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Architectural and Molecular Dynamics of Spermatogenesis in *Sarcophaga bravicornis* Ho, 1934

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Abstract:

Spermatogenesis in the flesh fly (*Sarcophaga bravicornis* Ho, 1934) is a complex biological process resulting in the formation of mature spermatozoa within the male reproductive system. This study examined the stages of spermatogenesis, including spermatocytogenesis, meiosis, and spermiogenesis, in *Sarcophaga braviconica* Ho, 1934. It was observed that germ cells undergo successive mitotic and meiotic divisions, surrounded by somatic cyst cells in the testes. The development culminates in the transformation of round spermatids into elongated, motile spermatozoa with a distinct flagellum and typical insect axoneme structure. This process was studied using microscopic techniques, revealing details of cell differentiation and organization in the testes. The findings contribute to understanding reproductive biology in flesh flies, which has practical relevance for forensic entomology and pest management.

Keywords- *Sarcophaga bravicornis*, spermatogenesis, testicular histology, spermiogenesis, germ cell differentiation, forensic entomology

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I. INTRODUCTION

The family Sarcophagidae, commonly known as flesh flies, is a cosmopolitan group of insects with significant roles in both natural ecosystems and human impacted environments. These flies are often medically and economically important, with their larvae playing a crucial role as necrophagous insects in forensic entomology for determining post-mortem intervals. They can also act as vectors for enteropathogens and cause myiasis in livestock and humans.

The reproductive strategy of Sarcophagidae is characterized by ovoviviparity, a trait known as larviposition, where females retain fertilized eggs internally and give birth to live, first-instar larvae directly onto a suitable host or substrate. This contrasts with the oviposition (egg-laying) seen in many other fly families and represents a specialized reproductive adaptation. A primary challenge in the study of this genus, and a central issue for the present investigation, is the difficulty of species identification. Sarcophaga species exhibit a remarkably uniform external appearance, characterized by a greyish body with three dark longitudinal stripes on the thorax and a checkerboard-like abdomen. This visual similarity means that reliable identification is often impossible without the microscopic examination of male genitalia or the use use of modern molecular techniques.

To understand the structure of the testes, it is essential to first place them within their broader anatomical context. The internal reproductive system of a typical male flesh fly, including those of the genus Sarcophaga, conforms to the general bauplan of higher Diptera. The system is designed for the production, storage, maturation, and delivery of sperm. It consists of a bilaterally symmetrical arrangement of organs located within the abdominal cavity, typically ventral to the alimentary canal. The primary components are a pair of testes, the male gonads responsible for spermatogenesis; a pair of ducts, the vasa deferentia, which transport sperm from the testes; a pair of accessory glands that produce seminal fluid; and a median ejaculatory duct and associated ejaculatory sac, which convey the final semen product to the external genitalia (aedeagus) for transfer to the female during copulation.

The testes of *Sarcophaga bravicornis* Ho, 1934 are paired, oval organs located in the posterior abdominal cavity, generally around segments 4–6, where they lie dorsolaterally in the haemocoel above the hindgut and anterior to the accessory glands. These are the primary male gonads of the species and remain free within the haemocoel, connected to the reproductive tract through the vasa deferentia. Externally, each testis is ovoid to slightly elongated and enclosed in a thin peritoneal sheath composed of connective tissue, which provides protection and maintains internal pressure. In newly emerged adults, the testes appear pale whitish or translucent, but gradually turn yellowish or light brown with age owing to pigment accumulation. The average size in adults ranges from 1.0–1.2 mm in length and 0.3–0.5 mm in width, though variation occurs depending on age, nutritional status, and reproductive activity. Internally, the testes are composed of numerous spherical or oval spermatocysts, each bounded by a

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cyst wall of somatic cells and enclosing a clone of germ cells derived from a single spermatogonium. Spermatogenesis follows a zonal arrangement, progressing from spermatogonia in the apical region, to primary and secondary spermatocytes in the middle, and finally spermatids and mature spermatozoa towards the basal region near the vas deferens. Spermatids undergo spermiogenesis, which includes elongation, nuclear condensation, and flagellum formation, ultimately producing long, filamentous, and highly motile sperm. From each testis arises a vas deferens, a narrow tubular duct that transports mature sperm, with both ducts converging into the ejaculatory duct leading to the aedeagus. Functionally, the testes ensure continuous sperm production throughout adult life and contribute partly to sperm storage, although larger reserves are maintained in the accessory glands and seminal vesicles. Morphologically, the testes of S. bravicornis closely resemble those of other congeners but show slight variations in size and cyst arrangement, features that may hold taxonomic and forensic significance given the role of flesh flies in forensic entomology (9)

