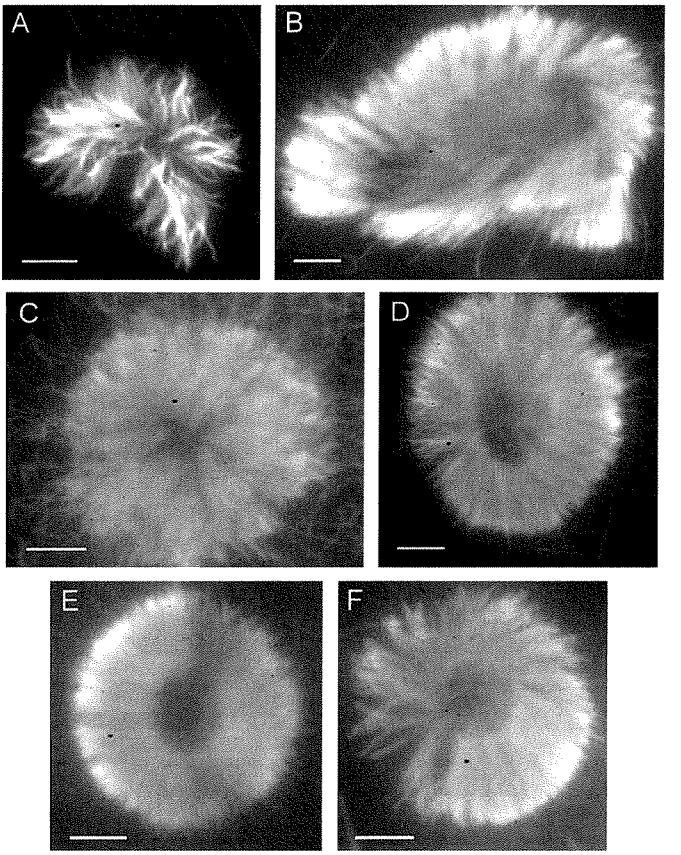


Fig. 1. Fluorescence images of DAPI-stained sperm nuclei in spermatodesmata of sawflies investigated: A, *Acordulecera* sp. (Pergidae); B, *Trichiosoma triangulum* (Cimbicidae); C, *Strongylogaster distans* (Tenthredinidae); D, *Dolerus tejoniensis* (Tenthredinidae); E, *Tenthredo xantha* (Tenthredinidae); F, *Cimbex americanum* (Cimbicidae).

(chromatin) is densely packed in sperm nuclei, and since we have no *a priori* reasons to expect differences in DNA density between taxa, we used sperm nucleus size as a surrogate for haploid DNA content.

RESULTS

The sperm heads are inserted throughout the cap of the spermatodesmata, with those sperm located more centrally being inserted more anteriorly (Figs. 1, 2). For Xyelidae, Anaxyelidae, Xiphydriidae and Orussidae, the spermatodesmata are elongate structures, in the case of *Orussus,* the sperm appear to be inserted in the cap in a spiral configuration rather as if a cylindrical roll of paper was 'pulled out' from the middle. Tenthredinoid spermatodesmata are far wider and the larger ones (i.e. those with a larger number of sperm; Fig. 1a,b,f) dry on to the slides as rosette like structures. Although the centres of these appear empty in DAPI-stained material, transmission electron micrographs (Newman and Quicke 1999a) suggest that this is the region where the acrosomes are inserted in the extracellular matrix of the cap.

Results of numbers of sperm per spermatodesm are shown graphically in Fig. 3. Visual inspection of numbers (which as explained above are expected to be integer powers of 2) suggests that for *Xyela,* the number is 16, for Orussidae, Anaxyelidae

