SYMPOSIUM

A Male Poecillid’s Sexually Dimorphic Body Plan, Behavior, and Nervous System

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Synopsis Here we review the literature of a male poecillid’s sexually dimorphic body plan, behavior, and nervous system, including work dating from the mid 1800s to the mid 1990s as well as work in press or in preparation for publication. Rosa-Molinar described the remodeling of the sexually dimorphic anal fin appendicular support, confirmed earlier claims about the development of the male and female secondary sex characteristics in the Western Mosquitofish, Gambusia affinis and provided for the first time direct embryonic evidence suggesting that remodeling of the sexually dimorphic anal fin appendicular support is biphasic. The first process begins in embryos and proceeds similarly in immature males and females; the second process occurs only in males and results in the anterior transposition of the anal fin and its appendicular support to the level of vertebra 11 [Rosa-Molinar E, Hendricks SE, Rodriguez-Sierra JF, Fritzsch B. 1994. Development of the anal fin appendicular support in the western mosquitofish, Gambusia affinis (Baird and Girard, 1854): a reinvestigation and reinterpretation. Acta Anat 151:20–35.] and the formation of a gonopodium used for internal fertilization. Studies using high-speed video cameras confirmed and extended Peden’s and others’ observations of copulatory behavior. The cameras showed that circumduction is a complex movement combining in a very fast sequence abduction, extension and pronation, S-start-type fast-start (defined as torque–thrust), and adduction movements. Recent work on the nervous system demonstrated dye-coupling between motor neurons and interneurons via gap junctions, suggesting an attractive substrate for the rapid motions involved in poecillid copulatory reflexes.

Revisiting the poecillid body plan

This review of work dating from the mid 1800s to the mid 1990s as well as work in press or in preparation for publication promotes the idea that the radical changes in the body plan of the male Western Mosquitofish, Gambusia affinis (Baird and Girard 1854; Rauchenberger 1989; Gambusia hereafter) are reflected in, and related to, sexually dimorphic behavior and nervous system organization and connectivity. It is worth keeping in mind that some aspects of the spinal neural circuit controlling Gambusia’s motor reflexes may be difficult to delineate. Further, how much spinal neural circuits in Gambusia will teach us about neural circuits of other poecillid fishes, whether or not Gambusia’s neural circuit can be further elucidated in a way described in this review, and whether or not these Gambusia spinal neural circuits bear a fundamental resemblance to other poecillid spinal neural circuits—only time will tell.

The primary ichthyological literature and textbooks as well as the primary comparative vertebrate anatomical literature and textbooks describe the proto-typical teleost axial skeleton vertebral formulae as being composed of two vertebral regions [i.e., anterior trunk (rib containing vertebrae) and posterior caudal] body plan (Rosen and Bailey 1963; Webster and Webster 1974; Kluge 1977; Lagler et al. 1977; Wake 1979; Parenti 1981; Romer and
Parson 1986; Rosa-Molinar et al. 1994; Kardong and Zalisko 1998; Helfman et al. 2009). However, the body plan of internal-fertilizing male poeciliid fish in the family Poeciliidae, specifically males of the genus Gambusia, have a three-part body plan consisting of anterior trunk, posterior caudal, and a third region known as the ano-urogenital region (Rosa-Molinar et al. 1994). In 1994 Rosa-Molinar and colleagues described the remodeling of the sexually dimorphic anal fin appendicular support, confirmed earlier claims about the development of the male and female secondary sex characteristics in this species, and provided for the first time direct embryonic evidence suggesting that remodeling of the sexually dimorphic anal fin appendicular support is biphasic, involving one) anteriorization of the most anterior caudal segments and two) growth and elongation of hemal spines of vertebrae 14–16 [note that in this review the 14th and 16th hemal spines previously termed “gonapophyses” will be referred to as hemal spines] (Rosa-Molinar et al. 1994). The first process, anteriorization, involves a sequential homeotic-like transformation of the hemal spines of vertebrae 11–13 through resorption of mineralized connective tissue, thus forming parapophyses that bear pleural ribs (Rosa-Molinar et al. 1994). This process begins in embryos and proceeds similarly in immature males and females (Rosa-Molinar et al. 1994).

