JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE
Volume 41, Number 1, January, 1966

THE MALE UROGENITAL SYSTEM OF GYRINOPHILUS DANIELSI DUNNI

PRISCILLA STRICKLAND [1]

Southwestern College, Memphis, Tennessee

The urogenital system is probably the most extensively studied system of the salamanders. A recent article by Baker and Taylor (1964) on several species of *Ambystoma* has renewed interest in this area. They point out the features of this system which may be of importance when considering phylogenetic relationships within the order. These include the degree of emancipation of the Wolffian duct from urine transport, the specialization of the epididymis, the presence of a longitudinal canal, the degree of development of the rudimentary Mullerian duct, and a decrease in the number of urinary tubules and vasa efferentia with development.

Although the literature does not lack sources (see Baker and Taylor, 1964), there has been no description of this system in the plethodontid salamanders, the family which includes the largest number of species. Therefore, it is the purpose of this paper to describe a plethodonid, *Gyrinophilus danielsi dunni* (Mittleman and Jopson), and to compare these findings to the other urodeles that have been studied.

MATERIALS AND METHODS

Gyrinophilus danielsi dunni (Mittleman and Jopson), commonly called the Carolina Spring Salamander, was chosen for study because it is relatively small, easy to handle, and little trouble to keep. Adult males were obtained from J. C. Nicholls, Jr., Murphy, North Carolina, early in December, 1963. They were kept in a twenty gallon glass aquarium furnished with a constant supply of fresh water. Each animal was sacrificed as needed for observation of living material and for permanent preparations.

For permanent preparations, the entire urogenital system was removed and fixed in Bouin's fluid. Several of these then were cleared, some for external observations as whole mounts and others for sections. Two complete systems were serially sectioned and others partially (approximately 35 micra thick). These were stained most effectively with standard haematoxylin and correlated with parallel studies of the whole mounts.

OBSERVATIONS

The urogenital system (Fig. 1) of *Gyrinophilus* is relatively long, approximately 5.34 cm and makes up about seventy per cent of the total length (snout-vent). The whole system except the caudal opisthonephros is heavily pigmented and very prominent. All components are paired, and, for the sake of convenience in the following discussion, reference usually is made to only one member of the pair.

Testis:

The testis of *Gyrinophilus* is a slender elongate structure approximately 1.79 cm long and 0.15 cm wide which begins as a pigmented band parallel to the vas deferens (Fig. 4) and ends either bluntly or in a slender extension called the caudal appendage. Slight pigmentation creates a pattern on the surface of the testis (Fig. 1). This organ is held in place by the mesorchium through which the blood vessels, genital arteries from the dorsal aorta and genital veins to the posterior vena cava, enter and leave the organ. The vasa efferentia, of which there is an average of five per testis, traverse this mesorchium in close association with the blood vessels.

An additional vas efferens was noticed leaving the testis at its most anterior portion (Fig. 2). This tubule was found to contain sperm and is believed to be an important connection between the testis and epididymis.

Three of the animals examined showed a caudal appendage while three others had slight constrictions in the middle of the gonad.

Epididymis:

In *Gyrinophilus* there is a definite epididymis anterior to the caudal opisthonephros. It is relatively small, rarely showing more than eight tubules in cross section. These tubules serve to transport sperm from the testis to the vas deferens.

The epididymal tubules are more numerous anterior to the testis where an average of six are seen in cross sections (Figs. 5 and 6); then they decrease in number and only one or two are present posteriorly (Fig. 8). These latter tubules are difficult to see except in cross sections because they are enclosed in the same tissue that surrounds the sperm duct. They often increase numerically in areas where the vas deferens is highly coiled. In the caudal opisthonephric region they

EXPLANATION OF ABBREVIATIONS

adrenal gland	ad
anterior testis	at
collecting duct	cd
dorsal aorta	da
epididymis	e
epididymal tubule	et
fat body	fb
genital opening	go
kidney	k
posterior cardinal vein	pcv
posterior vena cava	pvc
rudimentary Mullerian duct	rMd
testis	t
urinary opening	uo
vas deferens	vd
vasa efferentia	ve

[1] This research was submitted in partial fulfillment of the requirements for a degree with honors. Present address of the author is the Department of Biology, Emory University, Atlanta, Georgia, 30322.

