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REPRODUCTIVE ANATOMY AND GAMETOGENESIS IN *SHIPLEYA INERMIS* (CESTODA: DIOECOCESTIDAE)

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SUMMARY

Study of the reproductive anatomy in 65 strobilae of the dioecious cestode *Shipleya inermis* Fuhrmann, 1908 (Acoleata: Dioecocestidae) showed that a common genital duct, probably arising through fusion of the vas deferens and the proximal portion of the vaginal duct, compensated functionally for the loss of a patent vagina. Gonochorism was characteristic, but rudimentary genital organs of the opposite sex were present in 26 % of males and 9 % of females; two strobilae (3 %) were hermaphroditic. Hermaphrodites had normally developed male organs and were capable of cross-fertilization as males; their female organs were much reduced in size but were functional, and eggs or fertilized ova in the uteri indicated that self-fertilization occurred. Gametogenesis was traced, mainly in chromosomal preparations. The diploid chromosomal complement in embryos and germ-line cells consisted of four pairs of homologues ($2n$ = 8, n = 4, FN = 14). Based on observation in female cestodes of one pair of chromosomes having non-homologous or non-pairing segments due to influence of heterochromatin, the authors suggest that females produce gametes of two types relative to heterochromatic DNA, while males are homogametic, and that sex-determining effects are associated therein. In males, meiosis included chromosomal pairing and recombination, after which heterochromatin was eliminated from germline cells through fragmentation. Other biological characteristics of *S. inermis* in the hosts, *Limnodromus* spp. (Charadriiformes), are briefly discussed.

RÉSUMÉ : **L'anatomie reproductrice et la gamétogenèse de *Shipleya inermis* (Cestoda : Dioecocestidae).**

Une étude a été faite sur 65 strobiles du cestode dioïque *Shipleya inermis* Fuhrmann, 1908 (Acoleata : Dioecocestidae) concernant l'anatomie reproductrice et la gamétogenèse. Elle a montré que le conduit génital commun (résultant vraisemblablement de la fusion du canal déférent et de la partie proximale du conduit vaginal) sert à compenser la perte d'un vagin séparé. Chez ce cestode, le gonochorisme est typique, mais les auteurs ont observé des organes génitaux rudimentaires de l'autre sexe dans 26 % des mâles et dans 9 % des femelles. Deux des 65 strobiles (3 %) sont hermaphrodites, avec l'appareil mâle fonctionnel et capable de fécondation réciproque; les organes femelles sont réduits en taille. Les œufs des spécimens hermaphrodites ont atteint la maturité après autofécondation. La gamétogenèse a été observée principalement dans des préparations chromosomiques. La garniture chromosomique diploïde des embryons et des cellules reproductrices consiste en quatre paires homologues ($2n$ = 8, n = 4, NF = 14). Ayant observé une paire chromosomique avec des segments non-homologues ou non appariés en raison de la présence d'hétérochromatine, les auteurs suggèrent que les femelles de *S. inermis* produisent des gamètes de deux types en ce qui concerne le DNA hétérochromatique, tandis que les mâles sont homogamétiques; le déterminisme sexuel est lié à ce phénomène. Chez les mâles, tous les éléments chromosomiques sont homologues, sans segments différenciés; le DNA hétérochromatique a été éliminé de la lignée germinale par fragmentation des chromosomes. D'autres caractéristiques biologiques de *S. inermis* chez les hôtes, *Limnodromus* spp. (Charadriiformes), sont brièvement discutées.

The genera of dioecious cestodes in the order Cyclophyllidea have been arranged in either one or two families in the suborder Acoleata Skriabin, 1940. In a recent revision of the Acoleata, Ryzhikov and Tolkacheva (1981) recognized two families, Dioecocestidae Southwell, 1930, with a single genus, *Dioecocestus* Fuhrmann, 1900, and Gyrocoeliidae Yamaguti, 1959, holding the remaining genera. Schmidt (1986) placed all cyclophyllidean genera of dioecious cestodes in the family Dioecocestidae: *Gyrocoelia* Fuhrmann, 1899; *Dioecocestus* Fuhrmann, 1900; *Shipleya* Fuhrmann, 1908; *Infula* Burt, 1939; *Pseudoshipleya* Yamaguti, 1959 (listed as a synonym of *Infula* by Ryzhikov and Tolkacheva, 1981); *Neodioecocestus* Siddiqi, 1960 (listed as a synonym of *Dioecocestus* by Ryzhikov and Tolkacheva, 1981); and *Echinoshipleya* Tolkacheva, 1979. With the exception of *Gyrocoelia* spp., which apparently may be either dichogamous or dioecious, all cestodes in those genera are considered to be gonochoristic. Various modifications of the genital ducts have been reported in female strobilae. *Infula* spp. possess a patent genital duct in females; in *Dioecocestus* spp. the vaginal duct does not open to the exterior; and members of the remaining genera are regarded as lacking a vagina. Reproductive processes are not understood in these cestodes.

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We considered that dioecocestids might be especially suitable for the investigation of gametogenesis, and for that work, selected *Shipleya inermis* Fuhrmann, 1908, a characteristic component of the helminth-fauna of dowitchers, *Limnodromus* spp. (Charadriiformes). Our study revealed that some details of the reproductive system in females had been previously overlooked or misconstrued, and that hermaphroditic strobilae also exist, with a more complex arrangement of genital ducts. Herein we report our findings concerning anatomy, gametogenesis, and some other biological characteristics of *S. inermis*. This work is dedicated to the memory of our friend and colleague Gerald D. Schmidt.

