Chapter 8

The Basic Components of the Reproductive Strategy of the 'Typical' Vertebrate, as Illustrated by the Guppy, *Poecilia reticulata* (Peters)

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Introduction

The anatomical, physiological, behavioral, and ecological components that contribute to the successful reproduction of animals is increasingly being studied. This encompassing view is often referred to as the 'reproductive strategy' of the species. Considerable information exists about many different standard laboratory vertebrates such as the frog and mouse. However, these species do not lend themselves to laboratory activities that enable the student to obtain a complete view of reproduction in a period short enough to prevent breaking up the sequence of activities and thus the student's comprehension.

The interrelated series of exercises in this chapter adapt some of the results of a wealth of research with the guppy, *Poecilia reticulata,* to useful laboratory activities. These give the student an opportunity to appreciate the diversity of components and their interrelationships that contribute to the total reproductive effort of a 'typical' vertebrate. This species, as well as other members of the family Poeciliidae, is readily available, and easy to maintain. By virtue of its particular reproduction the guppy demonstrates a wide range of reproductive characteristics, from gonad structure to courtship behavior, in a single laboratory period.

The exercises are organized so that they can be performed together as a unit, or integrated with other activities, depending upon the instructor's interests and objectives. For example, the examination of gonads, gametes and embryos could be coupled with examination of comparable material in other vertebrates to illustrate similarity and differences.

The instructor or a technician will need an hour to accumulate and set out the necessary equipment and supplies. Assuming this prior preparation and organization, the students can accomplish the guppy exercises within a three-hour laboratory period. The exercises as a unit can be used to illustrate the various facets involved in reproduction. Dissection of the reproductive tracts and examination and preparation of gametes and embryos can be useful in a course in embryology. The behavior exercise can be appropriate to a course in ethology or as a separate behavior exercise in an introductory laboratory.

It must be remembered that the guppy is being employed as a vertebrate 'model'; both the instructor and student must keep in mind that teleosts possess a number of specializations which they must extrapolate from guppies to vertebrates in general.

Student Materials

Vertebrates exhibit a variety of reproductive modes depending upon the size (yolk content) of the egg, site of fertilization and site of development of the embryo/fetuses. The main reproductive modes are:

A. *Oviparity*—Eggs are deposited external to the body for embryonic development; fertilization may be external (most fish, frogs and toads) or internal if the egg is enclosed in a membranous or calcareous enclosure (reptiles, birds and monotreme mammals), or enclosed in jelly coats (newts and salamanders). These eggs will possess a large amount of yolk to sustain development of the embryo/fetus to birth.
B. *Ovoviviparity*—Eggs are fertilized internally and retained in the reproductive tract during development; the mother does not nourish the young directly, but they live off the food store in the large yolkey eggs. The young hatch internally and are born alive (certain bony fish, sharks and snakes).
C. *Viviparity*—The small and essentially yolkless eggs are fertilized internally, and the developing embryo quickly establishes an intimate 'placental' relationship with the maternal tissues to allow for nutrient and excretory exchange (certain bony and cartilagenous fishes, some reptiles and all marsupial and true placental mammals). A 'placenta' represents an intimate relationship between maternal and embryonic tissues to allow certain processes. Although this structural relationship is not restricted to mammals, in non-mammalian vertebrates it is frequently referred to as a 'pseudoplacenta'.

Vertebrates have evolved a reproductive system consisting of:

A. The primary sex organs called gonads (ovaries and testes) which produce and nourish the sex cells called gametes (eggs and sperm).
B. The secondary sex organs, ducts and their modifications which function to transport gametes. These may serve as a place of ova retention for development, and may possess specialized glandular areas in which the female may deposit accessory layers of substances (jelly coats, components of leathery or calcareous shells) around the eggs as they pass down the oviduct. In the male they may contribute volume to the spermatozoa.
C. The reproductive tracts of most male vertebrates are similar, but in the females greater variation is present in relationship to the specific reproductive mode which they possess.

In response to hormonal stimulation animals will also develop a variety of secondary sexual characteristics such as size differences, coloration, and other physical features. Behavioral changes are important for sex-recognition and mating. The species-specific nature of courtship behavior serves to attract members of the opposite sex, prevent interspecific matings and coordinate body movements so that gametes can be brought into the proximity of one another.

You will be using the common guppy, *Poecilia reticulata,* as a study model to become familiar with the different components that comprise reproduction in most, if not all, vertebrates. This prolific, live-bearing fish exhibits pronounced and colorful sexual dimorphism and courtship behavior.