Taxonomic Classification

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Diptera
- Family: Sarcophagidae
- Subfamily: Sarcophaginae
- Genus: *Sarcophaga*
- Species: *bravicornis* Ho, 1934

II. MATERIAL AND METHOD

The primary subject of this study is the flesh fly, *Sarcophaga bravicornis* Ho, 1934 using both larvae and adults. Raw meat is used to attract and breed the flies, ensuring a continuous supply of specimens. For rearing, a specialized insect rearing cage is used, with Petri dishes and sterile sawdust providing a clean and controlled environment. Forceps and brushes are essential for carefully handling the flies and larvae without causing them damage. For dissection, a stereomicroscope is used to provide a low-magnification, three-dimensional view, which is ideal for the delicate task of isolating specific organs. Standard dissection tools such as scissors, needles, and forceps are required. Once the tissue (e.g., testes) is dissected, it is immediately placed in a 10% formalin solution. This step, known as fixation, is crucial as it preserves the tissue's cellular structure and prevents degradation.

Histological processing and staining form the core of the procedure, preparing the tissue for slicing and microscopic observation. The fixed tissue is dehydrated by passing it through a series of increasing concentrations of ethanol solutions (30% to 100%), which removes all water. The tissue is then placed in xylene, a clearing agent that eliminates ethanol and makes the tissue transparent. The cleared tissue is infiltrated with molten paraffin wax, and as the wax cools and hardens, it forms a rigid block that supports the tissue for sectioning. Using a rotary microtome, the wax-embedded tissue is sectioned into thin slices of 5–10 micrometers thickness. These sections are carefully mounted on clean, grease free glass slides with the help of egg albumin as an adhesive. The wax is then dissolved using xylene, and the tissue is rehydrated with distilled water. Staining is carried out with Hematoxylin, which colors the cell nuclei blue-purple, and Eosin, which stains the cytoplasm and other structures pink. After a final dehydration step, the slides are mounted with a coverslip using DPX mounting medium for long-term preservation.

Finally, the prepared histological slides are examined under a compound light microscope to study the detailed cellular and tissue structures of *Sarcophaga bravicornis* Ho, 1934. This microscopic analysis allows the identification of various stages of spermatogenesis and the observation of characteristic features of the testicular tissue.

A. *Sample Collection and Rearing*

The process begins by using a simple but highly effective method to attract gravid female flies. A piece of raw meat serving as both a breeding substrate and a food source for the developing larvae was strategically placed in an open, outdoor environment. Flesh flies are known for their ability to quickly locate decaying matter, and female flies of the genus Sarcophaga are larviparous, meaning they deposit live larvae rather than eggs directly onto the meat. This rapid larval deposition provides an immediate start to the rearing process. The larvae quickly began to feed on the meat, undergoing several molts, and reaching their full larval stage within a few days. Once the larvae were fully developed (typically after 2 3 days), they left the meat to find a suitable location for pupation. This is a crucial transitional phase where the larval tissues are reorganized into adult structures. To prevent the pupae from dispersing and to facilitate their collection, they were carefully gathered from the meat and surrounding area. They were then transferred into a clean petri dish that had been pre-lined with sterile sawdust. The sawdust acts as a clean, dry medium that mimics the soil environment where pupation naturally occurs. This setup ensures that the pupae are contained and their environment is free from contamination, which could affect their development. The petri dish containing the pupae was then placed inside a secure insect rearing cage. This cage provides a controlled and protected environment for the final stage of metamorphosis. The pupal stage typically lasts for 3 to 4 days, after which the adult flies (imagoes) emerge. For this specific experiment, the timing of adult emergence was critical. The flies needed to be collected at precise intervals (48h and 72h post-emergence) to study the progressive development and maturation of their testes. This required constant vigilance and a methodical approach to ensure that the time points were accurately recorded for subsequent dissection and analysis.