MATERIAL AND METHODS

Sixty-one specimens of *Shipleya inermis* were obtained from 35 dowitchers, *Limnodromus* spp., as follows: Long-billed dowitcher, *L. scolopaceus* (Say): Beaufort Lagoon, arctic coast of Alaska, 1 bird (July 1970); St. Lawrence Island, Bering Sea, 6 (June 1984-1988); Vermilion Parish, Louisiana, 1 (April 1988); and Grant County, Washington, 6 (May 1988). Short-billed dowitcher, *L. griseus* (Gmelin) : Cameron Parish, Louisiana, 7 birds (May 1988); and Grays Harbor County, Washington, 14 (April 1990). Also studied were two permanently mounted specimens from *L. griseus,* Bristol Bay, Alaska, provided by Dr. G. D. Schmidt (Nos. 72 and 73), and two from *L. scolopaceus,* Texas, from the Helminthological Collection of the United States National Museum (Nos. 78896 and 78897).

Most of the cestodes were removed alive from the host. The birds collected in Louisiana were frozen soon after death and transported in that condition to the laboratory. At autopsy, the location (distance posterior to the pylorus) of each cestode was recorded, with the exception of those from birds from St. Lawrence Island and Beaufort Lagoon. Cestodes from the frozen birds were preserved in formalin. Strobilae to be used for cytological analyses were transferred immediately to Hank's basic salt solution buffered with 10 mM TES [N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid] and containing 100 U of penicillin/ml and 100 mg of streptomycin/ml. All strobilae were placed individually in vials. The cestodes collected on St. Lawrence Island were placed in the basic salt solution and refrigerated for up to seven days, until they could be taken to the laboratory. Prior to cytogenetic study, living cestodes were incubated at 37° C for varying periods (usually 3-4 hr) in the basic solution containing colchicine (1 μg/ml). Selected segments were cut finely and processed by standard methods for conventional Giemsa staining, and for G-banding (Seabright, 1972). C-banding was attempted on material from one male and two females, using barium hydroxide (Salamanca and Armendares, 1974). The reproductive organs were dissected from mature segments of two males and one female and stained in acetic orcein. The remaining portions of each strobila used for cytogenetic purposes, and those intended for morphological studies only, were fixed in a hot solution of 10 % formalin. Chromosomes from 17 specimens of *S. inermis* were studied (five males, ten females, and two hermaphroditic strobilae). For each, chromosomal components of 50 to 100 cells were counted and evaluated. Arm-ratio (AR) and fundamental number (FN; the total of major chromosomal arms) were calculated following Levan *et al.,* 1964, and Matthey, 1945, respectively. Karyograms were prepared from enlarged prints of photographs taken at 1000X.

For study of the reproductive anatomy, strobilae were usually stained in acetic carmine or acid hematoxylin, and cleared in terpineol. Other stains, including neutral red and pyronin, were used selectively. Before mounting on slides, all specimens were transferred to xylene. In most cases, with the aid of a dissecting microscope, the tegument and underlying layers of muscle-fibers were removed from areas of one strobilar surface. For preparation of thick sections (ca. 0.5 mm), series of segments were transferred from xylene to Permount (Fisher Scientific Company) on a slide, and cut by means of a razor blade under 30X magnification. Segments were also sectioned frontally, sagittally, and transversely at 10 to 18 μm after standard paraffin-embedding.

Most of the avian hosts collected were preserved as study skins or skeletons at the Burke Memorial Museum, University of Washington.

RESULTS

With respect to possible interactions between male and female strobilae, we recorded some observations on numbers and distribution of the cestodes in the intestine of the host. The mean length of the small intestine of the dowitchers (both species combined), from the pylorus to the openings of the caeca, was 409 mm. The location of individual cestodes was established by measuring the distance from the pylorus to the subserosal vesicle containing the scolex and anterior portion of the strobila, before the intestine was opened. In birds collected in April, some of the cestodes were quite small and either were entirely enclosed within the subserosal vesicle or extended their strobilae only a few millimeters into the intestinal lumen. All but one of 52 cestodes for which the data were obtained were attached within the first 230 mm of the small intestine; the one, a female, was situated 342 mm posterior to the pylorus. Of those attached within the first 230 mm, 23 (85 %) of 27 males were localized within the first 115 mm, and 17 (71 %) of 24 females were within the remaining expanse.

Sex-ratios of the cestodes in individual birds were uneven, as determined for 61 specimens from 35 hosts by classifying the cestodes according to predominant sexual characteristics, without regard for the presence of rudimentary organs of the opposite sex in some strobilae (see below). One male and one female occurred in 15 birds (42 %); single females in 11 (31 %); single males in three (8 %); and two males in two (5 %). The remaining combinations were two males and a female in two birds; a male and two females in one; and three males and a female in one. In pairs of one male and one female, the female was situated anterior to the male in two of 13 birds. The distance separating attached scolices of males and females in those birds ranged from 70 to 167 mm ($\bar{x}$ = 115 mm).