Instructors' Materials

Procedures

A. Observing external anatomy and secondary sexual characteristics.

1. Observe the male and female guppies in the community tanks. The male is smaller, exhibits more pigmentation and possesses a modified anal fin or gonopodium in which the fin rays are differentially elongated and recurved distally (Fig 8.1). This gonopodium is used as an intromittent organ to transfer masses of spermatozoa or spermatophores into the female reproductive tract. The female, by contrast, is larger, less colorful and possesses a convex anal fin.

   Most vertebrates with internal fertilization possess some type of intromittent organ with which to transfer sperm to the female reproductive tract. Which vertebrate taxa possess such a structure and with which reproductive mode(s) is its presence associated? Both the smaller size and more pronounced coloration of the male are characteristic of several other vertebrates as well. Can you think of other species in which one or both occur? What are the functions of pigmentation and size differences? Male guppies exhibit determinate growth once reproductive maturity is reached, whereas females continue to grow. How is this related to the reproductive potential of the female?

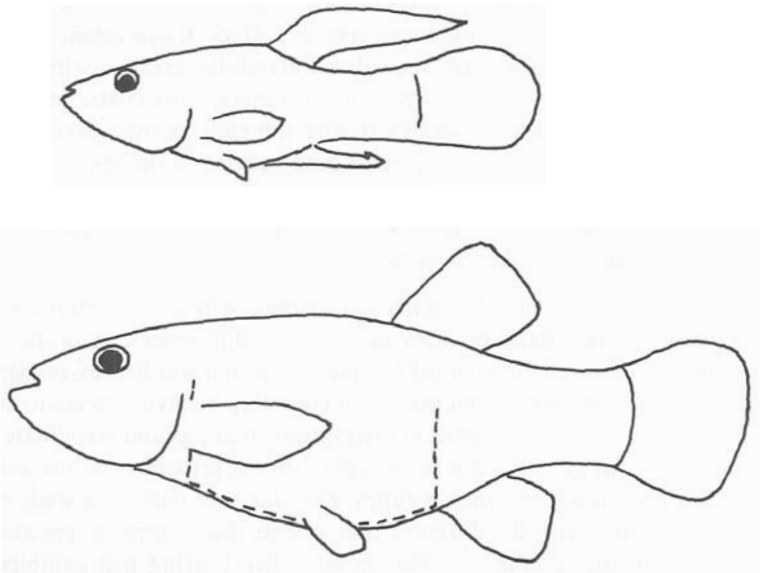

Figure 8.1. Male (top) and female (bottom) guppies showing body size and fin shape dimorphism. Female shows locations of cuts to provide "flap" to expose gonads. (Same location in male).

gonads. (Same location in male).

gonads. (Same location in male).

B. Exposing the reproductive tract
   1. The procedure for sacrificing the fish and dissecting out the reproductive tract is the same for both sexes. Decapitation of the fish just posterior to the operculum with a razor blade is preferable to over anesthetization in Finquel, in order to avoid reducing subsequent motile activity of spermatozoa or circulatory activity of developing embryos.
   2. Net a fish from the tank and place it on its side on a thin moist bed of absorbent cotton in a petri dish. Decapitate it and then with the fish on its back, open the abdominal cavity on one side with iris scissors by first extending a mid-ventral incision from the anal opening to the pectoral fins, followed by two cuts dorsally just behind the pectoral fin and just above the anus (Fig. 8.1). This will produce a flap which can be reflected to expose the body cavity. Bathe the specimen liberally with saline.
   3. In both sexes the gonad is suspended dorsally in the posterior part of the body cavity ventral to the swim bladder and above the gut, and is connected to the genital opening by a single genital duct.

C. Examining the testis, spermatophores and spermatozoa.
   1. The single bilobed testis is covered by a thin unpigmented peritoneal membrane. Internally it possesses a multi-branched sperm duct in each lobe; both ducts unite posterio-ventrally to form a short vas deferens. Remove the testis by snipping the suspending tissue and the vas deferens, and transfer the testis with a pair of forceps to a depression slide or watch glass.
   2. Cut up the testis with fine scissors or tease it apart with the forceps and needle. Note the numerous small whitish masses which are the sperm packets or spermatophores, many of which are transferred to the female during insemination. Can you think of what vertebrates transfer a single large spermatophore during reproduction and how this transfer is accomplished in those animals?
   3. Transfer a drop of the sperm suspension (avoid large particles of material) with an eyedropper to a clean slide and apply a coverslip. Examine the slide under a compound microscope and then a phase contrast microscope. Identify the structural components of the sperm and note their agitated motility. What differences in details of sperm structure were revealed by the phase contrast microscope? Teleost sperm do not possess an acrosome. What is the function of the acrosome? Does the 'micropyle' of the egg substitute in teleosts for the function of the acrosome?