B. *Dissection and Tissue Fixation*

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Before any microscopic analysis can take place, the specific organ of interest, in this case the testes, must be carefully isolated from the rest of the insect's body. A stereomicroscope is an essential tool for this because it provides a magnified, three-dimensional view, allowing for the precise and delicate dissection of such a small specimen. After removing the testes, they were immediately placed on a slide in a few drops of saline solution (0.75% NaCl). Immediately after dissection, the tissue was immersed in Carnoy's fixative (3 parts alcohol and 1 part acetic acid). This step, called fixation, is the most critical part of the entire process. Formalin works by cross-linking the proteins within the cells, which effectively "freezes" the tissue in its living state. Without this step, the cells would begin to break down (a process called autolysis), and the tissue's structure would be lost, making it impossible to study.

C. *Histological Preparation (Microtomy)*

This is a multi-step process designed to prepare the fixed tissue for sectioning and observation. The goal is to replace the water in the tissue with a solid medium (paraffin wax) without damaging the tissue's structure. Water is the most abundant component of biological tissues, but it is not miscible with paraffin wax. Therefore, the tissue must be progressively dehydrated by immersing it in a series of increasing concentrations of ethanol solutions (from 30% to 100%). Each series will be maintained for 30 minutes to ensure proper dehydration. This gradual increase prevents sudden osmotic shock that could shrink or damage the cells. After dehydration, the tissue is placed in xylene. Xylene serves as a clearing agent because it removes the ethanol and penetrates the tissue, making it transparent. Most importantly, xylene is miscible with both alcohol (which was just removed) and paraffin wax (which is about to be introduced), acting as a crucial intermediate step. The cleared tissue is then submerged in hot, molten paraffin wax. The wax slowly infiltrates all the spaces within the tissue, creating a homogenous block. When the wax cools and solidifies, it provides a rigid support structure, allowing the tissue to be cut into very thin, uniform slices without falling apart. The rotary microtome is a specialized instrument used to cut the wax block into these extremely thin sections (5–7 micrometers thick). This is necessary because light needs to pass through the tissue for it to be viewed under a microscope.

D. *Slide Preparation and Staining*

Once the sections are cut, they are prepared for staining to make the different parts of the cell visible. The thin sections are floated on a warm water bath to remove any wrinkles and are then picked up with a glass slide coated with egg albumin, a substance that helps the tissue adhere firmly to the slide. The slide is then run back through xylene to dissolve and remove the wax. Since the wax has been removed, the tissue needs to be rehydrated with a descending series of ethanol solutions before it can be stained with water-based dyes. The most common staining method in histology is Hematoxylin and Eosin (HandE). Hematoxylin is a basic dye that stains acidic components, like the cell nuclei, a blue-purple color. Eosin is an acidic dye that stains basic components, like the cytoplasm and proteins, a pink color. The different colors provide a clear contrast, making it easy to distinguish between various cell types and structures. After staining, the slides are dehydrated again and cleared with xylene. A coverslip is then applied using DPX mounting medium, which permanently seals the tissue section, protecting it from damage and allowing for long-term storage and observation.

E. *Microscopy*

The final step is to observe the prepared slides using a compound light microscope. The slides are now ready for detailed analysis of the cellular morphology and the various stages of spermatogenesis within the flesh fly testes.

Fig.1 Insect Rearing Cage

Fig. 2 *Sarcophaga bravicornis* Ho,1934 larva

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Fig. 3 *Sarcophaga bravicornis* Ho,1934 Pupal

Fig. 4 Adult *Sarcophaga bravicornis* Ho,1934

III. RESULTS AND DISCUSSION

The histological examination of testes at 48 hours post-emergence revealed pronounced spermatogenic activity, with well-defined zones of developing germ cells distributed within the seminiferous tubules. The tubules appeared elongated, compact, and structurally organized, reflecting an active state of gametogenesis. Along the periphery of the tubules, clusters of spermatogonia were observed, identifiable by their relatively small size and darkly stained nuclei, indicating their mitotically active nature. Progressing toward the lumen, larger primary spermatocytes in the prophase stage of meiosis were clearly distinguishable, exhibiting characteristic chromatin condensation patterns. In addition, a number of secondary spermatocytes and early spermatids were evident, providing strong evidence that spermatogenesis had already commenced within 48 hours of adult emergence. However, the presence of fully differentiated spermatozoa was comparatively limited, implying that the spermatogenic process at this stage was still in its early to mid-phase, and the testes were not yet at peak functional maturity.