Helminths of various species usually occurred with *S. inermis* in dowitchers. Those identified were *Catatropis verrucosa* (Froelich, 1789); *Parorchis avitus* Linton, 1914; *Plagiorchis fastuosus* Szidat, 1924; *Aploparaxis occidentalis* Prudhoe and Manger, 1967; *A. rissae* Schiller, 1951;

A. retroversa Spasskii, 1961; *A. brachyphallos* (Krabbe, 1869); *Dichoanotaenia bacilligera* (Krabbe, 1869); *Paricterotaenia rotunda* (Clerc, 1913) [= *Polycercus rotundus* (Clerc, 1913)]; *Echinocotyle* sp.; and *Arythmorhynchus petrochenkoi* (Schmidt, 1969).

Observations on gender of strobilae

Five types of strobilae of *S. inermis* were distinguished on the basis of functional and morphological characteristics that throughout each respectively typical strobila remained constant.

Type 1. Females (27 specimens; 41.5 %). Female strobilae were longer and wider than those of males. Present in each segment and communicating to the exterior through a genital pore was a large cirrus sac-like structure which, in agreement with D. R. R. Burt (1939), we designate the vagina. That structure was somewhat smaller than the cirrus sac of males and lacked a seminal vesicle at its proximal end. From that end of the vagina, and continuous with its internal duct, a genital duct initially formed a few small coils, then widened briefly, and extended posteromediad as a transparent, very thin-walled canal of small diameter (10-20 μm). The presence of spermatozoa in the duct helped to trace its course, at first dorsal to the uterus, and then ventral, to its distal expansion to form a seminal receptacle (large and distended with spermatozoa in mature segments) lying on the midline immediately anterior to the vitelline gland. A large, somewhat bilobed ovary occupied the central field of the mature segment ventrally. The reniform vitelline gland, with long axis directed transversely, was situated immediately posterior to the ovary. The uterus, with two major, arc-shaped limbs in the posterior 2/3 of the segment, and with the convexity directed anteriad, usually formed a third branch that extended anteriad at the midline. When fully developed, the uterus had numerous lateral projections and filled most of the gravid segment. Details of the female organs are shown in *figure 1.* A mounted strobila of a female has been deposited in the Helminthological Collection of the United States National Museum, No. 81484.

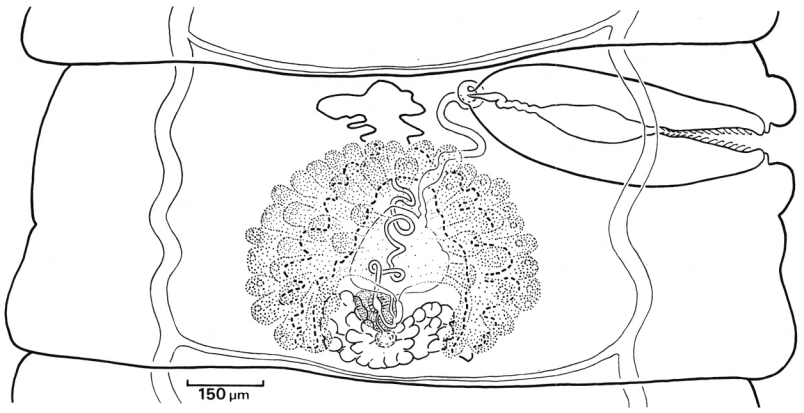
Posteriorly, a duct of very small diameter (approximately 5-8 μm) ran from the seminal receptacle to the common fertilization canal, entering therein a short distance from the point of entry of the oviduct. The vitelline duct, arising from two main branches within the vitelline gland, joined the common fertilization canal just posterior to the ootype, which was surrounded by a large Mehlis' gland. The uterine duct extended anteriad from the ootype, forming several convolutions in the area of Mehlis' gland before entering the uterus. Details of the female genital ducts are shown in *figure 2.*

Type 2. Males (12 specimens; 18 %). Male strobilae were shorter and narrower than those of females. Each segment contained a large cirrus sac, similar to the vagina in females, and a compact, prominent group of testes situated just posterior to the proximal end of the sac. Each testis gave rise to a short vas efferens that extended to a small chamber at the center of the group, from which chamber the vas deferens arose. That duct, equivalent to the genital duct in females, extended anterolaterad (often distended with spermatozoa), forming a few coils near the proximal end of the cirrus sac, which it entered. Within the cirrus sac, it enlarged to form an internal seminal vesicle (again often distended when filled with spermatozoa in mature proglottids). One additional male strobila was developed insufficiently to permit any determination about the possible presence of rudimentary female organs (see below).

Type 3. Incompletely gonochoristic females. In some typical, fully developed female strobilae (6 specimens; 9 %), small testes were present posterior to the proximal end of the vagina (in location like that in males; *i. e.,* in the area of the first small expansion of the genital duct). The underdeveloped testes occurred singly or in small groups (up to twelve per segment, each not more than 15-16 μm in greater diameter); in all cases, they lacked a discernible connection with the genital duct. Such female strobilae were identical in structure and function with those in which rudimentary testes were absent, *i. e.,* type 1 females.