During this same period, the second process, occurring only in males and probably mediated by male gonadal hormones, causes the addition of mineralized connective tissue at the hemal spines of vertebrae 14–16 (Rosa-Molinar et al. 1994). This second process elongates and bends the hemal spines of vertebrae 14–16 anteriorly (Rosa-Molinar et al. 1994) and results in the anterior transposition of the anal fin and its appendicular support to the level of vertebra 11 (Rosa-Molinar et al. 1994).

It appears that the developmental programs of female and male Gambusia lead to a third region of six vertebrae that are markedly different from any vertebrae anterior to 11 (anterior trunk region) or posterior to 16 (posterior caudal region) (Rosa-Molinar et al. 1994). The anterior transposition of the anal fin and its appendicular support in Gambusia represents a significant reorganization of the teleost axial formulae (Rosa-Molinar et al. 1994). Although data led to the proposition that the axial formulae of Gambusia is differentiated into three regions (i.e., anterior trunk, ano-urogenital, and posterior caudal) (Rosa-Molinar et al. 1994), it did not explain how, provide the mechanism for, or determine the critical size required for the vertebral column of Gambusia to differentiate into three regions, issues of some speculation since 1926 (Rosa-Molinar et al. 1994). Additional work by Rosa-Molinar et al. (1998) addressed these issues.


Rethinking poeciliid copulatory behavior

Research on Gambusia’s motor reflexes has been hampered by the speed and frequency of the male’s movements as well as by the limitations of photographic technology, including inadequate speed, inadequate depth-of-field and field-of-view, and poor or inappropriate lenses. Nevertheless, and remarkably, given the available technology, Peden (1970, 1972a, 1972b, 1975) provided one of the most detailed analyses of the courtship behavior of male and female Eastern Mosquitofish, Gambusia holbrooki (Girard 1859). Peden (1970, 1972a, 1972b, 1975) reported that male G. holbrooki approach females from behind and slightly below them and engage in “swing and thrust” behavior; the male swings the gonopodium forward, then thrusts it as he moves upwards toward the urogenital sinus of the female. Peden’s observations and those of others led to the conclusion that the pectoral fin supports the gonopodium during copulation; explanations of the placement of the gonopodium on the pectoral fin varied somewhat (Hubbs and Reynolds 1957; Warburton et al. 1957; Rosen and Tucker 1961; Peden 1970, 1972a, 1972b, 1973, 1975; Rosa-Molinar et al. 1996).

Three synchronized high-speed video cameras (1024 by 1024 pixel resolution) operated at
1000 frames/s–1 (1/1000 s shutter speed) provided clear, crisp, ventral, lateral, and frontal views of the gonopodium during circumduction (Fig. 1 and Table 1 for description of the movements) and during attempted copulations. In this review we use “circumduction of the gonopodium” versus “swing and thrust” to describe in an anatomically correct manner the movement of the gonopodium towards the female’s urogenital sinus (see Table 1 for description of the movements). The cameras clearly confirmed that male Gambusia circumduct the gonopodium without ever displaying precopulatory behaviors. The camera images made it possible to calculate the speed of the circumduction as well as that of the torque–thrust motion and to determine that circumduction occurs in ~1300 ms; the extremely rapid male torque–thrust motion takes 20–50 ms. The short duration of the torque–thrust motion of the circumduction of the gonopodium is one of the most rapid behaviors known in fishes, and it is similar in time-course to the C-type fast-start response.