In contrast, the testes examined at 72 hours post-emergence demonstrated a more advanced and differentiated stage of spermatogenesis. The seminiferous tubules were markedly more developed, containing a higher proportion of elongated spermatids and well-formed spermatozoa. These spermatozoa were observed in organized bundles, a typical feature of ongoing spermiogenesis, indicating the transition from late spermatid development to functional gametes. The chromatin within late spermatids showed intense condensation, a hallmark of nuclear maturation, while the cytoplasmic architecture revealed the presence of mitochondrial derivatives resembling the nebenkern, which play a critical role in energy supply for motility. The overall density and abundance of mature spermatozoa in the 72h samples was significantly greater compared to the 48h testes, demonstrating that the bulk of spermatogenic differentiation and maturation occurs during this time window.

Taken together, this comparative histological analysis highlights that spermatogenesis in *Sarcophaga bravicornis* Ho, 1934 is a time-dependent and progressive process. At 48 hours, the testes predominantly contain early germ cell stages, including spermatogonia, primary spermatocytes, and a limited number of early spermatids, representing the initiation of the spermatogenic cycle. By 72 hours, however, the germ cells have advanced considerably, with late spermatids and mature spermatozoa being the predominant forms, signifying the near completion of spermatogenesis and the availability of functionally competent sperm. This transition underscores the rapid progression of germ cell development post-emergence and reflects the reproductive strategy of the species, enabling early onset of reproductive capacity in the adult stage.

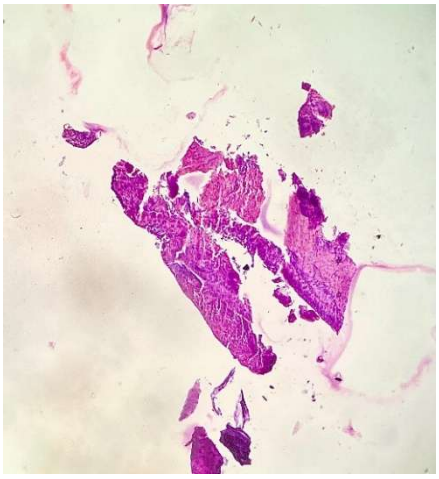

Fig. 5 Histological section of 48-h old testis of *Sarcophaga bravicornis* Ho,1934 (flash fly) showing spermatogenesis (10×)

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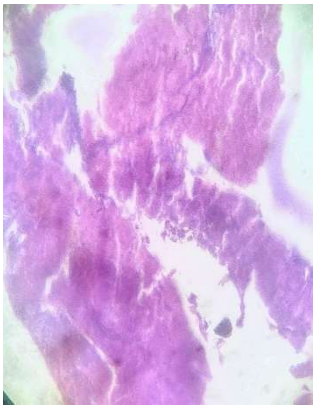

Fig. 6 Histological section of 48-h old testis of *Sarcophaga bravicornis* Ho,1934 (flash fly) showing spermatogenesis (40×)

Fig. 7 Histological section of 72-h old testis of *Sarcophaga bravicornis* Ho,1934 (flash fly) showing spermatogenesis (10×)

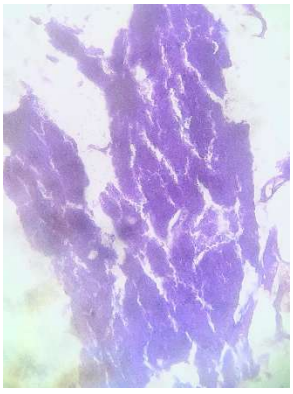

Fig. 8 Histological section of 72-h old testis of *Sarcophaga bravicornis* Ho,1934 (flash fly) showing spermatogenesis (40×)

The synthesis of research on the reproductive biology of Sarcophaga reveals a deeply interconnected system where cellular processes, genomic architecture, organismal morphology, and ecological strategy are woven together by powerful evolutionary forces. This discussion aims to integrate the key findings from the preceding sections, highlighting the causal links between different levels of biological organization and underscoring the value of this genus as a model for fundamental evolutionary questions.