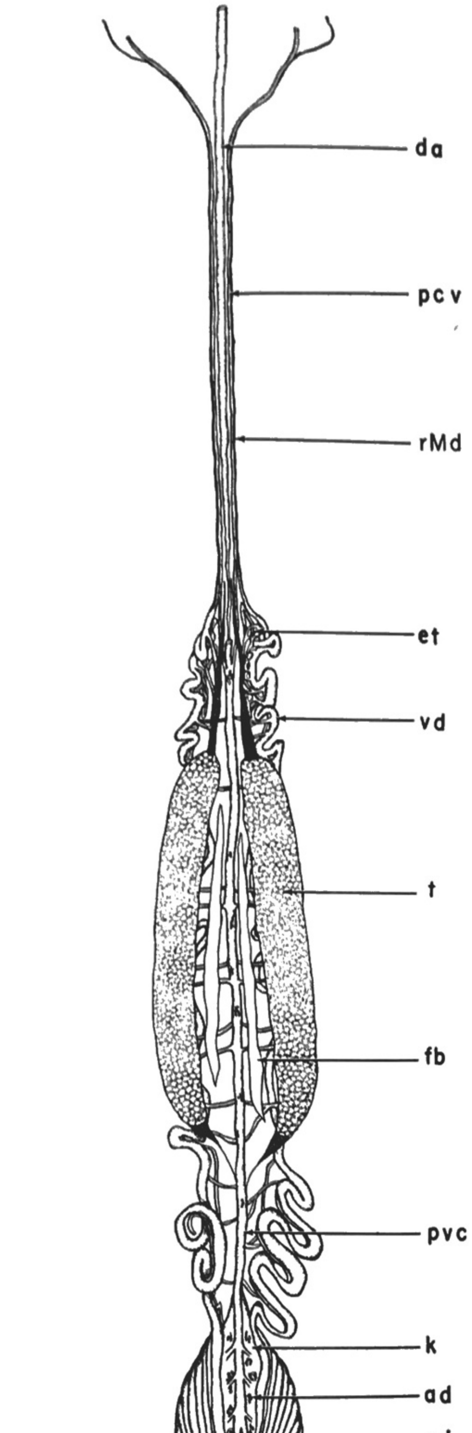


Fig. 1

Fig. 1. Urogenital system of **Gyrinophilus danielsi dunni.**

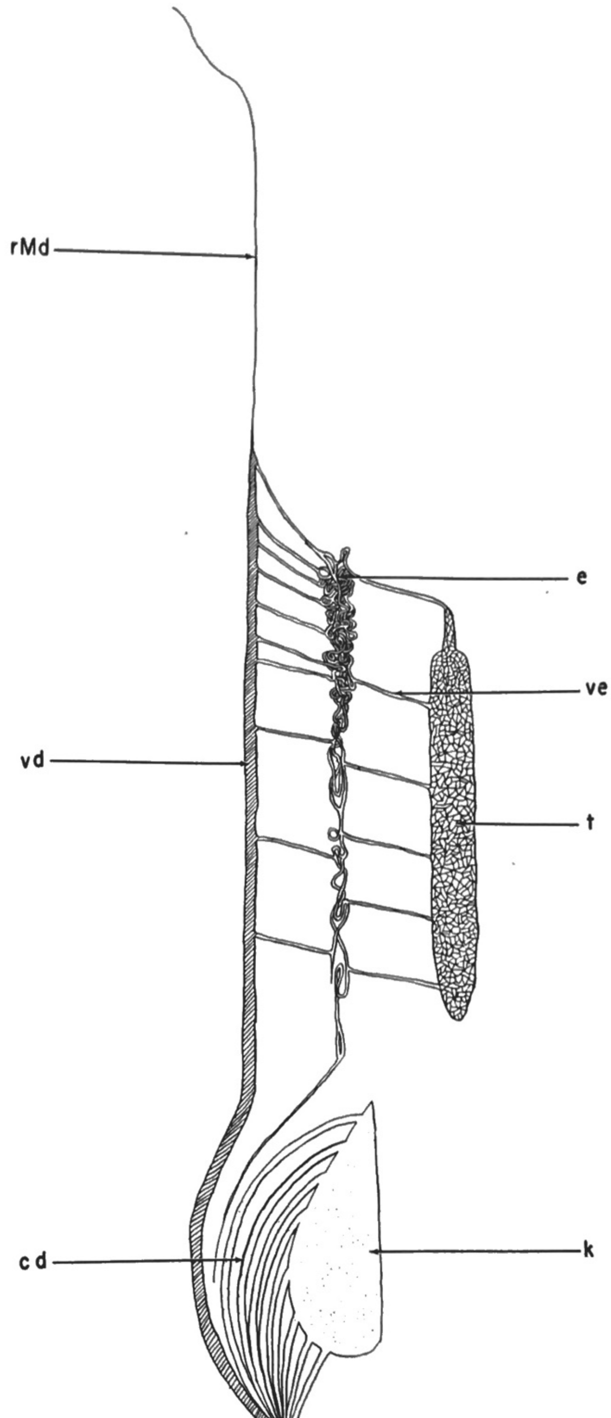
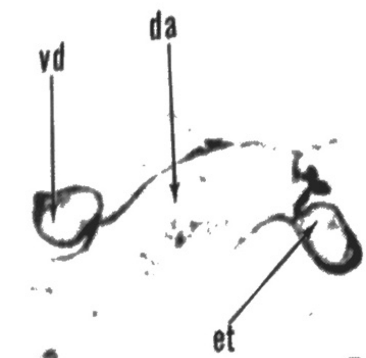


Fig. 2

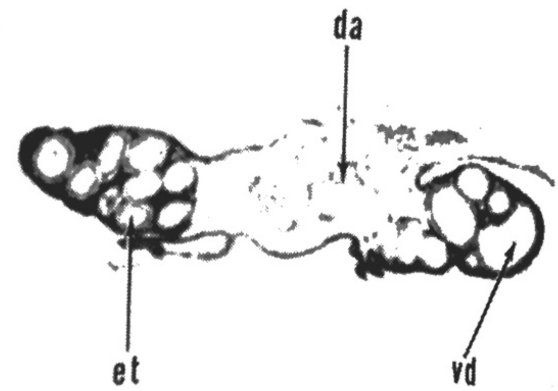
Fig. 2. Diagrammatic sketch of the right side of the urogenital system of the **Gyrinophilus** male with testis reflected to the left and the collecting ducts to the right.