D. Examining the ovary, ova and embryos.
   1. The single fused ovary is larger than the testis, orange-yellow in color and connected to the genital pore by a single oviduct. Cut the oviduct and suspending tissues and transfer the ovary to a petri dish with sufficient saline to cover it.
   2. Examine the ovary. In addition to many large, amber-colored ova and small whitish previtellogenic oöcytes, several embryos in a more or less synchronous stage of development will probably be present. The guppy (like all Poeciliids) exhibits intrafollicular fertilization and gestation. Sperm are stored for many months in a mid-dorsal 'sperm receptacle'. Sperm storage along with multiple inseminations assure a continued supply of sperm to father successive broods of young, even if the female is isolated from further contact with a male.
   3. Carefully tease the ovary apart with forceps and dissecting needle to release the eggs and embryos from their follicles. What EGG TYPE is seen here and what MODE OF REPRODUCTION does this species exhibit? Look for typical vertebrate embryonic features such as the pigmented retinal cups, contracting heart and circulating blood, and segmental somites. Near-term fry can be kept in saline or aquarium water and will continue normal development outside the mother. This illustrates the dependence of the developing embryo on its own nutrient rather than maternal nutrient, as in true viviparity.

E. Observing courtship behavior and insemination.
   1. Courtship in the guppy involves a series of reciprocal interactions between the male and female. Provided that the female is receptive, (greatest receptivity is immediately after the birth of a brood of fry) at the culmination of a courtship sequence the individuals will be so positioned that the gonopodium can be momentarily inserted into the genital pore of the female with the subsequent release of many spermatophores. Once in the tract, the sperm dissociate from one another and assume motile activity.
   2. Tables 8.1 and 8.2 summarize the primary movements exhibited by the male and female, and Figure 8.2 illustrates the common sequence of movements that you will observe. Also familiarize yourself with these movement sequences from the film, 'Reproductive Behavior of the Guppy, *Poecilia reticulata*'. Observe the fish in the community tank or in the individual observation tanks. Can you identify specific movements and sequences of movements from your sheet and the film? Is the behavior of the male or female more pronounced? If an isolated female has had a brood of young in the last few days, observe the male's behavior with her. Is it more or less pronounced than in the