Type 4. Incompletely gonochoristic males. A number of male strobilae (17 specimens; 26 %) had large, conspicuous male organs as well as rudimentary female organs that varied in the degree of their imperfect development. The female components consisted of small, nonfunctional elements (ovary, vitelline gland, Mehlis' gland, and uterus) situated at the midline near the posterior margin of the segment, and often the organ-sets were incomplete. Eggs were not observed in any uteri present in those strobilae.

Type 5. Hermaphrodites (2 specimens; 3 %). Two strobilae in our material were considered to be hermaphroditic. One, resembling a female in size, possessed in each segment normally developed male reproductive organs, as well as a complete set of female organs somewhat reduced in size. That the female organs were functional was indicated by the presence in the undersized uteri of terminal segments of numerous, ostensibly infective eggs. In number, the eggs were much fewer than in gravid uteri of gonochoristic females. Many contained completely developed embryos with embryonic hooks and other characteristic structures. In that specimen, the genital duct served as a vas deferens, extending from the testes to the cirrus sac, but it also extended posteriad from the group of testes, enlarging as in female strobilae to form a seminal receptacle (containing spermatozoa) and connecting with the common fertilization canal *(fig. 3).* The second hermaphroditic specimen was similar; the female organs were not so well developed, but small numbers of fertilized eggs were present in the uteri. Each of the hermaphrodites



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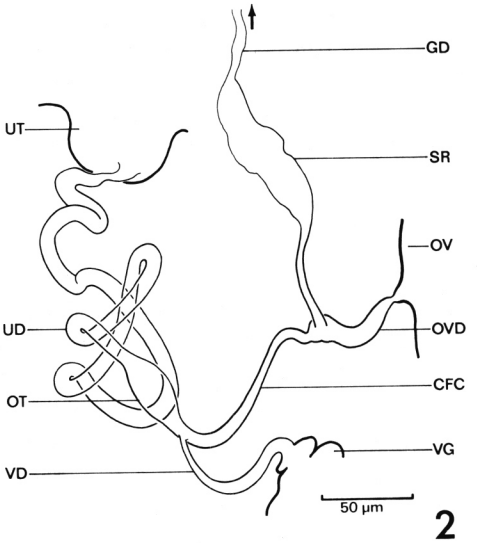

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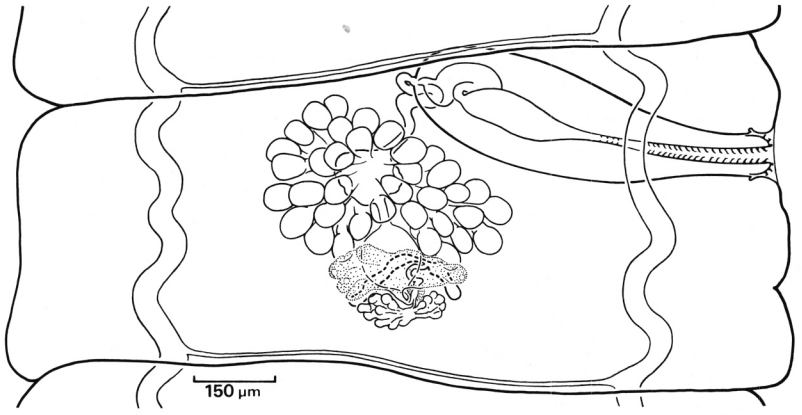

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FIG. 1 à 3. — *Shipleya inermis:* Anatomy of mature segment, gonochoristic female. *S. inermis:* Ducts of female genital system, from thick section. CFC, common fertilization canal; GD, genital duct; OT, ootype; OV, ovary; OVD, oviduct; SR, seminal receptacle; UD, uterine duct; UT, uterus; VD, vitelline duct; VG, vitelline gland. Mehlis' gland not shown. Arrow is directed porad. *S. inermis:* Anatomy of hermaphroditic segment, showing fully developed male system and underdeveloped female organs.

occurred in its host with a single type 1 female. A mounted strobila of a hermaphroditic specimen has been deposited in the Helminthological Collection of the United States National Museum, No. 81484.

OBSERVATIONS ON CHROMOSOMES

The chromosomal number in cells from embryos and reproductive tissues from gonochoristic males and females was eight, diploid ($n = 4$). The diploid set *(fig. 4)* consisted of four pairs of homologues: pair 1 -- two large submetacentric to subtelocentric chromosomes (the largest in the complement) (AR about 3.0 to 4.5); pair 2 -- two large subtelocentrics (AR about 4.5 to 6.5); pair 3 -- two medium-sized subtelocentrics (AR about 4.0 to 6.0); and pair 4 -- two small submetacentrics (AR about 1.5 to 2.0). The FN was recorded as 14. The germ-line karyotypes of males and females were similar in staining characteristics; each showed some degree of heteropycnosis in half of the diploid set, *i. e.,* in one of each pair were greater, but varying, amounts of heterochromatic DNA (interpreted) than in its homologue. In females, the chromosomes of pair 1 were slightly heteromorphic; one element was more nearly acrocentric, *i. e.,* with a higher AR value. Pair 1 also stained differentially (G-banded); the short arms of the more acrocentric chromosome were markedly heteropycnotic. In preparations made to demonstrate heterochromatin, all of the clearly intact mitotic nuclei found were condensed, and we could determine only that in such cells the large SM-ST chromosomes of pair 1 in females were unlike, in that the short arms of one (that more acrocentric) were prominently darker than those of the homologue, pointing to a quantitative difference in heterochromatin. In oocytic meiosis, all four bivalents showed chiasmata; the heterochromy became especially evident in the bivalent representing pair 1, one segment of which (involving the short arms) was achiasmatic *(fig. 5).* Very few intact mitotic cells were observed in males, due to their breakage during fixation and staining. The sequence of meiotic changes through metaphase I exhibited no unusual features, and included pairing and recombination in each bivalent (also in the short arms of pair 1) *(fig. 6).* After metaphase I, possibly just preceding metaphase II, a chromosomal fragmentation took place, through which the haploid elements were reduced in size and changed in composition; the dyads became small and euchromatic, and were observed in asso-