3

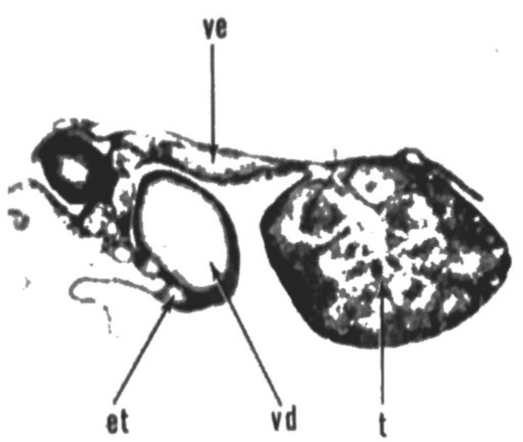
4



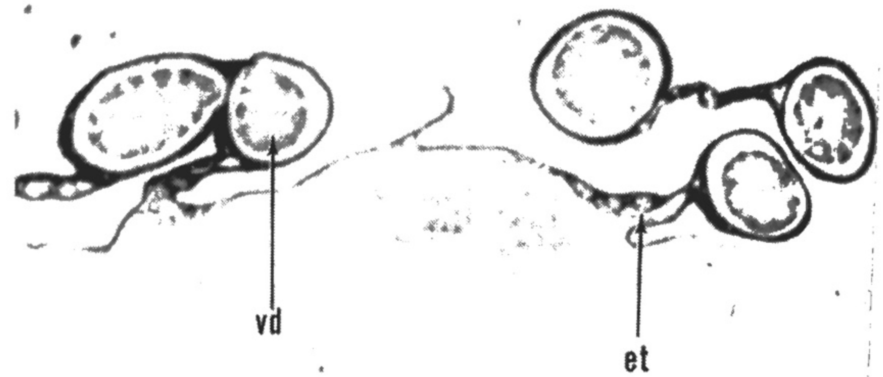
5



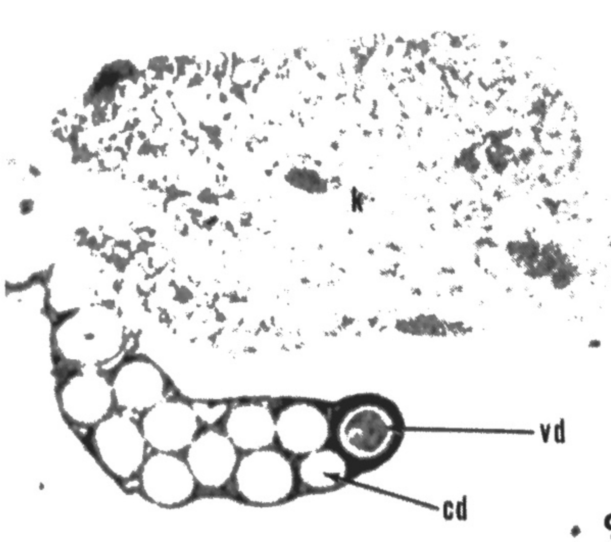
6



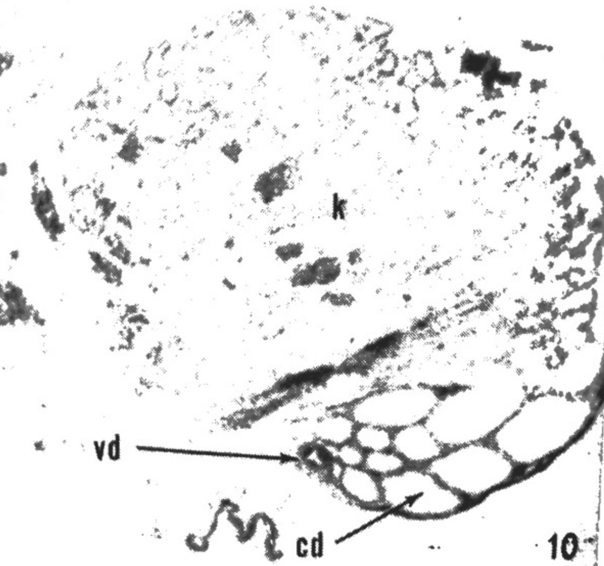
7



8



9



10

Fig. 3. Section posterior to the heart showing blood vessels and the pigmented band which represents the rudimentary Mullerian duct.
Fig. 4. Section showing the division of the pigmented band, one part of which is the anterior testis and the other the Mullerian duct.
Fig. 5. Section through the first epididymal tubules. Notice that the vas deferens cannot be distinguished from the epididymal tubules in such a section; this is done by retracing from sections in which it is filled with sperm.
Fig 6. Section through the epididymis anterior to the testis where the largest number of epididymal tubules can be seen.
Fig. 7. Typical section through the testis, epididymis and vas deferens. Notice a vas efferens leaving the testis.
Fig. 8. Section through the system posterior to the testis. Notice that the epididymal tubules are enclosed within the same tissue that surrounds the vas deferens.
Fig. 9. Section through the definitive kidney showing the collecting ducts and vas deferens filled with sperm.
Fig. 10. Last section showing coalescence of the collecting ducts

gradually disappear but remain distinct from the kidney tubules. No glomeruli are present in the epididymis.