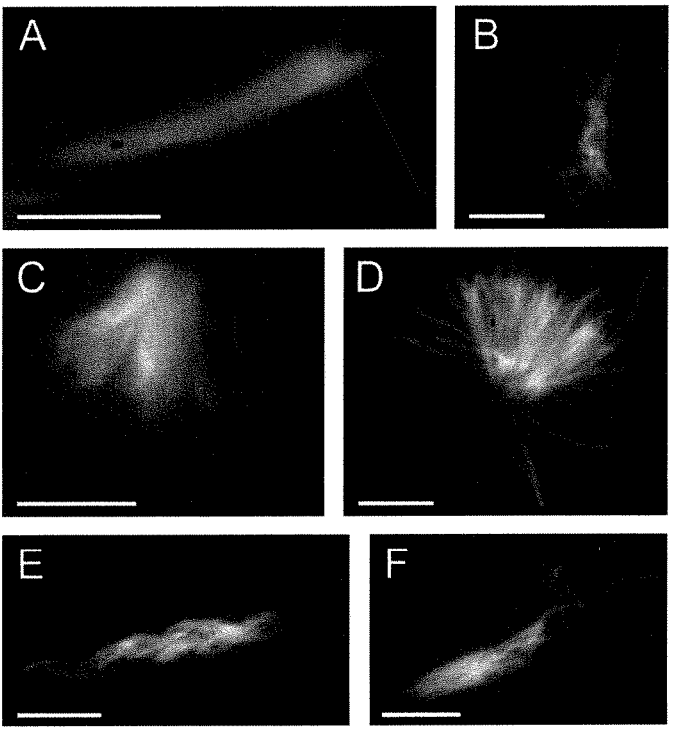


Fig. 2. Fluorescence images of DAN-stained sperm nuclei in spermatodesmata of sawflies investigated: A, *Xyela* (Xyelidae); B, *Syntexis libocedrii* (Anaxyelidae); C, *Xiphydria abdominalis* (Xiphydriidae); D, *Xiphydria maculata* (Xiphydriidae); E, *Orussus occidentalis* (Orussidae); F, *Orussus thoracicus* (Orussidae).

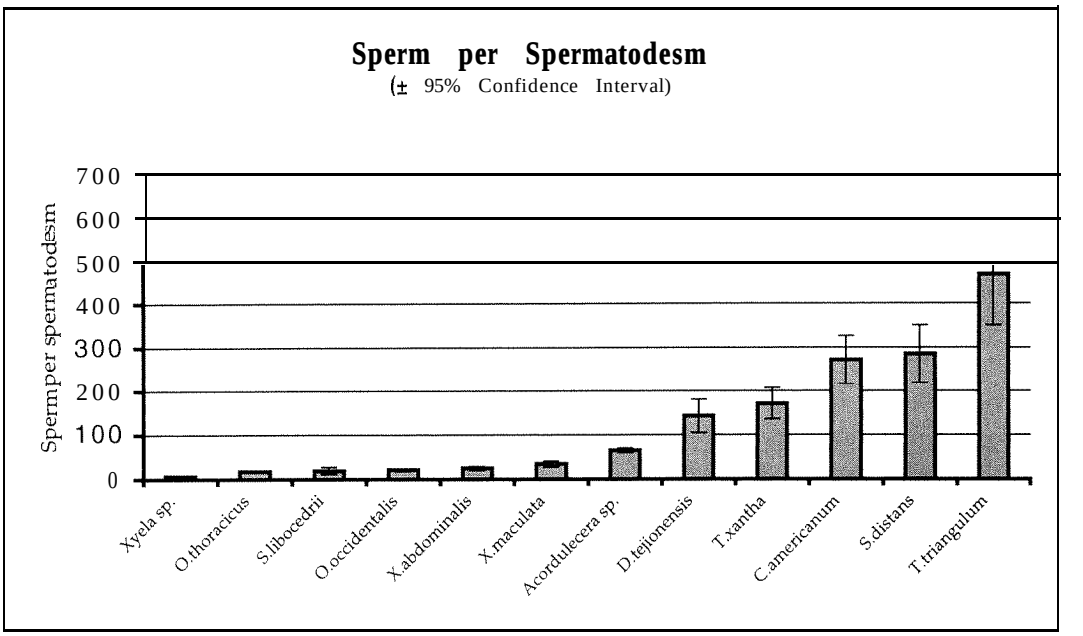


Fig. 3. Plot of numbers of sperm per spermatodesm for sawfly taxa ranked according to spermatodesm size.