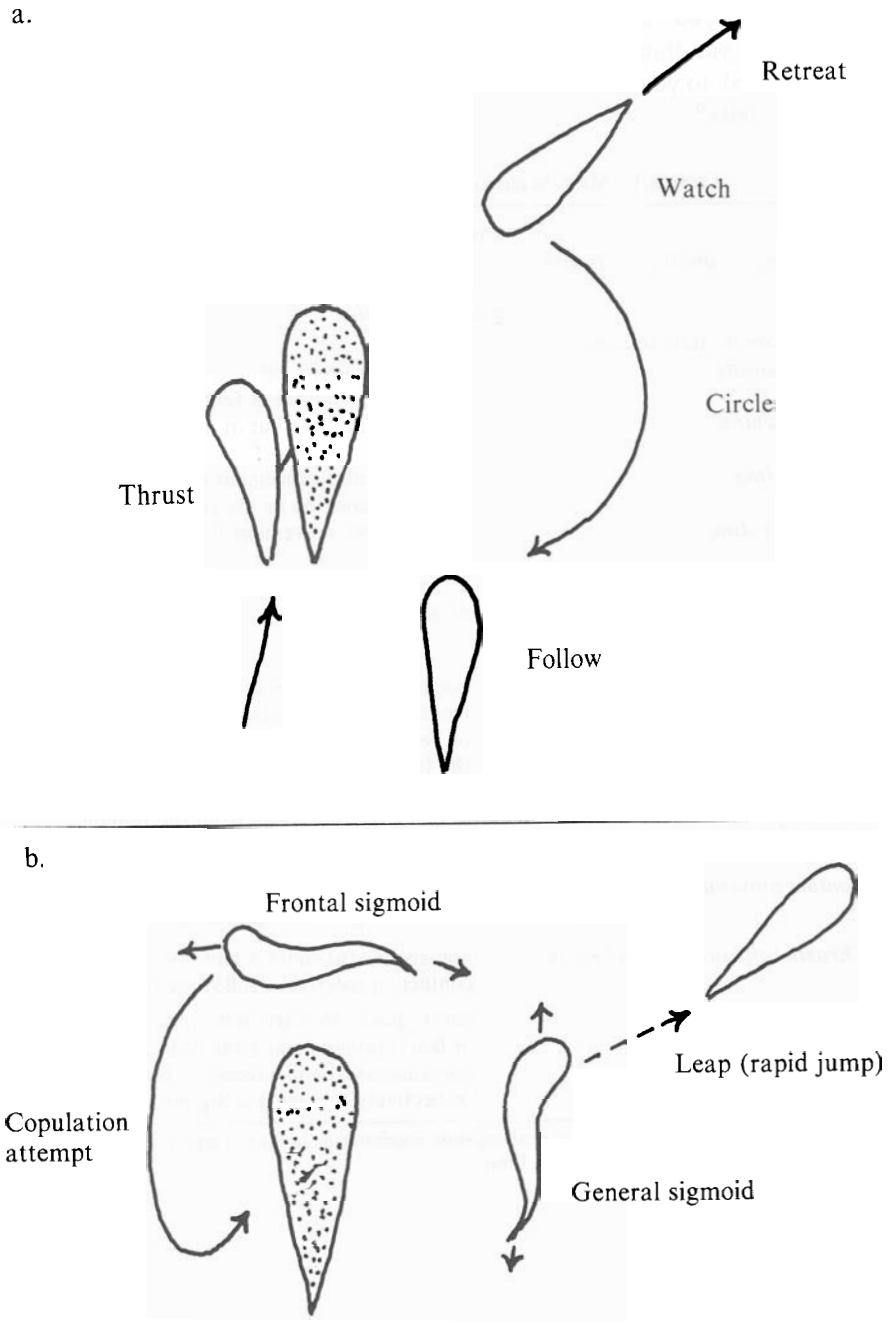

Figure 8.2. Patterns of (a) orientation and thrusting movements, and (b) display movements. Female stippled. Arrow indicates direction of male movement (after Liley, 1966).

Liley, 1966).

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pair you have been observing? If the male courts more actively, what do you think might be the cause? Transfer a cup of water from that tank to yours and observe your fish again. Is there any change in activity?

Table 8.1. Main behavioral movements of male guppies.*

Non-courtship Activity	
Swimming, 'standing' or feeding	
Courtship Behavior	
Orientation of male to female	
Following:	observing to rear, off to one side and usually slightly above level of female.
Watching:	observing in front but at an angle to the female.
Circling:	a semicircular movement from a following to watching position or the reverse.
Retreating:	a backwards movement from a watching position.
Gonopodial swinging:	a frequent forward rotation of gonopodium at any time.
Display of male to female	
Sigmoid Display:	body bent into an S or Sigmoid shape in front of and perpendicular (frontal position) or beside and parallel (general position) to the female. Body quivers and fins vary in degree of spread.
Leap:	A quick darting away from the female following frontal or general sigmoid.
Contact movements:	male activity resulting in actual or apparent contact of genital region by gonopodium.
Thrusts Intentions and Thrusts:	non-successful contact and noncopulatory contact, respectively, following orientation.
Jerks	short, quick, forward and upward movements of body; pronounced after copulation.
Copulation attempt and copulation	non-successful and successful insemination respectively following a Sigmoid display.

*Adapted from Liley, N. R. Ethological isolating mechanisms in four sympatric species of Poeciliid fishes. Behavior, Supplement 13:1966.

Table 8.2. Main behavioral movements of female guppies.*

General Behavior:	swimming, vigorous swimming, evasion, fleeing, attack, sparring and approach towards male.
Receptive Behavior	
Glide—	subtle movement towards male.
Arching—	raising of head and tail with anal fin folded.
Wheeling—	movement of female in circle as male attempts repeated gonopodial jabs, often resulting in gonopodial insertion or copulation.
Wobble—	pronounced lateral body movements following insemination.

*Adapted from Liley, N. R. Ethological isolating mechanisms in four sympatric species of Poeciliid fishes. Behavior, Supplement 13:1966.

3. Although the male may attempt to mate, actual insemination will be rare and difficult to observe. To increase your chances of observing the behavioral sequence leading to insemination and the pronounced subsequent activity of the male, do the following:
   a. Carefully net the female from your observation tank and anesthetize her in Finquel until muscular but not opercular movements cease.
   b. Next, carefully float the female out of the net at the surface of the water in the center of the tank.
   c. Resume quiet and patient observation. The male's response will range from indifference, to varying intensities of courtship behavior as the female drifts toward the bottom of the tank. Often, the male will immediately go through a normal sequence of movements culminating in insemination. Whereas under normal conditions the male is flipped away from the female after insemination, with an anesthetized female, because of her inertness, the two fish will noticeably 'tumble' in the water when the male removes the gonopodium. Following insemination the male will exhibit pronounced jerking of the entire body. You may also see released into the water white spermatophores that are evident against the black background.
   d. Sometimes anesthetizing the female will not work as expected. Don't hesitate to reanesthetize her, and if the male seems to have a lack of interest, try a different male established in another tank.