There are approximately ten efferent epididymal tubules connecting the epididymis to the vas deferens. The majority of these occur in the thick anterior portion of the epididymis (Fig. 2).

Caudal Opisthonephros:

The excretory system of *Gyrinophilus* is an excellent example of a caudal opisthonephros. It is short, approximately 1.26 cm long (Fig. 1), is pressed into the dorsal body wall, and is drained by a maximum of fourteen collecting duct or urinary tubules. Its anterior limit is definite, and it does not continue anteriorly parallel to the testis. The pigmented tubules of the epididymis are easily distinguished from those of the definitive kidney; there is no gradual merging of the two. The collecting ducts leave the opisthonephros obliquely on the side, become united posteriorly, and enter the cloaca independently of the vas deferens (Figs. 2, 9, and 10). The caudal opisthonephros is fed by the renal portal vein and drained by the posterior vena cava.

Vas Deferens:

The separate drainage tubules of the opisthonephros described above are needed because the highly specialized Wolffian duct is used completely for the transport of sperm. It is therefore more appropriate to refer to this genital duct as the vas deferens or sperm duct even though its original function was excretory. This duct has its origin within the epididymis anterior to the testis where it receives a vas efferens directly. Posterior to this, the vas deferens is easily distinguished from the epididymal mass by its large lumen which is often filled with sperm.

Opposite the anterior portion of the testis, the vas deferens courses posteriorly in a highly coiled manner. In cross sections usually one can see from four to six sections through it (Fig. 8). It is connected to the dorsal body wall by a mesentery through which the blood vessels and efferent tubules cross. Upon reaching the kidney, the vas deferens changes from a lateral position in relation to the system to a ventral one. In other words, it lies ventral to the kidney and parallels the collecting ducts which drain the kidney. The vas deferens then enters the cloaca anterior to and separate from these tubules.

Rudimentary Mullerian Duct:

Gyrinophilus possesses a rudimentary Mullerian duct whose regression is almost complete. This structure is represented by a thin closed cord of pigmented cells which parallel the posterior cardinal vein anterior to the epididymis (Fig. 3). At the level of the epididymis, this cord is lost in the pigmentation which accompanies that structure.

Longitudinal Canal:

No longitudinal canal, sometimes called Bidder's duct, is present in this species.

Fat Bodies:

In urodeles the fat bodies, housing reserve nutrients for the transforming germ cells, are always closely associated with the gonads. In *Gyrinophilus,* they are connected to the mesentery between the testis and the dorsal body wall (Fig. 1). They vary greatly in size, ranging from a thin thread to a large mass completely covering the testis.

Adrenal Glands:

Although in no sense a part of the urogenital system, the adrenal glands (Fig. 1) always occur in such close association with the kidneys that it seems appropriate to include them. They are easily located in *Gyrinophilus* living material, appearing as small white patches following the posterior vena cava forward to the liver. There are five of these patches on the kidney plus nine to eleven more anteriorly on the vena cava.

Discussion

A discussion of the male urogenital system of *Gyrinophilus* can be broken up into four main categories: the testes, the excretory organs, the duct system, and the phylogenetic significance of these.

The testes of the urodeles undergo various modifications such as multiple testes (Adams, 1940) and caudal appendages (Humphrey, 1925). *Gyrinophilus* exemplifies only the latter case. Although Humphrey reported a caudal appendage in practically every male *Gyrinophilus,* only one-third of those studied in this work showed such a structure. This variation may be explained as a seasonal effect of the sexual cycle since Humphrey's observations were made during the summer months.