ciation with numerous, separate, pale-staining chromosomal fragments, which probably had no further function in gametogenesis *(fig. 6)*. We did not identify further developmental change until spermatozoa appeared. The largest element of the haploid set at metaphase II was approximately 1 μm in total length (average of ten); at metaphase I, its length averaged about 1.7 μm. The remaining chromosomes of the male set also were somewhat reduced in size. Compa-

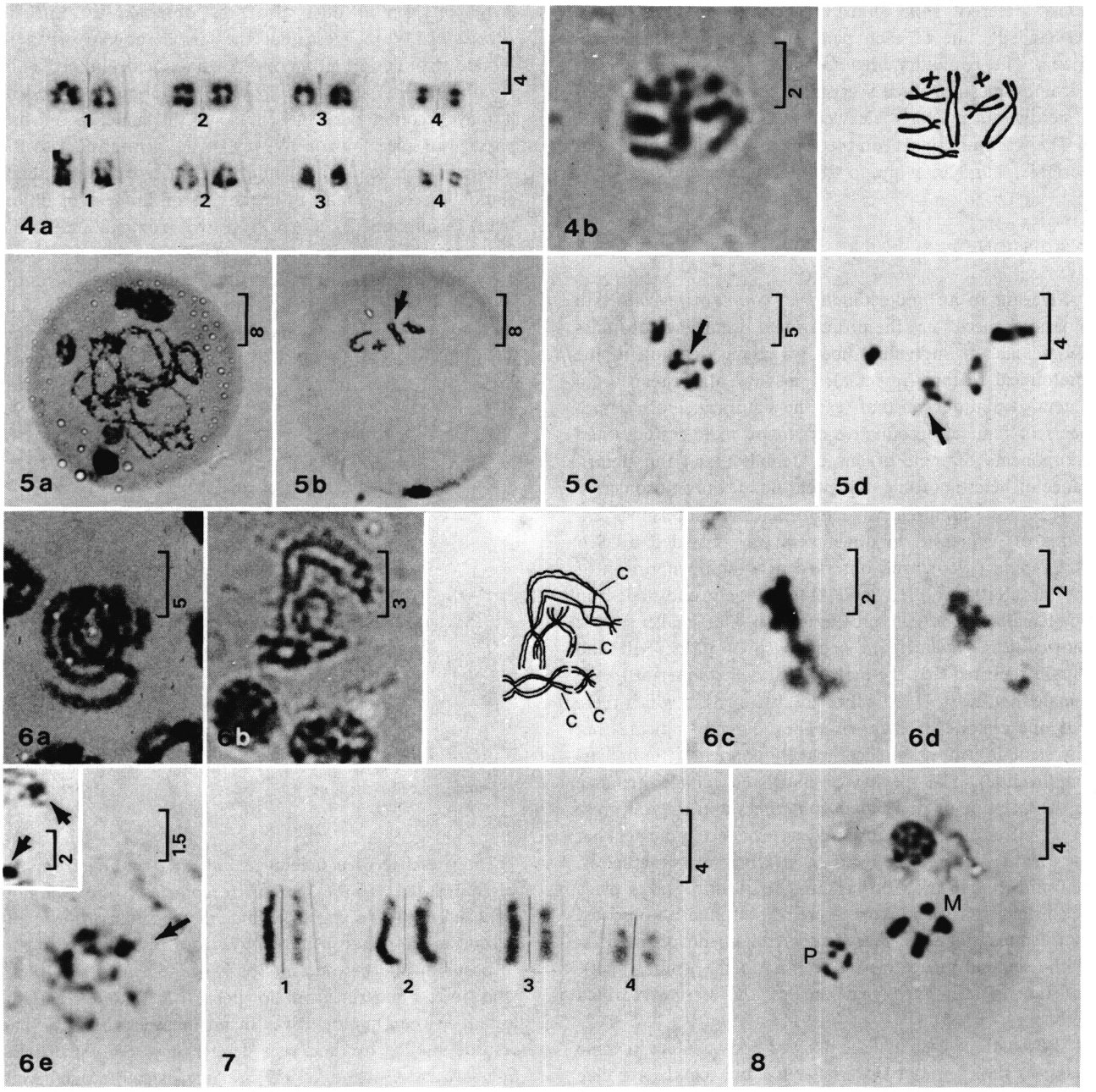

FIG. 4 à 8. — *Shipleya inermis:* Karyotypes and gametogenesis. Scale-values in micrometers.