As Peden observed, males approach females from behind and below them. High-speed images show that when males are directly below females, they circumduct the gonopodium. The images show that circumduction in G. affinis (and in G. holbrooki) is a complex movement in which abduction, extension and pronation, S-start-type fast-start, and adduction movements are combined in a very rapid sequence (see Fig. 1 and Table 1 for description of the movements). After extension and pronation of the gonopodium during circumduction, males’ bodies bend in an S-shape (the S-start-like portion of the circumduction of the gonopodium takes ~20 ms) that is similar to the rapid-acceleration S-start type of fast-starts, used by teleost fish during predator–prey interactions (Domenici and Blake 1997; Hale 2002;
Pronation: a rotational movement

Circumduction: the circular (or, more precisely, conical) movement of a body part, such as that of a ball-and-socket joint. It consists of a combination of extension, adduction, and abduction.

Reevaluating gap junctions and considering a spinal neural circuit linked to fast copulatory behavior

The development of neural circuits requires the establishment of millions of synaptic connections, and numerous studies have focused on the development of chemical synapses (Peinado et al. 1993; Mills and Massey 1999; Marin-Burgin et al. 2005; Szabo and Zoran 2007). Fewer studies have focused on the formation or roles of electrical synapses, with the exception of studies of their role in the retina and other systems in which the role of electrical coupling between cells has long been accepted (O’Brien et al. 1996, 2004; Söhl 1998). Recently, however, new interest has been shown in the sequential development of electrical (hereafter we use the term “gap junction”) and chemical synapses as well as in the role that gap junctions might play in helping to guide the formation of synaptic connections in neural circuits (Harris and Landis 1986; Peinado et al. 1993; Mills and Massey 2000; Szabo et al. 2004; Harris 2007; Szabo and Zoran 2007).

In invertebrates, a “switch” to chemical neurotransmission following an initial transient expression of gap junctional synaptic coupling has been observed (Szabo et al. 2004). In vertebrates, transient gap junctions have been proposed as being essential in the formation of neuronal networks (Gibson et al. 1999; Marin-Burgin et al. 2005; Szabo and Zoran 2007). Now, a growing body of ultrastructural and immunocytochemical evidence shows the presence and persistence of gap junctions at “mixed” (i.e., chemical and electrical combined) synapses in the adult CNS (Sotelo and Korn 1978; Vaney 1991; Kalb 1994; Rash et al. 1996, 1997, 1998, 1999, 2000, 2001, 2005; Kamasawa et al. 2006). This persistence has been taken to suggest that gap junctions provide a previously unrecognized means of communication between vertebrate CNS neurons (Rash et al. 2007). Recent advances in neuroanatomical tract tracing and imaging have made it possible to begin detailed analyses of neuronal cells and connectivity in the CNS of vertebrates.