In urodeles, the opisthonephros is long and thin. It may be defined as an adult kidney, not a developmental stage, which incorporates both mesonephric and metanephric materials (Jollie, 1962). The anterior portion is partially reduced and modified in the male into an epididymis which connects to the elongate testis while posteriorly it becomes generally expanded with a great multiplication of tubules, thus foreshadowing the amniote condition.

Gray (1932) described the development of this condition in *Triton vulgaris.* In the course of development there is a very distinct separation between the two sets of units. There is an early set differentiated throughout the whole length of the nephric tract whose anterior members persist as the sexual kidney or epididymis; and a later set, arising only in the region of the adult functional kidney to which they give rise. Witschi (1956) adds that in male salamanders the nephrostomes of the sex kidney detach from the tubules and regress at puberty.

Although distinct glomeruli are visible in epididymal cross sections of members of the Sirenidae (Willett, 1965), Ambystomidae (Baker and Taylor, 1964), and Salamandridae (Baker, 1965), none is present in the epididymis of *Gyrinophilus.*

The term epididymis can easily become confusing. Its more common usage is employed when speaking of amniotes. There the highly coiled anterior portion of the vas deferens, the vasa efferentia, sexual kidney, and collecting efferent tubules become closely associated with the testis under the name of epididymis (Wilder, 1923). In amniotes, however, only the anterior part of the opisthonephros, having lost its excretory function is designated as the epididymis (Smith, 1960). This structure in urodeles is reminiscent of that found in the elasmobranchs. Specifically, an epididymis has been identified in *Cryptobranchus* by Nelsen (1953), in *Necturus* by Walker (1954) and Jollie (1962), in *Ambystoma* by Baker and Taylor (1964), in *Siren* by Willett (1965), and in the Salamandridae by Baker (1965).

The relative size of the epididymis may be of some importance from a phylogenetic standpoint. Three genera, *Siren* (Willett, 1965), *Cryptobranchus* (Ratcliff, 1965), and *Necturus* (Chase, 1923), have prominent, well-developed epididymides. In the Salamandridae (Baker, 1965) and Ambystomidae (Baker and Taylor, 1964) the epididymis is smaller and more distinct while *Gyrinophilus* has only a few tubules that can be called an epididymis.

In the anurans the testis does not have a separate vas deferens to collect and transport sperm to the cloaca; instead rete tubules connect it to the kidney where the sperm use the urinary path to their destination (Huettner, 1949). In general, however, this dual function is unsatisfactory and there appears to have been an evolutionary struggle between urinary and genital system for possession of the archinephric duct. Thus in higher vertebrates, which have a metanephros, a separate duct develops to serve the kidney and the old urinary duct becomes entirely genital.

To see the process by which this change occurs one must understand the evolution of the kidney and its ducts. The urodeles offer an excellent opportunity to see the gradations that exemplify this change, since they themselves show various transition stages.

In some urodeles, there is a strong trend toward the development of many separate urinary ducts from the kidney; these again foreshadow the amniote development of a ureter leaving the old duct free for sperm transport. The salamanders show a wide range of development in this area from the primitive condition in *Necturus* (Romer, 1956), in which 60 opisthonephric tubules pass directly laterally into the vas deferens, to a more advanced condition shown in *Gyrinophilus* where 14 collecting ducts turn posteriorly, become elongated, and unite into a short ureter.

In all salamanders, the Wolffian duct shows a seasonal variation in size, becoming large at the height of reproductive activity. In the non-breeding season, it may be reduced to a mere thread, particularly at the anterior end (Goin, 1962).

The rudimentary Mullerian duct cannot be called vestigial because it has never been functional in the male. In 1853, Leydig (see Francis, 1934) demonstrated the tubular nature of this anterior prolongation and revealed it as the homologue of the Mullerian duct of the female, although he does not actually call it such. Thus the first to distinguish this structure as a Mullerian duct was Spengel (see Francis, 1934) when in 1876 he demonstrated that in *Proteus, Siren,* and *Necturus* this duct is never a continuation of the Wolffian duct, but runs along its ventral wall enclosed in the same connective tissue sheath.