*Fig. 4*a. Diploid chromosomal complements ($2n$ = 8) from two vitelline cells, gonochoristic female. Homologous pairs numbered 1-4. Giemsa-banding. *Fig. 4*b. Diploid complement, male (spermatogonium). Orcein. Diagram shows relationship of each element. *Fig. 5*a-d. Oocytes from gonochoristic females. G-banding. *a.* Zygotene stage. *b, c, d.* Diakinesis-prometaphase I. Achiasmatic portion indicated (arrows). *Fig. 6*a-e. Spermatocytic meiosis from gonochoristic males. *a.* Pachytene stage. G-banding. *b.* Diplotene stage. Orcein. Diagram shows conformation of homologues and location of chiasmata. C, centromere. *c, d.* Prometaphase I. G-banding. *e.* Fragmentation, germ-line nucleus. Four euchromatic chromosomes with numerous heterochromatic fragments in one cell, lower center (arrow). Inset shows the two largest euchromatic chromosomes (arrows) from another cell (part) following fragmentation. G-banding. *Fig. 7.* Diploid complement of male embryo from uterus of hermaphroditic strobila. G-banded. *Fig. 8.* Zygote from uterus of hermaphrodite, showing gonomeric association of chromosomes. M, maternal chromosomes; P, paternal chromosomes. G-banding.

ring males and females at metaphase I, average lengths, from ten cells of each, of the respective total haploid sets were 4.8 μm (males) and 7.8 μm (females).

The diploid set in embryos from the two hermaphrodites also numbered eight *(fig. 7)*. The respective homologues differed somewhat in staining characteristics (G-banded), one of each pair tending to be heterochromatic. The haploid number was four. Male and female chromosomes remained segregated following syngamy for a period during early cleavage *(fig. 8)*.

The chromosomal complement of somatic cells was variable, numbering up to 28 in intact groups.

DISCUSSION

Relevant to an understanding of *Shipleya inermis* as a dioecious species are the proportional occurrence of males and females in individual hosts; a reconsideration of the anatomical features in females, in view of existence of a patent genital duct in that sex; the significance of components of female reproductive organs in male cestodes and components of male organs in females; and the significance of heteromorphic, heterochromatic chromosomes.

The most comprehensive information concerning sex-ratios of *S. inermis* in dowitchers was provided by Self and Pipkin (1966), who observed in a high proportion of the birds (104 of 131, or 79 %) that only one male and one female cestode were present in each. Our smaller sample showed less regularity in occurrence of pairs. Self and Pipkin reported that the male was attached anterior to the female in at least 99 (95 %) of the 104 birds in which there was one cestode of each sex (They did not indicate that any strobila was either incompletely gonochoristic or hermaphroditic). The spacing of male and female strobilae in the intestine of dowitchers sometimes appears too great to permit cross-fertilization, and no evidence indicates that the cestodes change the locus of attachment. Nonetheless, Self and Pipkin implied that cross-fertilization takes place when single males and females are present, and we observed that female strobilae consistently contained spermatozoa in the seminal receptacle (as well as in the genital duct), and that in fully developed females, the uteri were filled with eggs.

The length of each cestode allowed body-contact in some cases; as shown in our material, male and female strobilae could have overlapped in the area between loci of attachment. Self-fertilization in females is not a possibility. In the total number of female *S. inermis* (33), rudimentary testes were present in only six, but all females were gravid. As noted, the testes seen in females were very small and nonfunctional, and lacked efferent ducts. Anatomically, the cestodes of both sexes evidently possess the requisite structures to enable cross-fertilization.

In reproductive anatomy, *S. inermis* closely resembles *Infula burhini* Burt, 1939, in which the terminal portion of the female genital tract is similar to the cirrus sac of the male, with a large, spinose, cirrus-like organ, extrusible but equally capable of intrusion. Burt (1939) concluded that the organ, when intruded, functioned as a vagina. A patent genital duct also was observed by Burt in *I. burhini.* We suggest that the cirrus sac-like organ in female strobilae of *S. inermis* is functionally like that of *I. burhini.* As in *I. burhini,* when the organ is intruded, it may accommodate the cirrus of the male, permitting passage of spermatozoa by way of the genital duct to the seminal receptacle. The genital duct in several individuals could be seen (usually by means of oil-immersion objectives) throughout its course from the proximal end of the vaginal organ to the seminal receptacle. The duct was extremely thin-walled and slender *(figs 2 and 9),* and was most difficult to trace across the central area between the ovarian lobes and uterus, which are dense and opaque even when delicately stained.

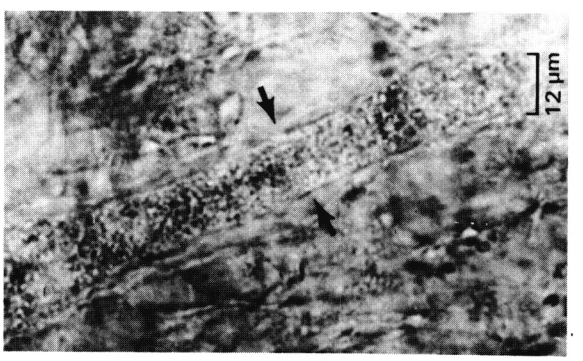

FIG. 9. — *S. inermis:* Common genital duct (arrows), containing spermatozoa, extending across uterus and ovary, gonochoristic female. Acetic carmine.