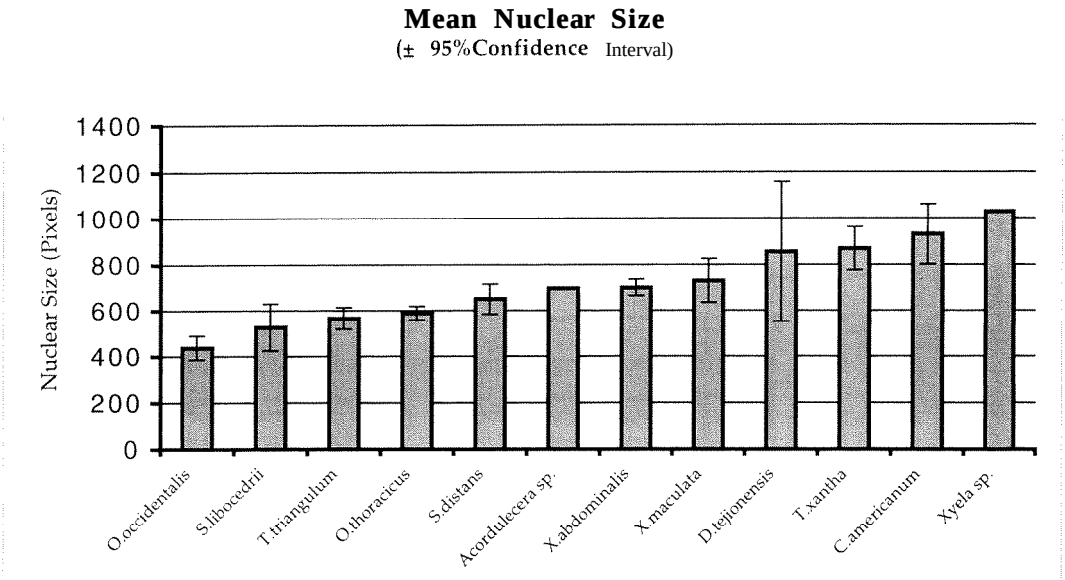


Fig. 4. Plot of mean nucleus size, a surrogate for DNA content, for individual sperm in each sawfly taxon.

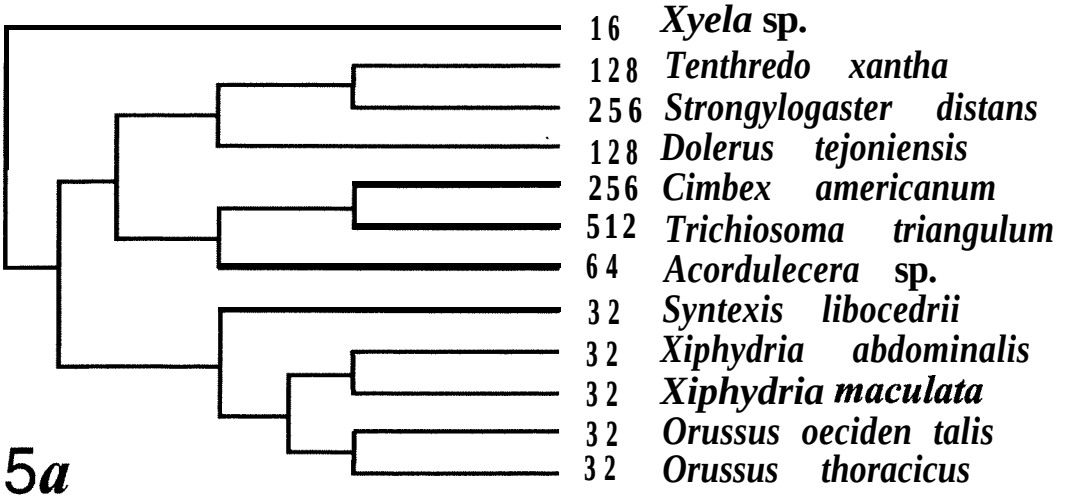
and Xiphydriidae, it is 32, for Tenthredinidae the number ranges from 128 to 256, for Pergidae 64 and for Cimbicidae, 256-512. Visual inspection of toluidine blue stained slides of two other tenthredinids (*Rhogogaster californica* (Norton) and a Ten*thredo* that was either *T. lacticirzcta* Cresson or *T. varipicta* Norton) suggests that they have the same number of sperm per spermatodesm as T. *xantha.*

Counts of the number of pixels occupied by isolated sperm nuclei (Fig. 4) allow us to estimate nuclear DNA content. The distribution is suggestive of a trend among the sawflies in that *Xyela,* the most basal genus, has the largest nucleus and *Orussus,* the most derived genus has the smallest nucleus.

Although we have not been able to utilise the present technique to quantify spermatodesm size in the Cephoidea or Siricidae, inspection of the stained light micrographs and of transmission electron micrographs for these two superfamilies respectively (Quicke et al. 1992, Newman and Quicke 1999a) indicate that both of these have rather large numbers of sperm per spermatodesm. Our best estimates were taken as the smallest power of two larger than the definite minimum number of individual sperm within a micrograph of a spermatodesm. The sperm tails are relatively straight for a distance after emerging from the sperm head and in our micrographs the spermatodesmata were reasonably isolated so we do not believe that there is any reason why that sperm number will have been over-estimated. For the cephid, *Cephus,* this is 128 (based on a count of 93 sperm tails), and for the siricid, *Tremex,* it is 512 (based on a count of c. 400 transverse sections of sperm in a largely complete micrograph section).