After this foundation was laid by Spengel, many others found such a duct: Francis (1934) in *Salamandra,* Witschi (1956) in *Triton* and Jollie (1962) in *Necturus.* Many general descriptions in textbooks include the vestigial Mullerian duct.

In some salamanders, *Necturus* for example (Moog, 1949), this duct is an open tube which extends the entire length of the system but neither receives nor gives off tubules. On the other hand, *Gyrinophilus* has a very small completely closed Mullerian duct.

The longitudinal collecting duct was first described in 1846 by Bidder (see Francis, 1934) and is consequently often called Bidder's duct. It has been described by both Spengel in *Triton* and Francis in *Salamandra* (Francis, 1934). Lying in the mesentery that holds the testis in place, it can be found on the ventro-medial side of the sexual kidney (McCurdy, 1931). The sperm follow the vas efferentia to the longitudinal canal which connects by means of a series of short ducts to the sexual kidney (Goin, 1962). As stated previously, no such canal is present in *Gyrinophilus.* This is substantiated by Spengel's statement that in the genera *Spelerpes, Batrachoceps,* and *Plethodon* the vasa efferentia run directly to the renal corpuscles without the intercalation of the collecting duct of the kidney and its transverse trunks. Willett (1965) also has reported the absence of a longitudinal canal in *Siren.*

Using the criteria proposed by Baker and Taylor (1964) one can place *Gyrinophilus* rather high in the phylogenetic structure of the urodeles. In the first place, the Wolffian duct has been completely emancipated from urine transport. The epididymis has lost all urinary structure and function and is reduced in size. There is no longitudinal canal and the rudimentary Mullerian duct has no lumen. Finally the urinary tubules which drain the kidney are reduced in number and course posteriorly to form a short ureter entering the cloaca independently of the vas deferens.

Summary

Most of the structures usually associated with the male urogenital system of urodeles are present in *Gyrinophilus* with the exception of a longitudinal canal. The possibility that the degree of development of each of these parts may be applied to the phylogeny of this group is discussed.

Acknowledgment

The author wishes to thank Dr. C. L. Baker for his continued interest and encouragement during the course of this research and also for his technical assistance with the preparation of the manuscript and photographs.

LITERATURE CITED

Adams, A. E. 1940. Sexual conditions in *Triturus viridescens,* *Am. J. Anat.,* 66:239.

Baker, C. L. 1965. The male urogenital system of the Salamandridae. *Jour. Tenn. Acad. Sci.,* 40:1-5.

---------- and W. W. Taylor. 1964. The urogenital system of the male *Ambystoma. Jour. Tenn. Acad. Sci.,* 39:1-9.

Chase, S. W. 1923. The mesonephros and urogenital ducts of *Necturus maculosus,* Rafinesque. *Jour. Morph.,* 37:457-532.
Francis, E. T. B. 1934. *The anatomy of the salamander.* Oxford at the Clarendon Press.
Goin, C. J. and O. B. Goin. 1962. *Introduction to herpetology.* W. H. Freeman & Co., San Francisco.
Gray, P. 1932. Development of the amphibian kidney II. The development of the kidney of *Triton vulgaris* and a comparison of this form with *Rana temporaria. Quart. Jour. Micr. Sci.,* 75.
Huettner, A. F. 1949. *Comparative embryology of the vertebrates.* The MacMillan Co., New York.
Humphrey, R. R. 1925. A modification of the urodele testis resulting from germ-cell degeneration. *Bio. Bull.,* 48:3.
Jollie, M. 1962. *Chordate morphology.* Reinhold Pub. Corp., New York.
McCurdy, H. M. 1931. Development of the sex organs in *Triturus torosus. Am. Jour. Anat.,* 47:367-403.
Moog, F. 1949. *Structure and development of the vertebrates.* Prentice-Hall, Inc., New York.
Nelsen, O. E. 1953. *Comparative embryology of the vertebrates.* The Blakiston Co., Inc., New York.
Ratcliff, M. A., Jr. 1965. The male urogenital system in *Cryptobranchus. Jour. Tenn. Acad. Sci.,* 40:52-57.
Romer, A. S. 1956. *The vertebrate body.* W. B. Saunders Company, Philadelphia, Penn.
Smith, Hobart M. 1960. *Evolution of chordate structure.* Holt, Rinehart and Winston, New York.
Walker, W. F. 1954. *Vertebrate dissection.* W. B. Saunders Company, Philadelphia, Penn.
Wilder, H. H. 1923. *The history of the human body.* Henry Holt & Co., New York.
Willett, J. A. 1965. The male urogenital system in the Sirenidae. *Jour. Tenn. Acad. Sci.,* 40:9-17.
Witschi, E. 1956. *Development of vertebrates.* W. B. Saunders Co., Philadelphia, Penn.