Retrograde labeling using 3000 MW Texas Red® dextran amine (3kDa TDA), previously presumed to be gap junction-impermeant because of its size and anionic charge, revealed extensive dye-coupling between motor neurons (MNs) and commissural primary ascending interneurons (CoPA INs; Hale et al. 2001; Fig. 3). In this review, “extensive dye-coupling” refers to the condition in which fluorescence permeates throughout the entire labeled neuron(s) but the relative fluorescence intensity is always higher in the parent neuron(s), in this case, MNs, than it is in the dye-coupled neuron(s), in this case CoPA INs (Fig. 3). Some of the dye-coupled neuronal somata are >105μm away from the somata of the nearest labeled parent MNs. In CoPA INs, 3kDa TDA revealed extensive dendritic...
arbors and branches associated with the bipolar “T-shaped” dorsal dendrites that extend rostrally and caudally; it also revealed a long axon of the CoPA INs (Fig. 3). Because of researchers’ skepticism of dye-coupling studies, the following should be noted: the dye-coupling technique used does not disturb distant regions of the spinal cord because the tracers were retrogradely introduced from a site ~5 mm away, representing ca. a quarter of the fish’s body length. Likewise, absence of tracer in the extracellular space around commissural primary ascending interneurons disallows extracellular transynaptic transport and large-scale endocytosis into INs. Artifactual labeling of commissural primary ascending interneurons by dye diffusion was thus unlikely.
Combining neural-tract tracing with confocal microscopy and freeze-fracture replica immunogold labeling (FRIL; Fujimoto 1995) confirmed spinal motor neuron-to-interneuron coupling and showed the presence of mixed synapses (Fig. 4). Given that mixed synapses offer a powerful means of synchronizing fast motor behavior (Galarreta and Hestrin 1999, 2001a, 2001b; Saint-Amant and Drapeau...
Fig. 4 Freeze-fracture replica immunogold labeling (FRIL) revealed gap junctions between dye-coupled motor neurons and commissural primary ascending interneurons in the ventral spinal cord of a male G. affinis. The Cx 35 gap junctions were labeled using a Cx35 antibody that recognizes the perch Cx 35. The Cx35 antibody does not cross-react with Cx 34, a very closely related Cx protein (O’Brien et al. 1998). The Cx35 labeling was visualized using immuno-collodial gold [colloidal gold particles range from 6 nm (black arrows) to 18 nm (arrows)], FRIL labeling and imaging: The freeze-fracture replica and immunogold labeling (FRIL) protocol used for this paper has been described in detail (Pereda et al. 2003; Rash and Yasumura 1999). The labeled spinal cord region associated with vertebral segments 7–17 (see the labeling section above for details) was first placed in a 3% low melting agarose, then transferred and embedded in 6% agarose, followed by refrigeration until fully gelled. Coronal sections were cut (100 μm) using a Lancer Vibrotome 3000 that maintained the tissue sections at 4 °C. Spinal cord sections were infiltrated with 30% glycerol, mounted on solid aluminum stubs, and frozen by contact with a liquid nitrogen-cooled metal mirror. Frozen samples were fractured and replicated in a JOEL/RMC 9010 freeze-fracture device, then bonded to gold “index grids by using 2.0% Lexan dissolved in dichloroethane. After solvent evaporation at −35 °C, the Lexan-stabilized samples were thawed; samples were viewed and digitally photographed using a 20× (0.50 N.A; 2.10 W.D.) objective in a Zeiss Meta Laser Scanning Confocal Microscope. Replicas were washed in 2.5% SDS detergent in 0.16% Tris–HCl buffer (pH 8.9) for 29 h at 48.5 °C. After the initial wash in 2.5% SDS (4.0 h), the samples were digested 1.25 h in 4.0% collagenase D in 0.15 M Sorensen’s phosphate buffer (pH 7.4), followed by an additional 18-24 h in SDS solution. The replicas were rinsed in “labeling-blocking buffer” (1 mg/mL LBB), then incubated for 1–1.5 h at 22–24 °C in 1:100 primary antibody solution in LLB (LBB consists of 1.5% fish gelatin plus 10% heat-inactivated goat serum in Sorensen’s phosphate buffer). The replicas were labeled for 12–16 h with species-specific secondary antibodies (goat anti-rabbit) coupled to 12- and 30-nm gold beads, or to 6-nm and 18-nm gold beads. All FRIL replicas were viewed with a JEOL 2000 EX-II transmission electron microscope operated at 100 kV. Stereoscopic images (8° included angle) allowed assessment of the “sidedness” of the gold beads and the level of background immunogold labeling. Cell-specific ultrastructural markers were used to confirm cell identifications. FRIL images were correlated with confocal microscopic images obtained before SDS washing.

Acknowledgments

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Conclusion

The work reviewed here is a small part in a continuum of studies of poeciliid fishes. Previous and ground-breaking studies provided fundamental knowledge of the anatomy, physiology, and reproductive behavior of poeciliids. Data reviewed here show the following: Gambusia have a three-part, not a two-part, body plan. Male Gambusia engage in extraordinarily fast copulatory behavior that appears to be linked to a remodeling of the CNS that accompanies the radical sexually dimorphic remodeling of the male body plan. Gap junctions link four different neural cell types (i.e., three distinctive and identifiable motor neuron types and one distinctive and identifiable interneuron type) in the Gambusia spinal cord. These neural cells work together and may have a role in a distinct neural circuit that controls male Gambusia’s rapid copulatory behavior. Building on the foundational work that began in the mid 1800s and using modern tools and technologies will continue to increase understanding of how sex-specific behaviors are encoded by neural circuits.
References


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