DISCUSSION

Given that we now have a robust phylogenetic hypothesis for the superfamilies of sawflies and at least an estimate of family level relationships within the tenthredinoid lineage (Vilhelmsen 1997, 2000a,b, 2001), we can consider the evolutionary pattern of spermatodesm size (number of



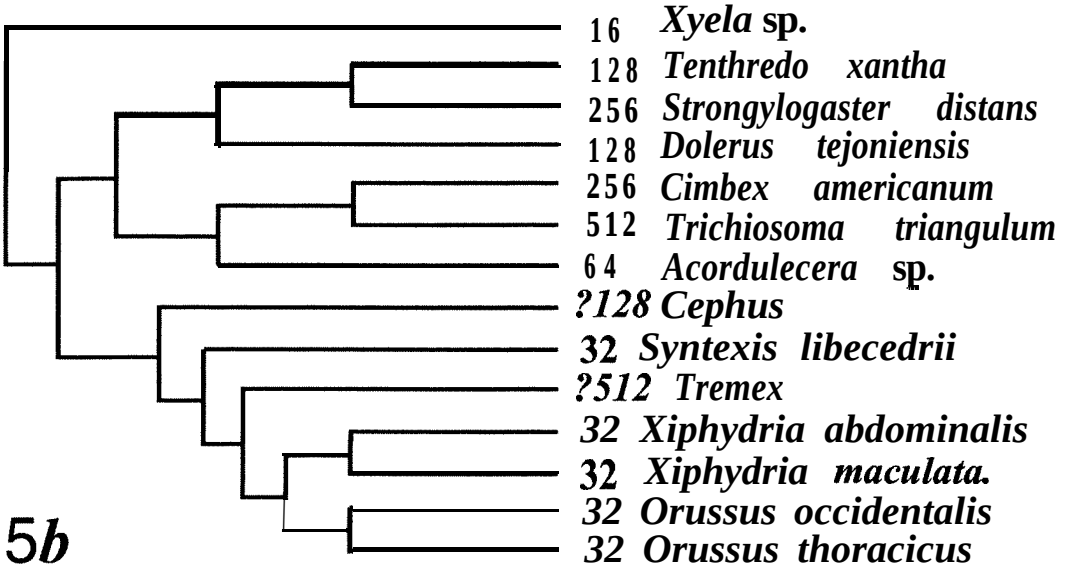


Fig. 5. Number of sperm per spermatodesm shown on the independently obtained cladogram of sawfly relationships (from Vilhelmsen 1997, 2001), showing in (a) only data obtained from DAPI-staining, and (b) with values for additional taxa based on other estimation techniques incorporated.

sperm included). Unfortunately, there is considerable uncertainty about what constitutes a suitable outgroup for the Hymenoptera, and if one accepts a currently common view that the order is the sister group of the remainder of the Holometabola, then there is too much variation with this putative sister group to use it as an outgroup for the purposes of the current analyses. Therefore we base our interpretations on the likely ancestral state in the order on the state shown by the Xyelidae which display the most putatively plesiomorphic character states of any of the extant Hymenoptera. Visual inspection of the DAPI sperm-quantification data mapped on to Vilhelmsen's (*loc.* cit.) independently derived sawfly phylogeny

(Fig. 5a) suggests that the groundplan spermatodesm size for sawflies is low (16 or 32), but that there has been a general increase within the Tenthredinoidea (range 64-512) and that particularly large numbers (256-512) have evolved at least twice within this superfamily. However, incorporating estimates of sperm number for *Ccphus pygmeus* (Cephoidea) and *Tremex* sp. (Siricidae) into Vilhelmsen's phylogeny (*loc.* cit.) (Fig. 5b) tends to confuse the picture in that it is equally parsimonious that there was a marked increase in sperm number per spermatodesm above the Xyelidae, and that there were reversals to lower numbers in the Anaxyelidae and Xiphydriidae + Orussidae as for multiple increases from a groundplan of 16 or 32 (viz in the Tenthredinoidea, Cephoidea and Siricidae).

Denser taxon sampling may help to clarify the above issues and may also provide additional phylogenetic evidence within some groups, especially within the Tenthredinoidea. Future work will examine nuclear DNA content across the Hymenoptera in more detail (Schiff, Flemming and Quicke in preparation).

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