The presence of a patent genital duct leading from the vaginal organ *via* the seminal receptacle to the common fertilization canal seems to exclude the possibility of « hypodermic insemination » in *S. inermis,* a process considered to occur in that cestode by Spasskii and Gubanov (1959), who studied material from dowitchers, *L. scolopaceus,* collected on the Omolon River in northeastern Siberia. The cestode studied by them was described as *Shipleya dioica* Spasskii and Gubanov, 1959, a name placed in synonymy with *S. inermis* by Ryzhikov and Tolkacheva (1981). Spasskii and Gubanov concluded that a vagina was lacking in their specimens. Coil (1970) also suggested that hypodermic insemination might occur in *S. inermis,* but found no direct evidence for it. We frequently observed in female strobilae that the spinose lining of the distal part of the genital tract (*i. e.,* the vaginal lumen -- as also described by D. R. R. Burt for *I. burhini*) had been detached, and protruded from the genital pore as strands of thin tissue

with the spines still intact and with strong basal attachment, which suggested that the membrane had been extracted when the spinose cirrus of the male cestode was withdrawn following copulation. If insemination through the strong, thick tegumental and muscular tissues occurred in *S. inermis,* some evidence of it should remain, such as perforations, perhaps equal to the diameter of the large cirrus of the male, in the tegument of females, possibly with embedded spines detached in the process (cf. Schmidt, 1969), but such marks have not been reported, nor has the means been explained by which spermatozoa in that case could reach the seminal receptacle.

Various explanations have been advanced concerning the occurrence of reproductive organs of the opposite sex in the respective female and male individuals of *S. inermis.* Baer (1940) (whose anatomical illustration shows all relationships save that of the genital duct, which he did not observe, and as a consequence he considered the cirrus sac-like organ in females to be other than a vagina) ranked *S. inermis* as protandrous, stating (p. 182) that « The testes have completely disappeared in the specimens in which the female genitalia have formed », which suggests that his material consisted of female strobilae wherein rudimentary testes were present but not discernible in post-mature segments. All such organs in the present study were very small and not easily seen in whole-mounts. Whether Baer studied male strobilae is uncertain, since he remarked that in *S. inermis* the testes were best studied in sections. In our male specimens, to the contrary, the testes were large and prominent organs (range 34-85 μm in greater diameter; $\bar{x}$ = 50 μm) forming a large aggregation, and unobscured by overlying tissues. Spasskii and Gubanov (1959) also in female strobilae observed rudiments of male reproductive organs, in all of which the vasa efferentia were incompletely formed. As well, they found components of female reproductive organs in male strobilae. Schell (1959) considered *S. inermis* to be protandrous and dichogamous; he observed that four of 10 strobilae studied had fully developed testes as well as some development of the female genital organs, and concluded that a rapid transition from male to female reproductive organs took place. Voge and Rausch (1956) determined that *S. inermis* is dioecious; they did not observe any evidence of incomplete gonochorism nor the genital duct in females. In 23 incompletely gonochoristic cestodes, we discerned no major changes in the underdeveloped organs of the opposite sex in either male or female strobilae; in both, rudimentary organs were constant in that condition, showing a degree of progressive change in the strobila compatible with their limited potential. In the hermaphroditic strobilae, the male reproductive organs were consistently prominent and became fully developed, indicating that maleness was predominant, whereas the female organs were smaller than normal, but functional, as demonstrated by the presence in the uteri of eggs containing embryos. By grouping all male and predominantly male strobilae, the sex-ratio in our material was approximately 1 : 1.

We observed that *S. inermis* was either wholly male or wholly female, without rudiments of organs of the opposite sex in either case; or that strobilae of predominantly one sex showed some degree of development of sexual organs of the opposite sex; or that strobilae (two in our material) were hermaphroditic, *i. e.,* five phenotypes were expressed in the cestode. It is assumed that the ancestor of *S. inermis* was monoecious, and that it was a hermaphrodite (sensu Bacci, 1965), maintained in that status through a genetic equilibrium in which genes influencing sexual characters were inherited, in every individual, in the same quantity and quality relative to the genome. Evidently, chromosomal mutation has altered the genetic balance, giving rise to a dioecious state in *S. inermis.* Heteromorphism and differential heterochromatin-constitution in the homologues of pair 1 in females may indicate that a chromosomal rearrangement (at present undetermined as to type) has taken place at some distant time. For *S. inermis,* that differential may represent the « evolutionarily youngest genome fraction » (Manfredi Romanini, 1973).

In male strobilae (type 2), nuclei of spermatocytes underwent fragmentation, a process similar to that described by Child (1907) in *Monezia expansa* (Rudolphi, 1810) (Cestoda). With reference to Child's work, Rosario (1964) studied other cestodes (*Hymenolepis* spp.) by electron microscopy but was unable to determine whether or not fragmentation occurred in them. Apparently, no other investigations have been concerned with the question (cf. Benazzi and Benazzi Lentati, 1976, for review), and Child's observations have remained unconfirmed. As Rosario noted, the syncytial nature of testicular tissue in the Cestoda makes a precise definition of the nuclear units difficult. In *S. inermis,* fragmentation evidently took place during the interval anaphase I, metaphase II, and it involved the reduction of germ-line chromosomes in size and DNA composition as the result of dissociation of pale-staining, heterochromatic elements, which possibly were eliminated as a cytoplasmic by-product (also suggested by Child), while the euchromatic portions were conserved. To what extent each chromosome was reduced could not be determined. The few measurements made were of the large submetacentric dyad, and they indicated that approximately 40 % of its DNA substance (judged to be heterochromatin) had been removed from the germ-line. The end-result of spermatogenesis was a single type of gamete, largely euchromatic. The genetic system operating in *S. inermis* appears to be similar in some respects to those of the coccids (Coccoidea) (cf. Brown and Nur, 1964, for review).