NEWS OF TENNESSEE SCIENCE

(Continued from page 25)

plants, especially those of its own family. There seem to be three major species of the Polypodium, yet botanists have not studied these diverse elements enough to show their evolutionary relationships between each other and other plants, Dr. Evans reports. He plans to study changes within plant substances which determine sex differences and govern the changes in generations.

The Atomic Energy Commission has made a $24,534 contract with the University of Tennessee to provide for a course in the fundamentals of radiation. The course will be coordinated by William Stafford of U T and classes will be held at the U T Center in Nashville. The program was started at the request of the Tennessee Department of Public Health primarily to train people who are responsible for handling radioactive materials. Guest experts from various institutions and government agencies will conduct lectures and laboratory experiments concerned with up-to-date techniques of handling radiation. The program, geared to the advanced undergraduate level, will cover a year of study in 4½ hour sessions per week. Twenty persons having a bachelor's degree or its equivalent will be admitted to the course which will cover such topics as radiation detector devices, sources of radiation, proper shielding techniques, methods of "cleaning" contaminated areas, and proper disposal of radioactive waste.

The final report of the three-week pilot institute on Nuclear Science and World Politics held April 12-30, 1965, in Oak Ridge is now available from the Oak Ridge Associated Universities, formerly ORINS. The institute was presented by ORINS and co-sponsored by the Carnegie Endowment for International Peace and the U. S. Atomic Energy Commission.

A description of the institute, together with sections concerning development of the program and comments and suggestions, comprise the main body of the report. In addition, a listing of the speakers and their topics and of the participants is included.

Purpose of the pilot institute was to increase understanding of some major concepts of nuclear physics and related technologies in order to examine the role science plays in a technologically advanced society and to explore international political problems having roots in contemporary developments in nuclear science. Twenty participants engaged in the field of international relations, selected from colleges, universities and government agencies, attended the conference.

A second unusual technical book for botanists by a University of Tennessee authority on mushrooms and a fellow scientist from the University of Michigan has been published. Dr. L. R. Hesler, dean emeritus of U T's College of Liberal Arts, has written "North American Species of Crepidotus" along with Dr. Alexander H. Smith, a University of Michigan botanist. Two of the leading scientists in the study of mushrooms, the writers collaborated for "North American Species of Hygrophorus" in 1963 and plan to publish a third volume next summer. Published by the Hafner Publishing Co. of New York, the new book examines the genus of mushrooms known as Crepidotus. It is the first book-length treatment of this rather large genus and contains descriptions of 125 species, of which 72 were discovered and described by the authors.

A $14,550 grant from the National Science Foundation will make research facilities at the University of Tennessee available to selected chemistry teachers from small southern colleges for 11 weeks next summer. Selection of the chemistry teachers to participate in the program will be based on their applications and recommendations of their deans, according to Dr. William E. Bull of U T's Department of Chemistry who will direct the program. The program is open to teachers

(Continued on page 34)