A central theme emerging from this review is the profound impact of sexual selection on sarcophagid evolution. The intense male–male competition observed in species like S. ruficornis, where nearly 40% of mating attempts are disrupted by rivals, creates a high-stakes reproductive environment.(2) This pre-copulatory conflict is a strong predictor of equally intense post copulatory sperm competition. The evolutionary consequence of this relentless selective pressure is vividly illustrated in the morphology of the male genitalia. The intricate, species-specific architecture of the phallic complex, with its elaborate hooks and lobes, is not merely a taxonomic curiosity but a functional toolkit shaped by sexual selection to maximize fertilization success, likely through mechanisms of rival sperm removal or enhanced stimulation of the female. Thus, the behavioural ecology provides the selective context that directly explains the extreme morphological diversification seen in the reproductive anatomy.

At a deeper level, the process of sperm production itself appears to be under strong stabilizing selection, which in turn has constrained the evolution of the entire genome. The discovery that Sarcophagidae, along with their sister family Calliphoridae, have almost universally retained the ancestral Dipteran X chromosome (Muller element F) for millions of years stands in stark contrast to the rapid sex chromosome turnover seen in the related Muscidae. This remarkable genomic conservatism is best explained by functional constraint.(11)(12)

This review also highlights how a foundational understanding of physiology can illuminate applied problems. The hormonal regulation of spermatogenesis, particularly the critical role of ecdysone in promoting the mitotic proliferation of early-stage germ cells, provides a clear mechanistic hypothesis for the observed effects of certain insecticides. The histopathological finding that insecticides cause pycnosis and degeneration specifically in the apical zones of the testis— the location of spermatogonia and spermatocytes—strongly suggests that these chemicals act as endocrine disruptors, interfering with the ecdysone signaling required for these specific developmental stages. This transforms a descriptive toxicological observation into a testable model of molecular disruption.

Finally, the ecological success of the Sarcophagidae is inextricably linked to their primary reproductive strategy, larviparity. By depositing live, first-instar larvae directly onto ephemeral resources, females give their offspring a critical head start, enhancing their competitive ability against other decomposers. This biological adaptation, honed by natural selection in a competitive ecological niche, has a direct and significant application in forensic science. The elimination of the variable and temperature-dependent egg incubation period

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makes the age of sarcophagid larvae a more direct and reliable indicator of the minimum post mortem interval compared to their oviparous counterparts. Thus, the evolutionary strategy that ensures reproductive success in nature also provides a more precise tool for medico-legal investigation.

IV. CONCLUSION

The integrative study of the reproductive biology of Sarcophaga highlights the intricate connections between histological processes, genomic stability, morphological adaptations, and ecological strategies. The cystic model of spermatogenesis, with its precise zonal organization from germarium to spermatozoa, demonstrates how cellular differentiation is finely regulated to ensure reproductive efficiency.(2)(3) The application of effective staining techniques, such as hematoxylin and eosin, provides critical clarity in tracking these developmental stages and offers a standardized protocol for entomological research.

From an evolutionary perspective, the remarkable conservatism of sex chromosome organization in Sarcophagidae reflects powerful functional constraints on genomic architecture, distinguishing this group from related Dipteran families that exhibit frequent sex chromosome turnover.(12) Such genomic stability underscores how molecular mechanisms shape evolutionary trajectories over millions of years.

At the ecological level, larviparity represents a unique adaptation that confers competitive advantages in ephemeral habitats. By bypassing the vulnerable egg stage, Sarcophaga species ensure larval survival while simultaneously providing forensic scientists with a reliable marker for estimating the post-mortem interval.(1) Furthermore, the sensitivity of early germ cells to insecticides, mediated through potential disruption of ecdysone signaling, links basic physiology with applied challenges in pest management and toxicology.(4) Taken together, these findings demonstrate that the reproductive biology of Sarcophaga is not only a subject of taxonomic and histological interest but also a valuable model for addressing broader questions in evolution, toxicology, and forensic entomology. By integrating cellular biology, evolutionary genomics, and ecological adaptation, Sarcophaga provides a comprehensive framework for understanding how selective pressures operate across multiple levels of biological organization.

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