Nuclear fragmentation was not observed in female *S. inermis.* The female germ-line karyotype included two heteromorphic homologues (pair 1), in one of which the

short arms were highly heterochromatinized. Chiasma-formation did not occur between the short arms in bivalents. We judged that the disparate arms were non-pairing, achiasmatic due to non-homology or to recombinational restriction associated with the presence of heterochromatin. The effects of heterochromatic blocks on chiasma-formation have been described (Southern, 1970; John and King, 1985; Nagl, 1985; Charlesworth *et al.,* 1986). That a non-pairing portion remained discrete suggests that females of *S. inermis* produce gametes of two types, one euchromatic and one bearing a substantial amount of heterochromatin that may act as sexual factor. The sexually differing occurrence of heterochromatin has not been investigated in other dioecious cestodes, but it has been reported in schistosomes (Digenea) (Grossman *et al.,* 1980; Short *et al.,* 1989).

Syngamy in *S. inermis* took place in the common fertilization canal and beginning uterine duct. As other investigators have noted in cestodes (cf. Rybicka, 1966, for review), maturation of oocytes in *S. inermis* progressed from early meiotic prophase I into reductional division after entry of spermatozoa. For a time, the maternal and paternal chromosomes remained segregated; such an orientation following fertilization has been observed in other invertebrates, including cestodes (Häcker, 1895; Douglas, 1963; Hossain and Jones, 1963; Vijayaraghavan and Subramanyam, 1980). In *S. inermis,* heterochromatinization must in some way re-commence in the male-line chromosomes during the gonomeric interval following syngamy. Heterochromatin-synthesis is known to take place at certain mitotic stages in various eukaryotes (White, 1973). We were not able to determine the possible occurrence of chromosomal diminution in mitotic cells in embryos, as known in *Parascaris* spp. and other invertebrates in establishment of germ-cell and somatic-cell lines (cf. Pimpinelli and Goday, 1989, for review), nor to define centromeric structure.

S. inermis as a species is not completely gonochoristic, as shown in our material by individuals that were sexually heterozygous to varying degrees. Some possessed normally developed male reproductive organs but female organs in them were very small and underdeveloped or the sets were incomplete (*e. g.,* a small uterus would be present but an ovary absent; some individuals lacked a vitelline gland, etc.). When the small-sized female organs were complete, as in the two hermaphroditic strobilae, oncospheres were produced. Evidently, hermaphrodites are able to self-fertilize, but are unable to receive spermatozoa from another strobila. On the other hand, they are able, anatomically, to cross-fertilize as males. Incompletely gonochoristic females had normally developed female organs and very small, rudimentary testes situated around the genital duct near the proximal end of the vagina. Vasa efferentia were lacking; fertilization of such females could occur only as in gonochoristic females, *i. e.,* through insemination by a male strobila.

We can only speculate about the derivation of strobilae of types 3, 4 and 5. Assuming that chromosomal rearrangement has occurred, along with synthesis and amplification of heterochromatin (Lohe and Roberts, 1988), one hypothesis would be that genetic control functions to produce such phenotypes. The activity of genes may be influenced when they are situated adjacent to heterochromatin, as happens in position-effect variegation (Moritz *et al.,* 1976; Reuter *et al.,* 1985). On the basis that the ancestral cestode was hermaphroditic, possessing only autosomal chromosomes, that concept might be tenable. Possibly, also, chiasmata might form in the short arms of pair 1 in females if distal euchromatin is ever present there bilaterally.

The evolutionary events leading to the acquisition of the dioecious state by *S. inermis* involved some fundamental modifications of the distal parts of the genital ducts. The cirrus sac, now functioning also as a vagina, was retained, whereas a separate vagina disappeared. The single duct that extends mediad from the cirrus sac/vagina in *S. inermis* seems clearly to represent a fusion of the vas deferens with the proximal portion of the former vaginal duct. The common duct so produced conveys spermatozoa from the testes to the cirrus sac in male strobilae; in females, following mating with a male strobila, it transports spermatozoa to the seminal receptacle. The dual function of the common duct was best exemplified in hermaphrodites, which like females lack a separate vaginal duct; the male system alone opens at the genital atrium. In hermaphrodites, the common duct that terminates in gonochoristic males at the small cistern at the center of the testicular group continues posteriad, as in females, and delivers spermatozoa to the seminal receptacle. The predominantly male hermaphroditic strobila is capable of cross-fertilization with a female strobila, but is limited to self-fertilization of its ova.

The selection pressures that led to the dominance of dioeciousness in *S. inermis* are not understood. The events through which one male and one female tend to occur in a single host also require explanation.

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