Suggestions

One of the advantages of these activities is that they all involve simple and straightforward procedures. With reasonable care and attention to directions any student can complete each activity without any problem.

The instructor plays an important part in the success of the laboratory in several ways. In addition to obtaining and organizing the necessary supplies and equipment, you can help pace the students through the exercises by demonstrating the simplicity of the procedures and by acting as a facilitator of discussion about different facets of the exercises.

Dissection

As indicated decapitation is preferable to anesthesia and should be accomplished by the student with no problem. Students should be cautioned not to exert too much pressure directly on the body of the fish. The procedure is straight-forward and invariably successful if performed with care. The only problem has arisen when a student has dropped the whitish testis on the cotton bedding on which the fish was placed!

Microscopy

Dissecting scopes are necessary for the dissection and observation of the gonads, ova and embryos. Standard compound and phase contrast microscopes are needed for examination of the sperm smears. At least one phase microscope should be available, but preferably more in a large class so that several students can examine their temporary sperm smears.

Preparation of Observation Aquaria

For observation of courtship and insemination each student should have available a small aquarium, preferably 2–3 gal., with at least one clean flat side. The other three sides should be covered with black poster board to reduce distractions to both the fish and the observer. The tank should contain neither gravel nor a filter.

Use of Audiovisual Aids

The nine-minute film "Reproductive Behavior of the Guppy, *Poecilia reticulata*" is a valuable adjunct to the behavioral exercise. This film will enable the students to more quickly recognize the primary types of sequences of movements in courtship behavior.

Promoting Discussion and Further Work

Some questions have been included in the Procedures to encourage the student to relate the exercise to vertebrates in general. They are not so numerous as to divert the student from completing the exercises, and leave the instructor discretion in directing discussion, depending upon the course in which this exercise is being used or how it fits with other laboratory exercises. In an

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embryology course emphasis might be on culturing and examining fry and considering similarities and differences in embryonic characteristics, such as egg and cleavage types in other vertebrates. If the emphasis is on behavior, discussion might center around the significance of pheromone secretion in the guppy (Crow and Liley 1979) and its occurrence in other vertebrates. Interested students might make observations and attempt to obtain insemination in other poeciliids. The instructor might wish to expand on such topics as the testis structure and spermatophore production (Pandey 1969) and what stimulates sperm motility in the female reproductive tract (Morisawa and Suzuki 1980).

Supplies and Materials

Microscopes—

1. Phase contrast—ideally one for every one or two students
2. Compound—one for every one or two students
3. Dissecting—one per student

Dissecting and other instruments—one set of the following per student

1. Scissors—1 pair fine tip (iris) dissecting
2. Forceps—2 straight tip, fine dissecting
3. Teasing needles—2 straight and/or bent
4. Glass needles—2
5. Eye dropper—1
6. Single edged razor blades—2 per student

Slides/coverslips

1. Plain glass slides—2 boxes
2. Coverslips—6 per student
3. Depression slides—double well if possible, two per student

Solutions

1. Teleost saline (5.50g NaCl, 0.14g KCl, 0.12g $CaCl_2$ per liter) Sufficient saline for 1 liter per student
2. Finquel anesthetic (1 gm of Finquel to distilled water to make 1 liter) Finquel (tricane methanesulfonate or MS222) is available from Kent Laboratories, Ltd., 1292 Franklin Street, Vancouver, V6A IKI or Ayerst Laboratories, 685 Third Ave, New York 10017.

Miscellaneous glassware

1. Watch glasses—2 per student 72 mm and 95mm
2. Petri dishes—one per student; glass and deep
3. One 250 ml narrow-mouthed bottle per student (containing teleost saline)

Miscellaneous materials

1. Fish nets: 1 2″ net per student
2. Absorbent cotton
3. Film projector and screen
4. Film: 'Reproductive Behavior of the Guppy *(Poecilia reticulata)*' available from: The Pennsylvania State University
   Audio Visual Services
   University Park, PA 16802
   (814-865-6315)

Fish aquaria

1. 1 aqaurium, 10–20 gallon per class
2. 1 2–3 gallon aquarium per one or two students

Fish—Two pairs mature male and female guppies per student

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